Anaerobic Treatment of Phthalates,

Microbiological and Technological Aspects

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Stellingen

1 "Patience is the key for a successful start-up".

McHarg, W. H., (1990) Start-up report of the Kaohsiung wastewater treatment system. *Internal report*. Amoco Petrochemical Inc., Chicago, USA

Dit proefschrift.

2 Sommige anaërob moeilijk afbreekbare verbindingen kunnen toch met hoge snelheid worden afgebroken.

Dit proefschrift.

3 De voordelen van een tweetraps boven een ééntraps anaërobe reactor worden sterk onderschat.

Dit proefschrift.

4 Door de specifieke substraat consumptie snelheid (q) te schrijven als resultante van de groeisnelheid (μ) en de biomass yield (Y), volgens $q=\mu/Y$, wordt geïmpliceerd dat substraat omzetting het gevolg is van groei. Groei is echter het gevolg van substraat omzetting en zodoende verdient het inzichtelijk de voorkeur om de groeisnelheid te beschrijven als $\mu=Y_{XS}\cdot q$.

5 De relevantie van de waarneming dat groeisnelheden van syntrofe propionaat, butyraat en benzoaat oxyderende organismen hoger is in co-culturen met sulfaat reducerende bacteriën dan met hydrogenotrofe methanogene archea is beperkt, zolang niet is vastgesteld of dit het gevolg is van een hogere specifieke substraat omzettings-snelheid (q) of een hogere biomassa yield (Y).

Auburger, G. & Winter, J. (1995). Isolation and physiological characterization of *Syntrophus buswellii* strain GA from a syntrophic benzoate-degrading, strictly anaerobic coculture. *Appl. Microbiol. Biotechnol.*, **44**, 241-248.

Boone, D. R. & Bryant, M. P. (1980). Propionate-degrading bacterium, *Syntrophobacter wolinii* sp. nov. gen. nov., from methanogenic ecosystems. *Appl. Environ. Microbiol.*, **40**, 626-632.

McInerney, M. J., Bryant, M. P., Hespell, R. B. & Costerton, J. W. (1981). *Syntrophomonas wolfei* gen. nov. sp. nov., an anaerobic, syntrophic, fatty acid-oxidizing bacterium. *Appl. Environ. Microbiol.*, **41**, 1029-1039.

6 Om aan de hand van pH-profielen in methanogeen korrelslib, gemeten in niet-gebufferd medium, uitspraken te doen over de microbiële samenstelling van korrelslib en praktijkaspecten van anaërobe zuivering, getuigt van een erg beperkt inzicht in de anaërobe zuiveringstechnologie (vooral als in 5 mM fosfaat-buffer een volkomen vlak pH-profiel wordt gemeten).

De Beer D., Huisman J.W., Van den Heuvel J.C., Ottengraf S.P.P. (1992) The effect of pH-profiles in methanogenic aggregates on the kinetics of acetate conversion. *Wat. Res.* **26**: 1329-1336.

7 De ontwikkeling van anaërobe zuiveringstechnologie wordt gehinderd door het blindelings toepassen van concepten afkomstig uit de aërobe zuiveringstechnologie, zoals Monod-kinetiek en een constante slibleeftijd.

8 Wiskundige modellen kunnen een belangrijke rol spelen bij het verkrijgen van inzichten in complexe processen door de experimenteel vaak moeilijk te verwezenlijken onderverdeling in de verschillende deelprocessen. Zonder deze inzichten verwordt experimenteel onderzoek aan complexe (milieutechnologische) processen al snel tot "schieten in het duister"

9 Het is bevreemdend dat een proefschrift geschreven door een familielid op een enigszins verwant vakgebied, zo totaal onbegrijpelijk is voor de auteur van dit proefschrift. Aanvullend geldt dat kruisbestuiving tussen verwante vakgebieden wordt beperkt door een overdaad aan vaktermen.

Kleerebezem M. (1995). Protein export in Escherichia coli: involvement of Psp proteins, ribosomes and Pplase A. *Ph.D. thesis*, University of Utrecht, Utrecht, The Netherlands

10 Promovendi moeten meer "algemene" en minder "specifieke" vakliteratuur tot zich nemen.

11 Het vergt de communicatievaardigheden van een tweedehands autoverkoper om in de academische wereld te overleven.

12 Het meest beangstigende aan de invloed van de mens op het haar omringende milieu, is het tijdsbestek waarin deze invloed plaatsheeft.

13 Alles is interessant, maar niets doet ertoe.

Stellingen behorende bij het proefschrift "Anaerobic treatment of phthalates, microbiological and technological aspects" van Robbert Kleerebezem.

Renkum, 10 September 1999.

Anaerobic Treatment of Phthalates,

Microbiological and Technological Aspects

Robbert Kleerebezem

Proefschrift
Ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
dr. C.M. Karssen,
in het openbaar te verdedigen
op dinsdag 9 november 1999
des namiddags te half twee in de Aula

Cover: Background: Crater Lake, Oregon, USA
Left picture: isophthalate degrading methanogenic culture
Right picture: Influent pumps and piping of the UASB-reactor designed by Grontmij n.v. and treating PTA-wastewater in Tainan, Taiwan.

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Subject headings: anaerobic/hybrid/methanogenic/phthalic acid/UASB.

Abstract

Kleerebezem, R. (1999). Anaerobic treatment of phthalates, microbiological and technological aspects. *Doctoral Thesis*, Wageningen University, Wageningen, The Netherlands. Pp. 191.

Phthalic acid isomers (dicarboxy benzenes) play an important role in our human environment as constituents of polyester fibres, films, polyethylene terephthalate (PET) bottles and other plastics. Due to the use and generation of water during phthalic acid production from the corresponding xylenes, a concentrated wastewater is generated. The generated wastewater consists of a mixture of phthalic acid isomers, acetic acid, benzoic acid, and toluic acids. The aim of the work described in this thesis was to elucidate if anaerobic biological treatment may represent an attractive alternative for conventionally applied aerobic treatment methods. With regard to the anaerobic biodegradability of the phthalate isomers it was demonstrated that all three phthalate isomers could be degraded by two types of methanogenic granular sludge and digested sewage sludge. Lag-phases prior to degradation ranged from 17 to 156 days. More reduced aromatic analogues of the phthalates were not degraded or only at extremely low rates. Kinetic properties of the anaerobic degradation of the phthalate isomers were studied using enrichment cultures obtained from the biodegradability experiments or bioreactor biomass. The phthalate isomers grown cultures were capable of degrading only one of the phthalate isomers and degraded benzoate without a lag-period. A three species kinetic model enabled the dynamic description of intermediate acetate and molecular hydrogen accumulation and final formation of methane from the phthalate isomers and benzoate. It was shown that the syntrophic biomass cultivated had a low growth rate on the phthalate isomers ($\mu^{max} \approx 0.09$ day^{-1}). The energetic efficiency for growth on the phthalate isomers was found to be significantly smaller when compared to growth on benzoate, suggesting that an energetic inefficiency prevails in the degradation pathway of the phthalate isomers. The cultures were furthermore strongly inhibited and even deactivated by co-incubation with acetate or benzoate, or a short period of a few hours without substrate. Despite these unfavourable microbiological characteristics, it was demonstrated that highly active terephthalate degrading biomass could be cultivated at high concentrations in both UASB (Upflow Anaerobic Sludge Bed) reactors and hybrid reactors, resulting in high terephthalate removal capacities (15-20 g-COD(Chemical Oxygen Demand)$\cdot l^{-1} \cdot day^{-1}$). High-rate terephthalate degradation in the UASB-reactors was strictly dependent on inoculation of the reactor with granular biomass. After demonstrating that terephthalate as sole substrate could be degraded at high-rates, we studied the feasibility of a two-stage reactor concept for the treatment of terephthalic acid production wastewater, consisting of a mixture of readily degradable substrates (acetate and benzoate) and slowly degradable substrates (terephthalate and *para*-toluate). It was demonstrated that through pre-removal of acetate and benzoate in the first stage the lag-phase prior terephthalate degradation in the second stage could be significantly reduced (from 300 to approximately 50 days) and the wastewater could be treated at high volumetric removal rates and short hydraulic retention times (25 g-COD$\cdot l^{-1} \cdot day^{-1}$ and 6 hours respectively). For start-up of a two-stage anaerobic bioreactor system for treatment of terephthalic acid production wastewater, a gradual transition from initial operation in parallel to operation in series is suggested.

Table of contents

General Introduction

Abstract

Production and application of the petrochemical bulk-chemical terephthalic acid, its isomers and corresponding esters are described. Microbiological and technological aspects of the anaerobic degradation of aromatic compounds present in the waste generated during production of the phthalate isomers are discussed. Based on this information, the scope and objectives of this thesis are presented.

Contents

1.1 Introduction

The twentieth century can just as well be referred to as the age of organic chemistry as it can the atomic age. The dramatic increase in the production of synthetic fibres, processed foods, pesticides, plastics, pharmaceuticals and a thousand other materials has completely altered our immediate human environment and provided a wealth of new materials [40].

Another consequence of the chemical and petrochemical industry is that it transformed our natural environment, both intentionally and unintentionally. The enormous production of synthetic organic chemicals resulted in the release of a large variety of chemicals into the environment that play no, or only a limited role in natural life-cycles. Herewith the chemical and petrochemical industries have the doubtful honour of being the principal supplier of a type of waste that, due to its composition, is principally different from agroindustrial wastes. The ecological impact of both controlled and uncontrolled discharge of these primarily anthropogenic (xenobiotic) compounds into the natural environment has received extensive research attention during the past decades.

With world-wide an annual production of approximately 4 kilogram per capita, phthalic acid isomers (dicarboxy benzenes) and the corresponding esters comprise an important fraction of the world-wide produced amount of aromatic chemicals (approximately 20 % in the USA, [4]). These aromatic compounds are used in a wide range of plastics, synthetic fibres and resins. The diffuse introduction of phthalic acids and esters into the natural environment results from leaching from products, or through the non-bound application of phthalic acid esters as pesticide carriers, components of cosmetics, fragrances and insect repellents.

Nowadays, it is generally accepted that the human environment is the sole source of phthalic acid esters [40, 95]. Due to their extremely long half-life in abiotic and biotic environments, phthalic acid esters may be regarded as highly persistent and they have been detected in remote marine atmospheres [5, 40, 117, 118]. Recent findings concerning the presumed carcinogenic and xeno-oestrogenic properties of phthalic acid esters, and their possible role in the decreasing quality of sperm in the western world, has led to an increased public discussion concerning the application of these compounds as plasticizers in polyvinyl chloride (PVC) [23, 81].

Ortho, meta and para oriented phthalic acid isomers are ranked among the group of compounds which represent a low hazard potential for the general population and for the environment due to their relatively low level of toxicity and short half-life in biotic environments. The strong recalcitrance of the products that contain phthalic acid isomers,

furthermore results in a very slow diffuse release into the environment. During production of phthalic acid isomers, however, concentrated liquid and solid wastestreams are generated that may create severe environmental pollution around production plants. Soil, ground water, or surface water pollution occurs due to uncontrolled spillage or discharge of the generated waste. The work described in this thesis i focused on the treatment of these wastestreams.

The major research question underlying this research is whether anaerobic biological treatment represents an attractive alternative for, or contribution to, the conventionally applied aerobic treatment methods for wastestreams generated during the production of phthalic acid isomers. Anaerobic treatment methods offer a number of distinct advantages over aerobic treatment methods, such as lower nutrient requirements, lower sludge production, and energy production in the form of methane gas, instead of energy consumption due to aeration. However, so far only limited information is available concerning the fate of the aromatic constituents of these wastestreams in anaerobic bioreactors. This thesis is therefore focused on the anaerobic degradation of phthalate isomers, with emphasis on (i) the microbiological characterisation and kinetic optimisation of phthalate isomers degrading biomass, and (ii) the development of high-rate anaerobic bioreactors for treatment of wastewaters from production plants of phthalate isomers.

In this chapter various aspects of phthalic acid isomers will be discussed. The production and application of phthalic acids and esters are described in paragraph 1.2 and 1.3, showing that in our human environment we are surrounded by these compounds. Based on the chemical production processes, the properties of the generated wastestreams are presented in paragraph 1.4. Available information concerning the anaerobic biodegradability and toxicity of aromatic acids, as well as the biochemistry of the different steps in anaerobic degradation of aromatic compounds, are described in paragraph 1.5. Paragraph 1.6 provides a general introduction in anaerobic wastewater treatment with emphasis on its application to wastestreams from chemical industries. Based on the information described in the preceding paragraphs, the scope of this thesis is presented in paragraph 1.7.

1.2 Production

Phthalic acids and the corresponding esters are produced world-wide in massive amounts. The total annual production of phthalic acid isomers amounts to 18.5 million tons (Table 1.1 [97, 99]). Production of phthalic acids is generally based on the oxidation of their xylene analogues, derived from crude oil.

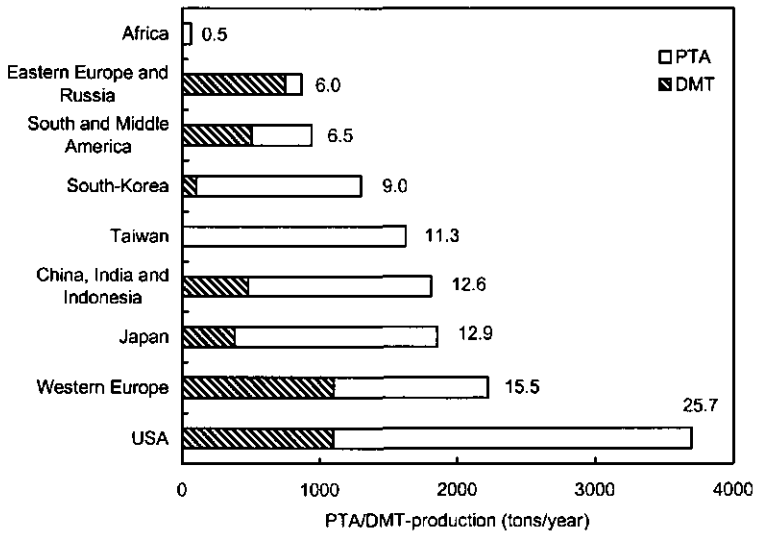


Figure 1.1: Geographical distribution of the production of Purified Terephthalic Acid (PTA) and Dimethyl Terephthalate (DMT). Data-labels represent the percentage of the total PTA and DMT production of 14,931 thousand tons (31 % DMT) in 1993 [99].

Purified terephthalic acid (PTA) and dimethyl terephthalate (DMT) did not become important industrial chemicals until after World War II. Since that time, the demand for polyethylene terephthalate fibres rapidly increased, initially resulting in a rapid growth of DMT production. After reaching an annual production of approximately 3 million tons per year by the end of the 1970's, production of PTA and DMT seemed to stagnate due to limitations in the polyester fibre market in the early 1980's. However, as new markets were found (see Paragraph 1.3), the production increased again with an annual growth of approximately 5 % since 1983. During the past 10 years production mainly increased in south-east Asia, resulting in the current dominance of this region in the production of PTA and DMT, besides the USA and Western Europe (Figure 1.1).

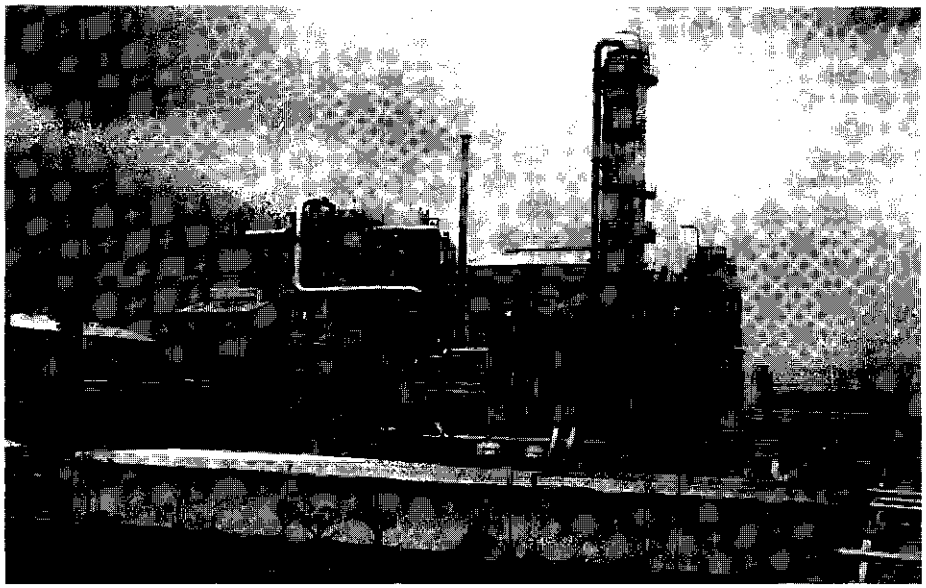


Figure 1.2: CTA-production unit from Tuntex, located in Tainan, Taiwan

Initially, commercial production of polyethylene-terephthalate fibres was based on esterification of DMT and ethylene glycol. DMT remained the dominant chemical for polyester production until the 1980's. The Hercules Inc./Dynamit Nobel A.G. process represents the dominant technology for the production of dimethyl terephthalate. The process involves four steps which alternate between liquid-phase oxidation and liquid phase esterification. The principal raw materials are *para*-xylene and methanol.

The use of PTA instead of DMT for the production of polyethylene terephthalate offers distinct cost advantages to the manufacturer, because polyester production processes using DMT must be designed for recovery of methanol, while processes based on PTA do not need these facilities. Furthermore, PTA provides a higher yield of polyester per kilogram of starting material, and less ethylene glycol is needed in the polyesterification which improves the final polymer quality. For these reasons PTA has become the dominant intermediate in polyester production, with an annual production of approximately two times that of dimethyl terephthalate [99].

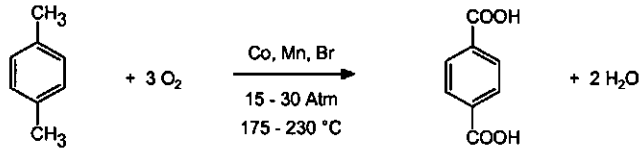


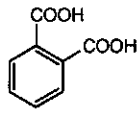
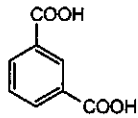
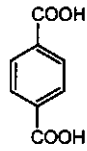
Figure 1.3: Reaction equation for chemical oxidation of para-xylene to terephthalic acid and water with molecular oxygen. The catalytic system consists of cobalt and manganese ions, and a bromine based promoter. Acetic acid is used as solvent.

The commercial production of PTA is based on catalytic, liquid phase air oxidation of *para*-xylene according to the reaction equation shown in Figure 1.3. Most production processes are based on the widely accepted Amoco technology [30, 37, 89]. A typical flow-sheet for a process based on Amoco technology is shown in Figure 1.4. In the first step of the process crude terephthalic acid (CTA) is produced. Acetic acid, *para*-xylene and catalyst are supplied continuously to the oxidation reactor. The catalytic system consists of cobalt and manganese and a bromine based promoter. The oxidation reactor is operated at a temperature of 175-230 °C and a pressure of 1500-3000 kPa. Air is added in excess to the stoichiometric amount to minimise the formation of by-products and the heat of the reaction is removed by condensing and refluxing acetic acid. The yield of CTA exceeds 90 mole %. The effluent from the oxidation reactor consists of a slurry due to the poor solubility of terephthalic acid. The CTA is recovered from the slurry by centrifugation and subsequently the crystals are washed with water and dried. The mother liquor from the centrifuge step is purified in a residue still and a dehydration tower and recycled to the oxidation reactor.

CTA is not suitable as raw material for polyester production. Therefore a separate purification step should be applied to obtain polymer grade terephthalic acid (PTA). In the Amoco purification process, CTA and water are fed into a mixing tank. The slurry is pumped into a pre-heater, operated at temperatures higher than 250 °C. The resulting solution flows through a hydrogenation reactor, which contains a noble-metal catalyst on a carbon support. The overall effect of the hydrogenation step is conversion of impurities to forms that remain in the mother liquid during subsequent crystallisation. After crystallisation, PTA is recovered through centrifugation and drying.

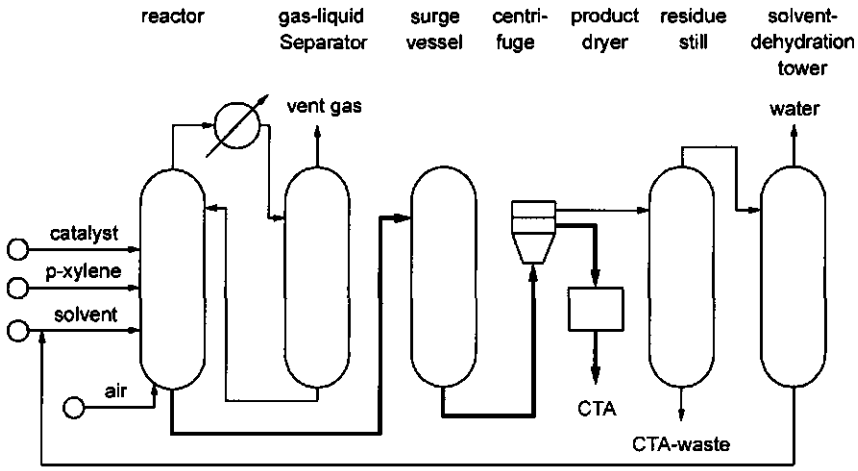
The oxidative production of isophthalic acid from *meta*-xylene proceeds very similar to that of terephthalic acid. Because isophthalic acid dissolves better than terephthalic acid in reaction solvents (Table 1.1), crystallisation equipment is more important.

Table 1.1: Properties of the phthalic acid isomers [9, 11, 40, 78, 97, 99].

chemical properties				
structure				
common name		phthalic acid	isophthalic acid	terephthalic acid
IUPAC name		1,2-benzene-dicarboxylic acid	1,3-benzene-dicarboxylic acid	1,4-benzene-dicarboxylic acid
CAS registry no.		[88-99-3]	[121-95-5]	[100-21-0]
pK_1		2.95	3.62	3.54
pK_2		5.41	4.60	4.46
solubility, g/100 g water	25 °C	0.7	0.013	0.0019
	100 °C	19.0	0.24	< 0.04
production:				
raw material		*o*-xylene (naphthalene)	*m*-xylene	*p*-xylene
commercial product		phthalic anhydride → phthalic acid esters (PAE)	isophthalic acid	terephthalic acid (dimethyl terephthalate)
main use		plasticizer in PVC	unsaturated polyester resins	polyethylene terephthalate
annual production, million tons (1991-1993)		3.3	± 0.8	9.8 (4.6)[1]
environmental aspects				
acute LD_{50} rats, g/kg		7.9	12.2	> 15 (15.3)[1]
explosibility index		>10	4	6.9 (>10)[1]
environmental distribution		widely distributed (anthropogenic: PAE's)	-	traces in air and domestic sewage
natural occurrence		intermediate in biodegradation of PAH	traces in lignite and the rhizome of the iris plant	traces in *Zizyphus sativa*

[1] Values between brackets represent values for dimethyl terephthalate

Step 1: Crude, or technical-grade, Terephthalic Acid (CTA) production by catalytic, liquid-phase air oxidation of *p*-xylene.



Step 2: The Amoco purification process for production of Purified, or polymer-grade, Terephthalic Acid (PTA).

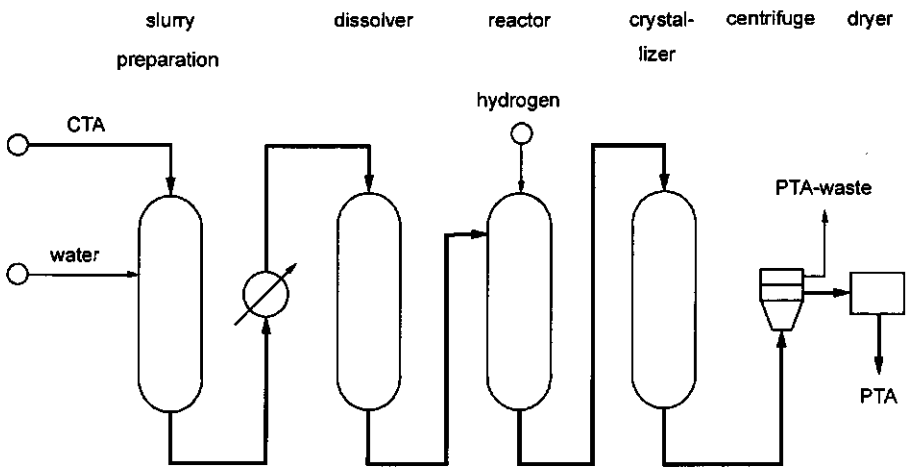


Figure 1.4: Two-step production process of Purified Terephthalic Acid (PTA). Thick lines indicate the flow of the substrate/product.

Commercially, phthalic acid is produced in its anhydride form. Until World War II, phthalic anhydride was manufactured by BASF's method for oxidation of naphthalene, obtained from coal tars, by sulphuric acid in the presence of mercury salts. This process was already patented in 1896. Due to a shortage in naphthalene since the late 1950's, many companies started to use *ortho*-xylene for phthalic anhydride production. Since then, fixed-bed vapour-phase oxidation of *ortho*-xylene has become the dominant production process. Multitubular fixed bed reactors, containing vanadium oxide and titanium dioxide catalyst, operated at a temperature of 380 - 400 °C are generally used. Phthalic anhydride is recovered from the cooled reactor effluent through switch condensers, where 99.5 % of the phthalic anhydride solidifies on cooled tubes. During the next step, hot oil is charged through the switch condensers in order to melt the anhydride, and the product is drained into a tank. Crude phthalic anhydride is heated at atmospheric pressure in order to dehydrate traces of phthalic acid and convert impurities to high boiling compounds. Purification is obtained in two continuous distillation columns for light-ends and product fractionation respectively.

1.3 Application

Dimethyl terephthalate (DMT) and polymer-grade terephthalic acid (PTA) are petrochemicals that are used interchangeably as raw material for the production of polyester. The principal outlet for polyester is in textile fibres. Other applications for terephthalic acid resins, however, increase rapidly and start to count in terms of their consumption. In particular this applies for polyethylene terephthalate (PET) bottles, that are now widely used for carbonated drinks. The production of PET from terephthalic acid and ethylene glycol involves two steps according to the reaction equations shown in Figure 1.6. The first step leads to monomer formation in an uncatalyzed direct esterification reaction. The monomer is polymerised in the second step in the presence of an antimony catalyst. Chain extension is promoted by removal of excess glycol from the viscous melt with carefully controlled agitation [22, 41].

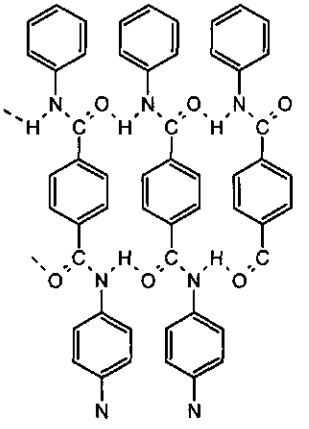


Figure 1.5: chemical structure of Kevlar, Du Pont.

Compared to bottles manufactured from polyvinyl-chloride (PVC), PET-bottles offer the advantages that they retain the pressure, can be recycled, and can be incinerated without the

production of hydrochloric acid (HCl). The PET-market is continuing to grow at an annual rate of 8 to 12 % and represents a world consumption of terephthalic acid of more than 1 million tons per year. Another important use of PTA and DMT is in polyester films, which are applied in the audio-visual, photographic, computer, packaging and decorative fields. Finally there are the technical plastics: polyethylene and polybutylene terephthalates, polyarylates, elastomers and various resins. Within these relatively new outlets, high tensile strength fibres (Kevlar, Du Pont and Twaron, Akzo) comprise a special group. These extended chains aramids are the condensation product of terephthaloyl chloride and *para*-phenylenediamine (see Figure 1.5) and they have a higher modulus and tensile strength on an equal weight basis than glass or steel. Due to these exceptional properties they are used in reinforcing composite structures, including tires, bullet-proof vests, boat hulls and drilling platform anchor cables [37, 91]. These outlets are still small in volume, but grow rapidly [11, 98].

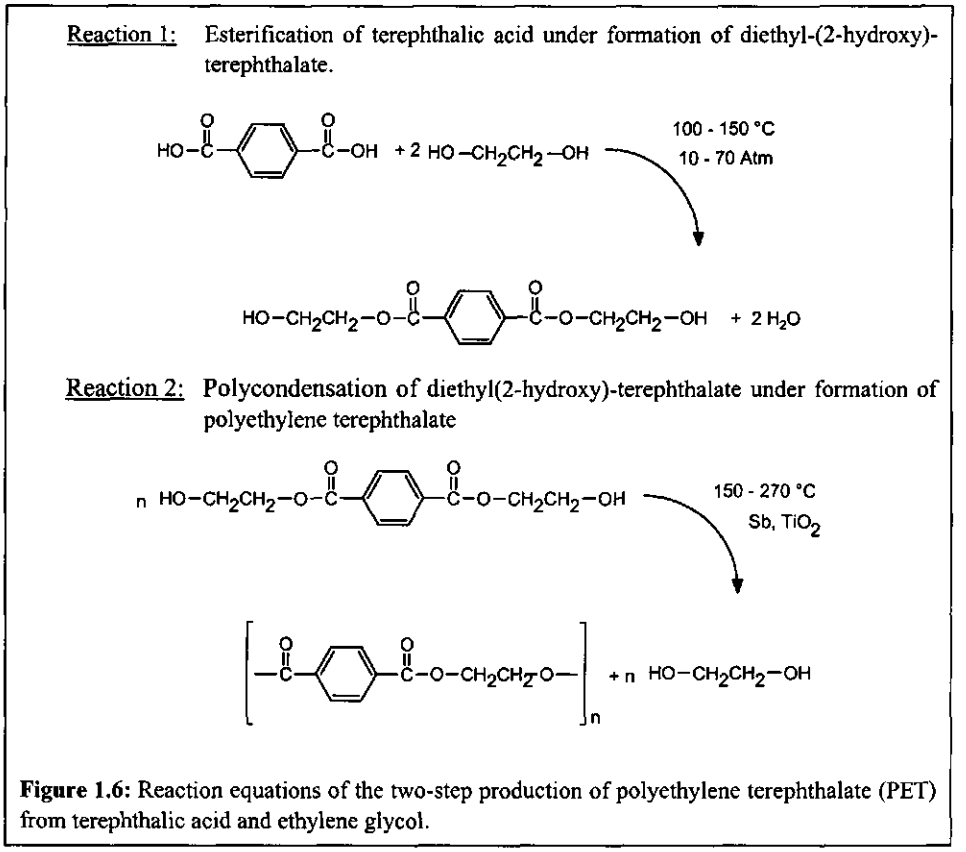


Figure 1.6: Reaction equations of the two-step production of polyethylene terephthalate (PET) from terephthalic acid and ethylene glycol.

Isophthalic acid is produced in much smaller quantities than the *ortho* and *para* oriented isomers (Table 1.1). More than 50 % of the isophthalic acid produced is used to prepare unsaturated polyester resins. These are polymers of isophthalic acid and unsaturated dibasic acids, e.g. maleic anhydride, and a glycol. Compared to *ortho*-phthalic acid based polyesters, isopolyesters offer improved water, chemical and impact resistance, but they are more expensive. They can be used with glass-fibre reinforcements in chemical-storage tanks, piping, swimming pools and boats, or without glass fibre reinforcements in coatings and vessel linings. The increase in the consumption of isophthalic acid during the past ten years is mainly due to chemically resistant, unsaturated polyester resins [11].

Approximately 60 % of the downstream market for phthalic anhydride is in plasticizers and 35 % in alkyl and unsaturated polyester resins [97]. Phthalic anhydride is converted to phthalic acid esters through reaction with alcohols for its application as plasticizer, mainly in polyvinyl chloride (PVC). The most widely applied phthalic acid esters are di-2-ethylhexyl phthalate and dibutyl phthalate. The demand for phthalic anhydride in plasticizers depends on the polyvinyl chloride market, which is large but restricted by concern about the toxicity of vinyl chloride monomers. Resins based on phthalic anhydride are used widely in the construction, marine, and synthetic marble industries [11, 40].

1.4 Generated waste

A general description of (petro)chemical wastewater can not be provided. They range from non-complex, with volatile fatty acids as main pollutants, to complex, with high concentrations of organic solvents or aromatic compounds. A general characteristic of petrochemical wastewater, however, is the strong fluctuations in pH, temperature and strength, that may occur due to maintenance activities or upsets in the production process. Normally, chemical wastewaters contain no macro- and micronutrients as required for biological treatment.

During production of phthalic acids, both solid and liquid wastestreams are generated. All wastestreams can be regarded as high strength and complex due to the high concentration aromatic compounds. Besides the generation of these wastestreams, soil contamination due to accidental spillage around production facilities is frequently encountered [57, 82]. In this paragraph the wastestreams generated during production of the different phthalic acid isomers will be described in relation to the corresponding production processes described in paragraph 1.2.

In both steps of purified terephthalic acid (PTA) production liquid wastestreams are generated (Figure 1.4). During production of crude terephthalic acid (CTA), condense water from the solvent dehydration tower is generated, which contains mainly acetic acid and small amounts of volatile solvents like methylacetate. Furthermore a solid residue is generated during distillation of the mother liquid. This waste consists mainly of terephthalic acid and, when dosed to the wastewater, it may contribute to more than 50 % of the generated organic pollution. During purification of crude terephthalic acid a wastewater is released, containing mainly terephthalic and *para*-toluic acid. Both wastestreams generated during crude terephthalic acid production are characterised by a low flow, and a high level of contamination, while during the purification step more dilute wastewater is generated at higher flows. Table 1.2 presents the generalised characteristics of the combined wastewaters generated during both steps of PTA production. Large differences in the composition of the wastes generated at different PTA production plants may be due to (i) differences between the production processes applied, (ii) addition of the solid residue to the wastewater or (iii) pre-removal of terephthalic acid in an acidification-sedimentation step. The wastewater may contain a large variety of organic compounds, but the four major compounds indicated in Figure 1.7 normally represent 80-95 % of the total Chemical Oxygen Demand (COD) load. In addition to the minor compounds shown in Figure 1.7, brominated aromatic compounds, di- and tri phenyl carboxylic acids and isomers of the compounds shown can be present [96]. Besides the organic pollutants, cobalt, manganese and bromine from the catalytic system are present in the wastewater in concentrations up to 30 ppm.

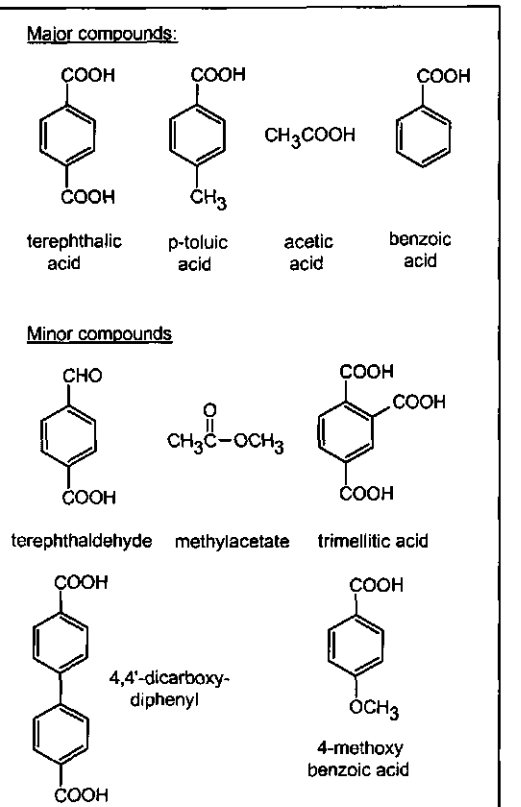


Figure 1.7: Major organic pollutants in effluents generated during terephthalic acid production, and some examples of minor organic pollutants.

Due to the highly comparable production processes for isophthalic acid and terephthalic acid, wastestreams generated during these processes are comparable in composition. Obviously, the main difference is that the major amount of aromatic compounds in the waste generated during isophthalic acid production is *meta*-oriented, while *para*-oriented aromatics pre-dominate in waste generated during terephthalic acid production [26].

Table 1.2: Generalized characteristics of wastewater generated during production of purified terephthalic acid (PTA) and dimethyl terephthalate (DMT) [21, 63, 68, 76, 90, 92, 94, 102, 119].

production	PTA	DMT
COD, ($g \cdot l^{-1}$)	4 - 20	15 - 140
COD/BOD_5, (-)	1.4 - 1.7	1.1
pH (-)	4 - 5	1.5 - 3
Temp. (°C)	40 - 60	40 - 60
composition[1]		
TA ($g \cdot l^{-1}$)	1.0 - 5.0	0.4 - 0.5
BA ($g \cdot l^{-1}$)	0.1 - 1.5	0.1 - 1.5
PT ($g \cdot l^{-1}$)	0.3 - 1.0	1.0 - 2.0
C2 ($g \cdot l^{-1}$)	0.5 - 7.0	2.5 - 40
MOH ($g \cdot l^{-1}$)	-	0.5 - 76
FOA ($g \cdot l^{-1}$)	-	1.5 - 7.5
C1 ($g \cdot l^{-1}$)	-	1.2 -- 13

[1] TA: terephthalic acid, BA: benzoic acid, PT: *p*-toluic acid, C2: acetic acid, MOH: methanol, FOA: formaldehyde, C1: formic acid

A major difference between the production of phthalic acids and dimethyl terephthalate (DMT) is the use of methanol in the latter process. This difference has a large impact on the generated waste during dimethyl terephthalate production, as can be seen from the data presented in Table 1.2. Major organic pollutants in DMT-wastewater are methanol, acetic acid and formaldehyde, while the concentrations of aromatic compounds are comparatively low. Depending on the efficiency of methanol recovery, the wastewater may contain very high concentrations methanol.

To limit transport expenses, PTA- or DMT-production plants are frequently located close to polyethylene-terephthalate (PET) production plants. Recently a combined PTA/PET-production plant has been built in Europoort, The Netherlands, by Eastman Petrochemical. Wastewater generated in both production processes will be treated in an activated sludge plant, followed by slow sand filtration for polishing of the aerobic effluent. Solid waste generated during distillation of acetic acid, as well as surplus sludge produced in the aerobic treatment will be incinerated in a fluidized bed incinerator. Like during PTA-production, a concentrated wastestream is generated during PET-production. The main source of this waste

is the water produced during two-step esterification of terephthalic acid with ethylene glycol (see Figure 1.6). Approximately 0.5 m^3 wastewater is generated per ton PET produced with a COD-concentration of 10-25 $kg \cdot m^{-3}$, a pH of 2-7 and a temperature of 35-40 °C. Dominant organic pollutants in this wastestream are ethylene glycol, acetic acid, acetaldehyde and/or methyl acetate. Like in the case of PTA and DMT production, the wastewater strength and composition depend strongly on the type of production process applied, as well as the efficiency of the recovery units and/or physical-chemical pre-treatment steps.

During production of phthalic anhydride, vent gases are generated in the switch condensers (see paragraph 1.2). These vent gases contain by-products and traces of phthalic anhydride and therefore, they are usually scrubbed before being vented to the atmosphere. The wastewater produced in the scrubber contains maleic acid (*cis*-1,2-dicarboxy ethene) and small amounts of phthalic, benzoic and citraconic acids (*cis*-1,2-dicarboxy-methyl ethene). During purification of crude phthalic anhydride in two continuous distillation towers, concentrated wastestreams containing maleic anhydride, benzoic acid, toluic acids and high boiling residues are produced [11]. No information is available regarding concentrations of the different compounds, as well as wastewater quantities produced.

1.5 Anaerobic microbiology

Biodegradability. During the past decades the fate of aromatic compounds in methanogenic environments has been studied extensively. It has been demonstrated that most mono-aromatic compounds with at least one phenolic or carboxylic group can be degraded completely to methane and carbon dioxide using inocula from different methanogenic habitats [8, 18, 47, 53, 71]. Recently, it even has been shown that some aromatic hydrocarbons like toluene and *ortho*-xylene can be mineralised under methanogenic conditions [27].

Interest in the environmental fate of phthalic acid esters arises from their ubiquity in the environment and their potential carcinogenic and pseudo-oestrogenic properties [40]. The most frequently identified phthalic acid esters in the environment are di-2-ethylhexyl phthalate and di-n-butyl phthalate. These compounds were found to be widely distributed in sediments, natural waters, plants and aquatic organisms [5, 40]. Because the abiotic removal of phthalic acid esters is very slow, with half lifes for chemical hydrolysis ranging from 4 months to over 100 years [117, 118], the principal method for removal from the environment is microbial activity. Both in aerobic and anaerobic environments organisms are available with the ability to metabolise several phthalic acid esters. Complete anaerobic mineralization has been demonstrated for dimethyl phthalate, diethyl phthalate, dibutyl phthalate and butyl-

benzyl phthalate. Anaerobic degradation of these compounds was observed using digested sewage sludge [86, 104, 123], anaerobic freshwater or salt marsh sediments [55, 88] and municipal solid waste under landfilling conditions [28]. No, or only partial anaerobic degradation was obtained with di-2-ethylhexyl phthalate.

The anaerobic biodegradability of phthalate isomers has hardly been studied. However, as phthalate is the principal intermediate in the anaerobic breakdown of phthalic acid esters, it is generally assumed that phthalate degrading organisms are widely available in methanogenic environments [8, 28, 105]. Information concerning the anaerobic biodegradability of isophthalate and terephthalate is only available from technological studies [26, 90]. Van Duffel [26] showed that both terephthalate and isophthalate were degraded in a low-loaded down flow fixed film reactor (see paragraph 1.6).

Toxicity. Until recently methanogenic organisms were considered to be highly susceptible to a large variety of toxic organic chemicals. Blum and Speece [13], however, demonstrated that aerobic heterotrophs and methanogenic bacteria show similar sensitivities to most organic toxicants. The common method applied for determination of the toxicity of organic chemicals to methanogenic bacteria is usually based on the method described by Owen et al. [87].

Table 1.3: Methanogenic toxicity of 4 industrially important phthalic acid esters at a concentration of 20 $mg \cdot l^{-1}$ [86] related to their chemical properties.

phthalates	sol. [ppm]	log K_{OW}	inhibition [%]
dimethyl	4.3	1.53	40
diethyl	900	2.35	94
dibutyl	11	4.57	6
di-2-ethylhexyl	0.04	9.64	63

The toxicity of aromatic compounds to methanogens was demonstrated to be strongly correlated to the lipophilic character of the compounds, expressed as the logarithm of the octanol-water partition coefficient (log K_{OW}) [25, 106]. Lipophilic compounds preferentially reside in the cytoplasmic membrane and interfere with essential membrane functions [50, 108]. The toxicity of aromatic compounds furthermore increases by the presence of reactive functional groups, like nitro or aldehyde-groups [13, 25].

The only data found dealing with the methanogenic toxicity of phthalic acid esters are summarized in Table 1.3. From the data in this table can be seen that no clear relationship exists between the log K_{OW} and the percentage inhibition obtained with the different phthalic acid esters. Therefore other mechanisms of toxicity besides interference with the cytoplasmic membrane (e.g. water solubility) appear to play a role.

In pH-neutral environments, the well soluble salt form of the phthalic acid isomers prevails (for pK_a-values, see Table 1.1). These phthalate isomers are more polar than benzoate and therefore are expected not to cause severe toxicity to methanogenic organisms (IC_{50} for benzoate is > 40 mM [25, 106]). This has been confirmed by Fajardo et al. [33], who observed no toxicity to the acetoclastic methanogenic activity in methanogenic granular sludge at a terephthalate concentration of 10 mM, and only 40 % inhibition at 100 mM for the hydrogenotrophic methanogenic activity. Since the concentration of phthalate isomers in the wastestreams generated during their production, do normally not exceed approximately 20 mM, little if any toxic effects are expected in anaerobic bioreactors treating these wastestreams. Also the more toxic terephthalic acid production wastewater constituents, *para*-toluate and terephthaldehyde, are expected to cause no toxic effects in anaerobic bioreactors, due to their lower concentrations in the generated wastewater [33].

Metabolic pathways and energetics. The basic difference between the aerobic and anaerobic metabolism of aromatic compounds exists in the way the highly stable aromatic ring is cleaved [3]. In aerobic environments, aromatic compounds enter peripheral pathways through removal of functional groups and introduction of hydroxyl functions to the aromatic ring. The aromatic ring of the resulting central intermediates catechol, protocatechuate and gentisic acid, are relatively easy to cleave oxidatively, through the action of dioxygenases [39, 48, 101].

In anoxic environments the aromatic ring cannot be cleaved by dioxygenases, and ring cleavage therefore proceeds only after reduction of the aromatic ring, as proposed by Evans [32]. Prior to ring cleavage, aromatic compounds enter peripheral pathways through removal or modification of functional groups as shown in Figure 1.8. The most common central intermediate in the anoxic degradation of aromatic compounds is the thioester of coenzyme A with benzoate, benzoyl-CoA. During the past two decades several metabolic pathways have been proposed for the anoxic degradation of benzoate [29] and one of the latest versions was described by Koch et al. [48, 59] as shown in Figure 1.9. Under standard conditions the overall conversion of benzoate into acetate and hydrogen is energetically unfavourable:

$$C_7H_5O_2^- + 7H_2O \longrightarrow 3C_2H_3O_2^- + 3H^+ + 3H_2 + HCO_3^- \qquad \Delta G^{0'} = 63.1 \text{ kJ/reaction}$$

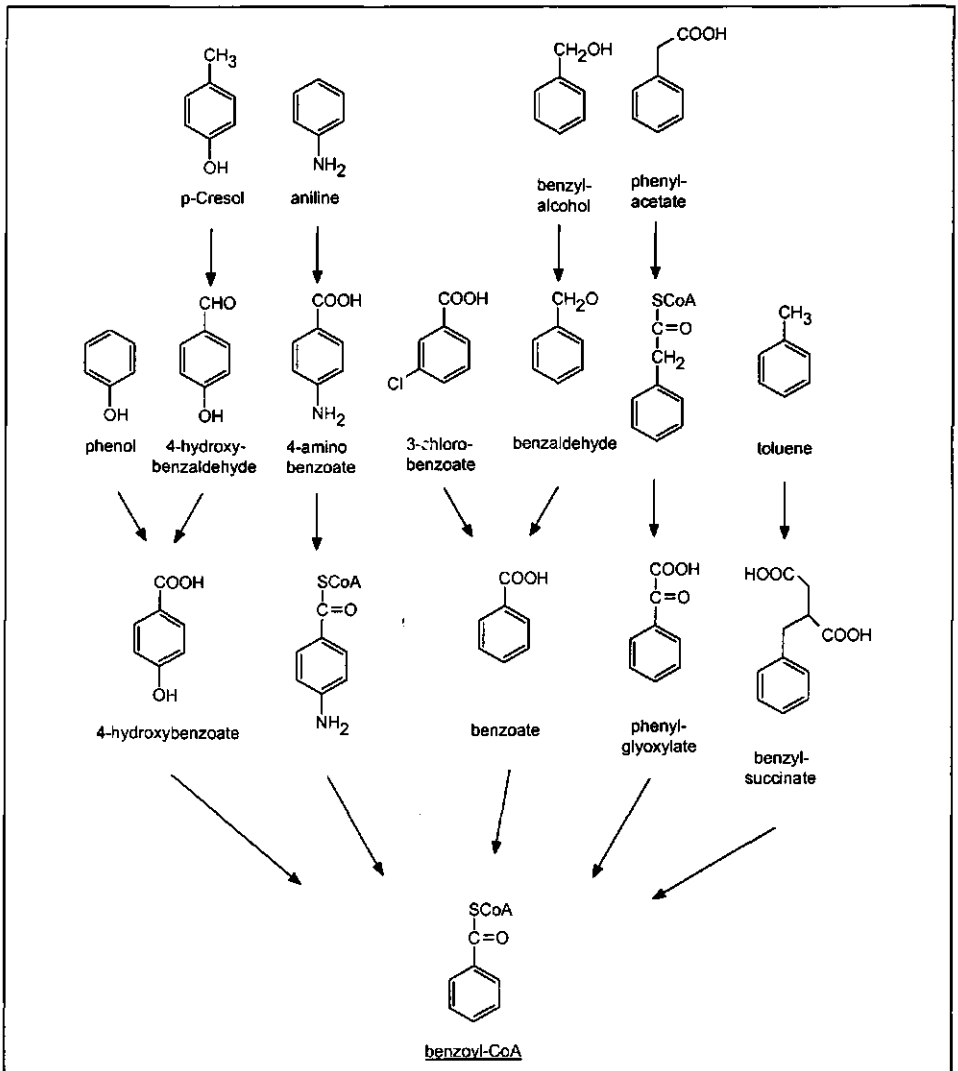


Figure 1.8: Channelling reactions transforming some aromatic compounds into the central intermediate benzoyl-CoA (after Heider and Fuchs [48]).

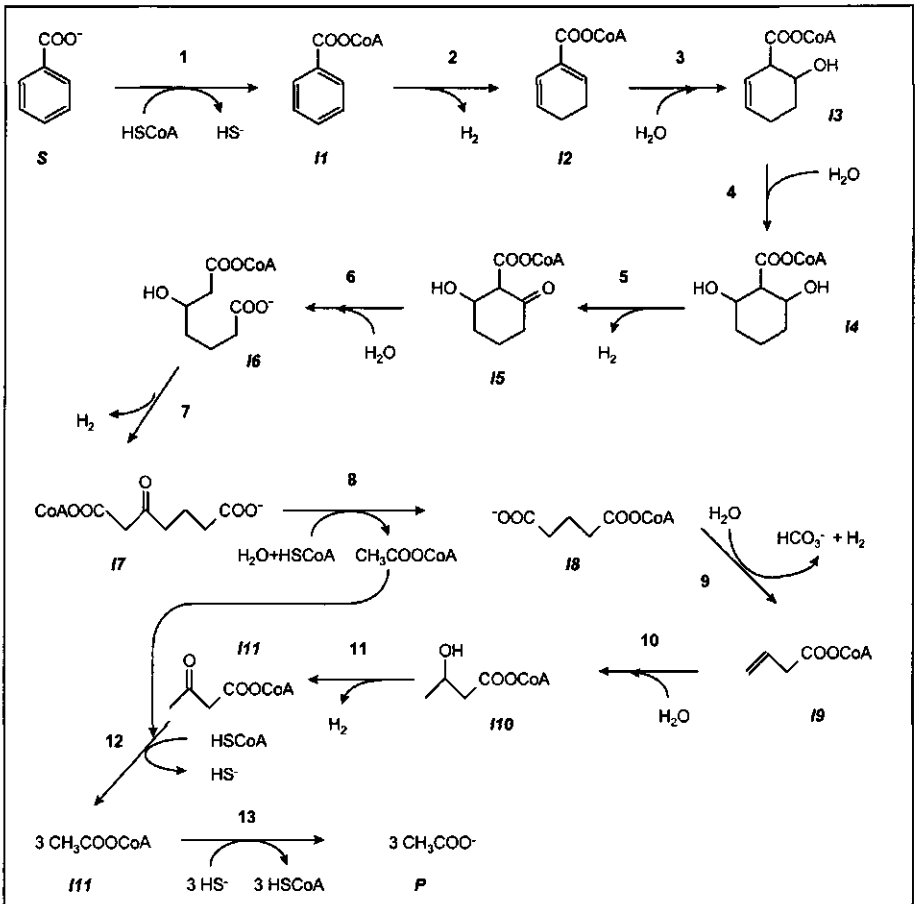


Figure 1.9: Pathway of anaerobic benzoate degradation in *Thauera aromatica* [48]. S = substrate, I = intermediate and P = product.

As a result of the mineralization of the products acetate and hydrogen in nitrate, sulfate or iron reducing organisms, the reaction can be pulled to the product-side. For nitrate reduction the overall mineralization of benzoate therewith becomes:

$$C_7H_5O_2^- + 6NO_3^- + 6H^+ \longrightarrow 6CO_2 + HCO_3^- + 5H_2O + 3N_2 \quad \Delta G^{0'} = -3011 \text{ kJ/reaction}$$

Likewise, the complete conversion of benzoate into a mixture of methane and carbon dioxide as observed in methanogenic environments is an energetically favourable reaction under standard conditions:

$$C_7H_5O_2^- + 4.75H_2O \longrightarrow 3.75CH_4 + 2.25CO_2 + HCO_3^- \qquad \Delta G^{0'} = -128.3 \text{ kJ/reaction}$$

In methanogenic environments, however, no organisms are available that are capable to catalyse this reaction, because the substrate spectrum of methanogenic bacteria is restricted to molecules with 1 to 3 carbon-atoms [77, 116]. The degradation of benzoate therewith depends on the presence of both fermentative organisms, converting benzoate into a mixture of acetate and hydrogen according to the pathway shown in Figure 1.9, and methanogens, converting acetate and hydrogen into methane. Only if the methanogens can keep the intermediate concentrations sufficiently low, the fermentation of benzoate becomes energetically favourable (see Figure 1.10). In view of their mutual dependency, these mixed methanogenic consortia are referred to as syntrophic cultures [100, 109].

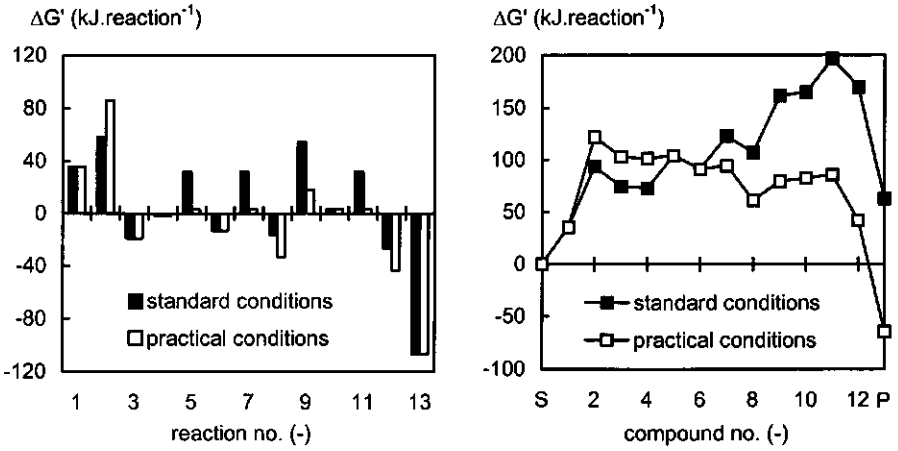


Figure 1.10: The (Gibbs) free energy change of the different steps of anaerobic benzoate degradation, shown in Figure 1.9. The left graph shows the free energy change of the individual steps of the pathway and the right graph the cumulative free energy change. "Practical conditions" are characterised by liquid concentrations of 1 mM and a hydrogen partial pressure of 1 Pa. The free energy changes were calculated as described by Thauer *et al.* [113]. If no data were available for specific compounds, values were calculated from known compounds using group contribution methods [24, 79, 80]

From the free energy changes of the individual reactions in Figure 1.9, as shown in Figure 1.10, it can be seen that the initial activation and reduction of the aromatic ring are the main biochemical bottlenecks in the anaerobic fermentation of benzoate. To overcome the activation energy for the initial aromatic ring reduction, benzoate is activated with CoA to obtain an active site for the benzoyl-CoA reductase enzyme and to allocate the π-electrons in the aromatic ring. This step has been demonstrated for several organisms including the benzoate fermenting culture *Syntrophus buswelli* strain GA [6, 7]. This first step requires energy input in the form of 1 ATP (Adenosine Tri-Phosphate) per benzoate ($\Delta G^{0'} \approx 44$

kJ/mole [7, 48]). The next step is reduction of the aromatic ring with benzoyl-CoA reductase ($\Delta G^{0'} \approx 55$ kJ/mole [101]). In the denitrifying organism *Thauera Aromatica* this step requires 2 ATP per benzoyl-CoA [14]. The initial investment of 3 ATP per benzoate imposes no problem in denitrifying bacteria, because the final product of benzoate fermentation, acetyl-CoA, enters the citric acid cycle and through anaerobic nitrate-respiration a multiple amount of ATP is generated.

Whether a similar energetically expensive method for aromatic ring reduction is applied in benzoate fermenting organisms is doubtful, because only 3 ATP is generated during substrate level phosphorylation of acetyl-CoA (reaction 13, Figure 1.9 [101]). This suggests that no net-ATP is generated, making microbial growth impossible. Therefore, either the initial reduction of the aromatic ring requires less ATP, or more ATP is generated during the later steps of the fermentation of benzoate. A one step reduction of benzoyl-CoA (involving 4 or 6 electrons) to carboxycyclohexene or carboxycyclohexane, would require less energy input and cannot be excluded to occur in benzoate fermenting cultures. An alternative method for energy generation in benzoate fermenting bacteria may be through generation of electron motive force induced by acetate excretion. In summary it is suggested that a lot of questions remain to be solved with regard to benzoate fermentation in syntrophic cultures.

Besides benzoyl-CoA, aromatic compounds with two or three phenolic hydroxyl groups in *meta*-position to each other (resorcinol and phloroglucinol) are susceptible to aromatic ring reduction. Due to their low level of aromaticity, these compounds do not require energy input for reduction [19, 20, 114].

The initial step in the mineralization of phthalic acid esters is hydrolysis of the ester side chains, resulting in formation of monoalkyl phthalate and phthalate. The esterases involved in the initial attack of the ester side chain, have no requirement for oxygen, and they therefore occur in aerobic, denitrifying and methanogenic environments [12, 31, 95, 104, 112].

Aerobic mineralization of phthalate isomers has been reported for several genera of bacteria, like *Pseudomonas, Micrococcus* and *Nocordia*. The main pathway for the aerobic degradation of ortho-phthalate (Figure 1.11) is characterised by an initial hydroxylation of the aromatic ring in the 3-4 position (or 4-5 position). The next step is decarboxylation, resulting in formation of protocatechuate, one of the central intermediate in the aerobic mineralization of aromatic compounds. Terephthalic acid and iso-phthalic acid are metabolized in a similar pathway, converging at protocatechuate [95].

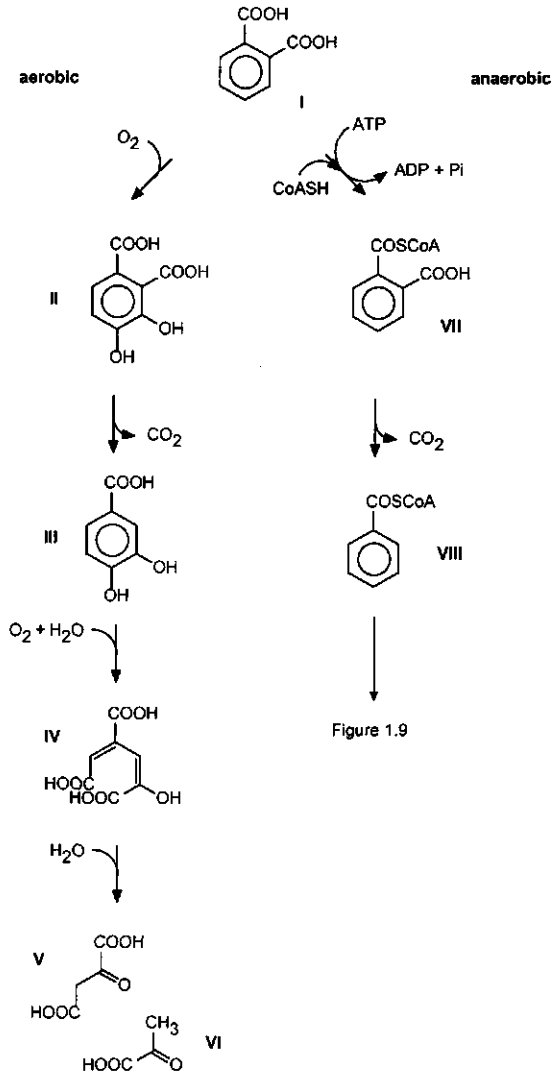


Figure 1.11: Main pathways for aerobic and anoxic mineralization of ortho-phthalate by *Micrococcus* sp. strain 12 B [95] and *Pseudomonas* sp. strain P136 [84] respectively. Compounds shown are o-phthalate (I), 3,4-dihydroxy-phthalate (II), protocatechuate (III), 4-carboxy-2-hydroxy-muconate (IV), oxalacetic acid (V), pyruvate (VI), o-phthalyl-CoA (VII), benzoyl-CoA (VIII).

The anoxic metabolism of phthalate isomers has only been studied with denitrifying bacteria [1, 2, 84, 85, 112]. Using a *Pseudomonas* sp., Nozowa and Maruyama [84, 85] demonstrated that all three phthalate isomers are first activated with Coenzyme A, followed by decarboxylation resulting in the formation of benzoyl-CoA, the central intermediate in the anoxic degradation of aromatic compounds (Figure 1.11). Even though the decarboxylation of the phthalate isomers to benzoate is an exergonic process under standard conditions ($\Delta G^{0'}$ ≈ -20 kJ/mole), some controversy exists concerning the mechanism of this step. Taylor and Ribbons [112] suggested that the decarboxylation of phthaloyl-CoA to benzoyl-CoA may involve two steps: an initial partial reduction of the aromatic ring (either through a one or two electron mechanism), followed by oxidative decarboxylation (Figure 1.12). Like the initial reduction of benzoyl-CoA, however, the reduction of phthaloyl-CoA would require energy input and whether this invested energy can be regenerated during oxidative decarboxylation, remains to be elucidated.

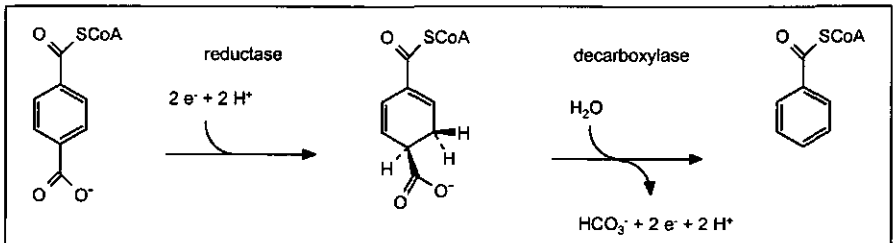


Figure 1.12: Two step decarboxylation of terephthaloyl-CoA to benzoyl-CoA as proposed by Taylor and Ribbons [112]

Hardly any information is available about the anaerobic mineralization of phthalate isomers under sulphate reducing or methanogenic conditions. It has been suggested that in methanogenic environments, degradation of *ortho*-phthalate proceeds analogue to the degradation in denitrifying environments, because methanogenic enrichment cultures grown on ortho-phthalate were capable to degrade benzoate [101].

1.6 Anaerobic wastewater treatment

During the past decades anaerobic wastewater treatment has evolved into a "proven technology" for treatment of medium to high-strength, non-complex wastewaters from agro-industries. The commercial success of the anaerobic wastewater treatment technology is based on a number of principal advantages of anaerobic treatment over conventional aerobic treatment methods: due to the lower microbial yield, anaerobic wastewater treatment systems require less nutrients (i) and less surplus sludge is produced (ii); during anaerobic wastewater treatment, energy is generated in the form of methane gas, instead of energy consumption

due to aeration during aerobic wastewater treatment (iii) and anaerobic bacteria can form dense biofilms, resulting in high biomass concentrations and volumetric conversion rates (iv). The disadvantages of anaerobic wastewater treatment are strongly related to the kinetic properties of anaerobic bioconversions: due to the low microbial yield, start-up of anaerobic bioreactors may require several months, depending on the reactor-type applied and the source of the inoculum (i), similarly, the recovery may be slow, after a severe process upset due to e.g. toxicity (ii), and due to the lower substrate affinity of anaerobic bacteria, effluent COD-concentrations are higher and consequently post-treatment is in most cases required.

The previously mentioned ability of anaerobic bacteria to form dense biofilms, has led to the development of high-rate anaerobic bioreactors with the capacity to uncouple hydraulic and solid retention times. Anaerobic biofilms can either be developed on a stationary support in up- or down-flow reactors [52, 120, 121], on a mobile support in fluidized bed reactors [49, 110], or be based on self-immobilisation in Upflow Anaerobic Sludge Bed (UASB) [64-66] or Hybrid reactors [44, 51]. More recent developments in anaerobic bioreactor technology, such as the expanded granular sludge bed (EGSB) reactors, focus on an improved sludge-water contact [56, 93, 122], and on application of staged bioreactors for spatial separation of the different anaerobic conversion steps and to enhance a plug-flow pattern [15, 42, 43, 70]. The mentioned references are just examples of the massive amount of literature available on the different reactor types, as applied for the treatment of a large range of wastestreams.

World-wide the most applied reactor system is the UASB-reactor, with currently approximately 600 reactors in operation. Biomass retention in this reactor concept is based on the formation of microbial aggregates (methanogenic granular sludge) with excellent settling abilities. Biomass retention in UASB-reactors is furthermore enhanced by an internal three-phase (gas-liquid-solid) separator in the top of the reactor. The three phase separator functions furthermore as an internal settler through creation of a laminar flow pattern in the top part of the reactor. In the settler compartment, biomass is allowed to settle, and slide back into the highly turbulent digester compartment of the reactor.

Since anaerobic treatment has successfully been applied for the treatment of medium to high-strength, non-complex wastewaters at mesophilic temperatures (30 - 40 °C), the ongoing research shifted to the applicability of the process at psychrophilic [93] and thermophilic temperatures [69, 70], the treatment of low-strength wastewaters [56], and the treatment of a variety of complex types of wastewaters. Prominant among these complex wastewaters are domestic sewage [46], wastewater from the pulp and paper industry [36, 107] and (petro)chemical industries [72, 73].

Petrochemical wastewaters frequently contain high concentrations of aromatic compounds. From all aromatic compounds in petrochemical wastewaters, phenolic compounds received most research attention. Despite their moderate toxic nature, phenol, catechol, resorcinol and

hydroquinone were all shown to be degraded at volumetric rates ranging from 5 to 15 kg-COD·m^{-3}·day^{-1} at temperatures ranging from 30 to 37 °C, in different types of high rate anaerobic bioreactors [34, 54, 61, 111, 115]. The maximum volumetric removal rate of benzoate in a UASB-reactor was found to be 17 kg-COD·m^{-3}·day^{-1} with a maximum specific conversion rate of benzoate of 1.0 g-COD·g-VS^{-1}·day^{-1} [67]. Herewith benzoate can be ranked among the rapidly degradable substrates. Contrary to the high removal rates obtained with phenolic compounds and benzoate, the measured maximum removal rates of *para*-toluate in a UASB reactor were found to be extremely low; 0.2 kg-COD·m^{-3}·day^{-1} [74, 75]. *Ortho*- and *meta*-cresol-isomers were only partially removed (± 10 %) in a UASB-reactor treating benzoate and sulfate as primary substrates [35].

Results obtained during lab- and pilot-scale studies with terephthalic acid and dimethyl terephthalate production wastewaters are summarised in Table 1.4. The data in this table reveal that strong controversy exists concerning the applicable loading rates of the anaerobic bioreactors for anaerobic treatment of terephthalic acid wastewater, and that the removal of terephthalate and *para*-toluate is poorly documented. Herewith should be mentioned that the composition of the (artificial) terephthalic acid wastewater applied during the various studies, may differ strongly. In case of low concentrations of terephthalate and *para*-toluate in the (artificial) influent, the COD-removal capacity of the reactors will primarily be based on the removal of acetate and benzoate. These compounds can be regarded as rapidly degradable, and high removal rates and treatment efficiencies can be expected. Herewith the high removal rates observed by Liangming et al. (Table 1.4, [68]) can be explained, because these authors worked with terephthalic acid wastewater consisting for 90 % of the COD-load of acetate and benzoate. Since all other removal rates shown in Table 1.4 for terephthalic acid wastewater are low, it may be concluded from literature information that wastewaters containing high concentrations of terephthalate and/or *para*-toluate can only be treated at low rates. Wastestreams generated during dimethyl terephthalate production commonly contain only a small fraction of aromatic compounds (less than 20 % of the COD-load, see Table 1.2). Therefore high treatment efficiencies can be achieved at high loading rates through removal of rapidly degradable compounds like, acetic acid, formic acid, methanol and formaldehyde (see Table 1.4), although the highly toxic disinfectant formaldehyde may severely inhibit the process [10]. Formaldehyde toxicity can be overcome through applying effluent recirculation, which will result in sufficiently low influent and reactor concentrations formaldehyde. Using this approach, wastewaters containing high concentrations formaldehyde can be treated at high rates [124]. It furthermore should be emphasised that due to its high strength, the effluent of anaerobic bioreactors treating dimethyl terephthalate wastewater may contain up to 3 g·l^{-1} of unremoved aromatic acids, despite treatment efficiencies of approximately 90 %.

Table 1.4: Overview of the lab and pilot scale technological experiences with the anaerobic treatment of terephthalic acid and dimethyl terephthalate production wastewaters.

reactor type[1]	reactor volume	biomass origin[2]	Influent COD	VLR[3]	η_{COD}	degraded[4]		ref.
	l.		$g \cdot l^{-1}$	$g\text{-COD} \cdot l^{-1} \cdot d^{-1}$	%	TA	PT	
terephthalic acid:								
UASB	3.0	DSS, AS	6.5	2.5	45	?	?	[45, 76]
DFF	4.8	AS	6.5	1.9	75	?	?	[76,83]
Fed Batch	0.1	MS	20	0.5	94	-	-	[119]
UASB	7.5	DSS	5.0	48	± 85	? (-)	NW	[68]
Hybrid	6.5	DSS	5.0	24	± 85	? (-)	NW	[68]
Fed Batch	0.3	DSS	± 20	± 2.2	?	? (-)	NW	[60]
UASB	19	DPM, MS	4.7	2.5	60	+	-	[21]
dimethyl terephthalate:								
FlB	?	?	52	17	88	-	-	[94]
2 * FB in series	?	?	52	26	92	-	-	[94]
BSP	2.5	DSS	140	3.3	95	-	-	[102]
BSP	2.5	DSS	153	20	95	-	-	[92]
UASB	8.5	MGS	30	4	0[5]	-	-	[10]
Hybrid	28	DSS	23	28	95	-	-	[68]

(1) UASB: Upflow Anaerobic Sludge Blanket, DFF: Downflow Fixed Film, Hybrid: upflow reactor with carrier material in top reactor, FB: Fluidized bed, BSP: completely stirred tank reactor with biomass support particles.

(2) AS: Activated Sludge, DSS: Digested Sewage Sludge, MGS: Methanogenic Granular Sludge, MS: Methanogenic Sludge, DPM: Digested Pig Manure

(3) VLR: Volumetric loading rate

(4) TA: Terephthalic Acid, PT: *para*-Toluic Acid, +: biodegradation observed, -: no biodegradation observed, ?: unknown, ? (-) unknown, but biodegradation is unlikely, NW: Not present in the Wastewater

(5) the capacity of the reactor was completely lost due to formaldehyde toxicity.

Currently, at least 42 full scale anaerobic bioreactors are in operation or under construction for treatment of a large range of (petro)chemical wastewaters [72]. Many of these wastestreams may be regarded as non-complex, although some of them contain phenolic compounds or benzoate [16, 17, 38]. Among these 42 reactors eight treat terephthalic acid production wastewaters and at least three dimethyl terephthalate wastewater. Operational parameters are only available for two installations for the pre-treatment of terephthalic acid wastewater. These will be described in more detail in the following paragraphs.

Amoco, world-wide the most important producer of terephthalic acid, may be considered as the pioneer in anaerobic treatment of terephthalic acid production wastewater [26, 30, 103]. Since the early 1980's this company studied the feasibility of anaerobic treatment for terephthalic acid production wastewater. This has led to the construction of at least three full-scale anaerobic bioreactors in the USA, Belgium and Taiwan, replacing the traditionally applied three-stage activated sludge system [62]. The layout of the treatment plant in Geel, Belgium, for the combined treatment of both the solid and liquid waste of a terephthalic and isophthalic acid production plant, is shown in Figure 1.13. The applied reactor system is a down flow fixed-film reactor with a volume of 15,000 m^3. Herewith this reactor is among the biggest anaerobic biofilm reactors in the world. The reactor is operated at a volumetric loading rate of 4 kg-COD·m^{-3}·day^{-1} and at a hydraulic retention time (HRT) of 4.5 days. Compared to the normally applied loading rates for high-rate anaerobic treatment of non-complex wastewater of 10-20 kg-COD·m^{-3}·day^{-1}, this may be regarded as a relatively low loading rate. A remarkable feature of this system is the installed buffer tank with a volume of 22,000 m^3, which results in a HRT of 6.5 days. This enormous buffer capacity is considered necessary to cope with peak-loads that may occur due to upsets in the production process. The involvement of chemical engineers in the design of the reactor can be deduced from the applied operational control strategy: temperatures are controlled at 37 ± 0.2 °C and the reactor pH to 7.0 ± 0.1 through addition of sodium hydroxide. The biogas produced in the anaerobic reactor (1200 Nm^3·$hour^{-1}$) is applied in the boiler of the energy production plant. In addition to the previously mentioned advantages of anaerobic treatment over aerobic treatment, Amoco mentions the efficient removal of cobalt and manganese by precipitation as sulphide salts in the anaerobic bioreactors.

The reactor in Geel was inoculated with digested sludge from the aerobic post-treatment plant. The start-up of the installation took approximately 1 year. Since then, the reactor operates at a COD-efficiency of approximately 80 %. All compounds present in the wastewater, except *para*-toluic acid, are at least partially degraded.

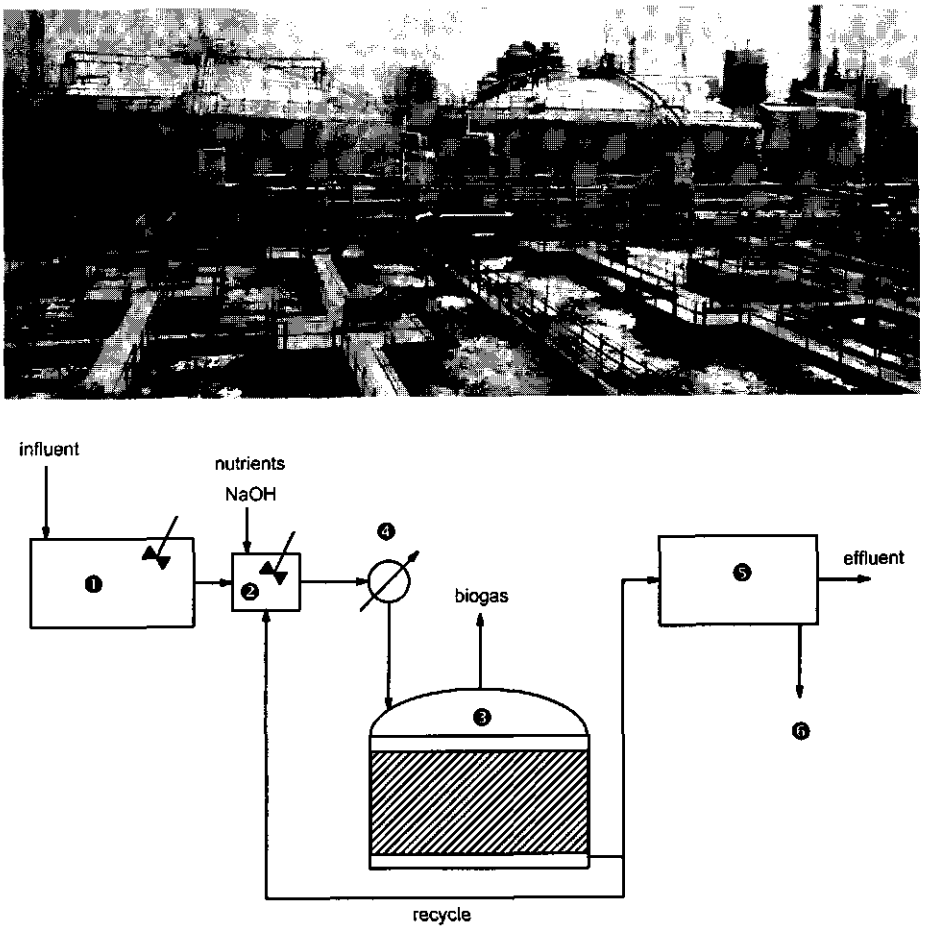


Figure 1.13: Flow sheet of the anaerobic-aerobic wastewater treatment plant for the terephthalic acid and isophthalic acid production plant from Amoco, at Geel, Belgium. The anaerobic bioreactor is a down-flow fixed-film reactor (DFF) with a volume of 15,000 m^3. Operational data are described in the text [26]. Numbers stand for ❶ buffer tank ❷ conditioning tank, ❸ anaerobic down-flow fixed-film reactor, ❹ heat exchanger, ❺ aerobic post-treatment, ❻ sludge treatment. The picture above the flow sheet shows the two anaerobic (background) and aerobic (foreground) reactors constructed by Amoco in Kaohsiung, Taiwan.

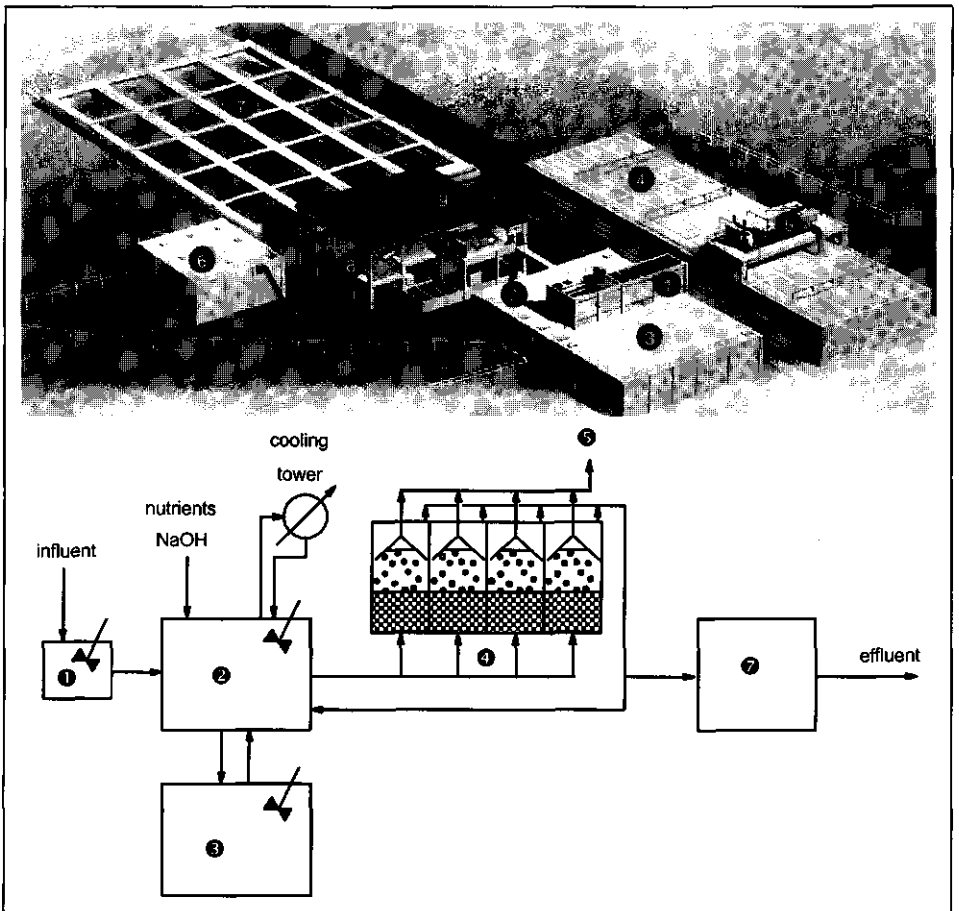


Figure 1.14: Flow sheet of the anaerobic-aerobic wastewater treatment plant for the terephthalic acid production plant from Tuntex, at Tainan, Taiwan. The anaerobic bioreactor is a four compartment UASB-reactor with a volume of 7,000 m^3, designed by Grontmij, De Bilt, The Netherlands. Operational data are described in the text [90]. The picture above the flow-sheet shows an artist impression of the treatment plant. Numbers stand for ❶ chemical pretreatment, ❷ influent buffer tank, ❸ calamity tank, ❹ UASB-reactor, ❺ biogas compressors, ❻ anaerobic sludge storage tank, ❼ aerobic post-treatment, ❽ sludge dewatering installation, ❽ control room.

Figure 1.14 shows the process layout of the treatment plant for terephthalic acid production wastewater of Tuntex at Tainan, Taiwan. The core of the treatment plant is a UASB-reactor consisting of 4 compartments of 1,700 m^3 each, which are operated in parallel. Due to a lack of a sufficient amount of seed-material during the initial start-up of the reactor, only two compartments were operated. Herewith, the initial volumetric loading rate of the reactor was

relatively high at 8 kg-COD·m^{-3}·day^{-1}. During this initial period of approximately two years, the COD-based efficiency of the reactor was restricted to 50 % as a result of the absence of terephthalate and *para*-toluate degradation [90]. The start-up of the reactor was furthermore hampered by strong fluctuations in the wastewater strength, pH and temperature. In this respect it should be noted that the applied HRT in the buffer of this system (1.5 days) is short, when compared to the previously described system of Amoco in Geel, Belgium (6.5 days). After approximately two years of operation and addition of digested pig manure as additional seed material, all four compartments of the system were in operation. Herewith the volumetric loading rate imposed to the system decreased to approximately 5 kg-COD·m^{-3}·day^{-1}. Probably due to these lower volumetric loading rates, and the higher concentrations of biomass in the reactor, terephthalate was degraded for approximately 70 % after two years of operation. Herewith the COD-removal efficiency of the treatment system increased from 50 to 80 %. Degradation of *para*-toluate was still not achieved.

1.7 Outline of this thesis

Even though approximately ten full-scale reactors are currently in operation or under construction for the anaerobic pre-treatment of terephthalic acid production wastewaters, it is evident from the information provided in the previous paragraphs that a great deal of controversy exists concerning the feasibility of anaerobic treatment for phthalic acids containing wastewaters. The reported applicable loading rates in high-rate anaerobic bioreactors range from 2 to 48 g-COD·l^{-1}·day^{-1}, at COD-based efficiencies between 45 and 94 % (Table 1.4). It therefore is obvious that, at this stage, it is impossible for engineers to decide whether or not anaerobic treatment may represent an attractive option for pre-treatment of these wastewaters.

The controversy between the different studies on anaerobic treatment of phthalic acid wastewaters can mainly be attributed to the lack of microbiological understanding concerning the fate of phthalic acid isomers in anaerobic environments. The contrary is true for the aerobic degradation of phthalate isomers, and also some information is available for phthalate isomer degradation by denitrifying organisms. Concerning the fate of phthalic acid isomers in environments where these strong electron acceptors are absent, like sulfate reducing or methanogenic environments, almost no information is available.

From the results of preliminary studies [58], dealing with the anaerobic treatment of terephthalic acid production wastewater, we concluded the following:

- The anaerobic biodegradation of terephthalate is the rate limiting step in the anaerobic treatment of a mixture of acetate, benzoate and terephthalate in lab-scale UASB-type reactors,
- The low degradation rates of terephthalate in UASB-type reactors can be attributed to the very low growth rate of the terephthalate degrading methanogenic biomass (estimated $\mu^{max} = 0.04\ day^{-1}$ at 30 °C),
- The anaerobic degradation of terephthalate is strongly inhibited by benzoate, acetate and sucrose.

The work described in this thesis is based on these preliminary studies and the (lack of) information presented in literature. The investigations deal with both microbiological and biotechnological aspects of the anaerobic treatment of wastewaters generated during production of phthalate isomers.

The anaerobic biodegradability of phthalic acid isomers and related compounds will be described in Chapter 2. The main objective of this study was to asses whether or not the source of the inoculum has a big impact on the length of the lag phase preceding the degradation of the tested compounds. Microbiological aspects of enrichment cultures grown on *ortho*-phthalate, isophthalate and terephthalate are described in Chapters 3 to 5. The following characteristic properties of these cultures are described: The kinetic properties of the phthalate isomers grown enrichment cultures (Chapter 3), the influence of short periods of starvation and the influence of acetate and benzoate on terephthalate degradation (Chapter 4), and the energetics of product formation (Chapter 5). Chapter 6 and 7 deal with anaerobic bioreactor studies focused on the treatment of terephthalic acid production wastewater. The research described in Chapter 6 was aimed at optimising the rates of terephthalate degradation in anaerobic hybrid-reactors, while in Chapter 7 the anaerobic treatment of a mixture of acetate, benzoate and terephthalate in a two-stage UASB-reactor is described. A general discussion concerning some engineering aspects of the anaerobic degradation of phthalates, is presented in chapter 8. Chapter 9 summarises the results obtained (In English and Dutch).

1.8 References

1. **Aftring, R. P., B. E. Chalker, and B. F. Taylor.** 1981. Degradation of phthalic acids by denitrifying, mixed cultures of bacteria. Appl. Environ. Microbiol. **41:**1177-1183.
2. **Aftring, R. P., and B. F. Taylor.** 1981. Aerobic and anaerobic catabolism of phthalic acid by a nitrate respiring bacteria. Arch. Microbiol. **130:**101-104.
3. **Aihara, J.** 1992. Why aromatic compounds are stable. Sci. Am. **266:**62-68.

4. **Anonymus.** 1988. Facts and figures for the chemical industry. Chem. Eng. News. **66:**34-44.

5. **Atlas, E., and C. S. Giam.** 1980. Global transport of organic pollutants: ambient concentrations in the remote marine atmosphere. Science. **211:**163-165.

6. **Auburger, G., and J. Winter.** 1996. Activation and degradation of benzoate, 3-phenylpropionate and crotonate by *Syntrophus buswellii* strain GA.; evidence for electron-transport phosphorylation during crotonate respiration. Appl. Microbiol. Biotechnol. **44:**807-815.

7. **Auburger, G., and J. Winter.** 1992. Purification and characterization of benzoyl-CoA ligase from a syntrophic, benzoate-degrading, anaerobic mixed culture. Appl. Microbiol. Biotechnol. **37:**789-795.

8. **Battersby, N. S., and V. Wilson.** 1989. Survey of the anaerobic biodegradation potential of organic chemicals in digesting sludge. Appl. Environ. Microbiol. **55:**433-39.

9. **Behret, H.** 1991. Terephthalic acid. *In* E. Bayer (ed.), BUA Reports, vol. 68-70. S. Hirzel, Wissenschaftliche Verlagsgesellschaft, Stuttgart, Germany.

10. **Bekker, P. d., T. Jans, and P. Piscaer.** 1982. Anaerobic treatment of formaldehyde containing waste water. Presented at the European Symposium AWWT, The Hague, The Netherlands, 23-25 November.

11. **Bemis, A. G., J. A. Dindorf, H. B., and S. C.** 1982. Phthalic acids and other benzenepolycarboxylic acids, p. 732-777. *In* H. F. Mark, D. F. Othmer, C. G. Overberg, G. T. Seaborg, M. Grayson, and D. Eckroth (ed.), Kirk Othmer Encyclopedia of Chemical Technology, vol. 17. John Wiley and Sons, New York, USA.

12. **Benckiser, G., and J. C. G. Ottow.** 1982. Metabolism of the plasticizer di-n-butylphthalate by *Pseudomonas pseudoalcaligenes* under anaerobic conditions with nitrate as the only electron acceptor. Appl. Environ. Microbiol. **44:**576-578.

13. **Blum, D. J. W., and R. E. Speece.** 1991. A database of chemical toxicity to environmental bacteria and its use in interspecies comparisons and correlations. Res. J. WPCF. **63:**198-207.

14. **Boll, M., and G. Fuchs.** 1995. Benzoyl-coenzyme A reductase (dearomatising), a key enzyme of anaerobic aromatic metabolism. ATP dependence of the reaction, purification and some properties of the enzyme from *Thauera aromatica* strain K172. Eur. J. Biochem. **234:**921-933.

15. **Boopathy, R., and A. Tilche.** 1992. Pelletization of biomass in a hybrid anaerobic baffled reactor (HABR) treating acidified wastewater. Bioresource Technol. **40:**101-107.

16. **Borghans, A. J. M., A. v. Driel, and H. d. Gronckel.** 1993. Anaerobe zuivering in een Biothane reactor; praktijkvoorbeelden uit de alkoholindustrie en de chemische industrie. Presented at the Zijn komplexe industriele afvalwaters anaerob behandelbaar?, Breda, The Netherlands.

17. **Borghans, A. J. M. L., and A. Van Driel.** 1988. Application of the Biothane UASB reactor to a chemical wastewater, containing phenol and formaldehyde. Presented at the 5th Int. Symp. on Anaerobic Digestion, Bologna, Italy.

18. **Boyd, S. A., D. R. Shelton, D. Berry, and J. M. Tiedje.** 1983. Anaerobic biodegradation of phenolic compounds in digested sludge. Appl. Environ. Microbiol. **46:**50-54.

19. **Brune, A., and B. Schink.** 1992. Phloroglucinol pathway in the strictly anaerobic *Pelobacter acidigallici*: fermentation of trihydroxybenzenes to acetate via triacetic acid. Arch. Microbiol. **157**:417-424.

20. **Brune, A., and B. Schink.** 1990. Pyrogallol-to-Phloroglucinol Conversion and other hydroxyl transfer reactions catalyzed by cell extracts of Pelobacter acidigallici. J. Bacteriol. **172**:1070-1076.

21. **Cheng, S. S., C. Y. Ho, and J. H. Wu.** 1997. Pilot study of UASB process treating PTA manufacturing wastewater. Presented at the 8th Int. Conf. on Anaerobic Digestion, Sendai, Japan, May 25-29, 1997.

22. **Davis, G. W., and E. S. Hill.** 1982. Polyester fibres, p. 531-549. *In* H. F. Mark, D. F. Othmer, C. G. Overberg, G. T. Seaborg, M. Grayson, and D. Eckroth (ed.), Kirk Othmer Encyclopedia of Chemical Technology, vol. 18. John Wiley and Sons, New York, USA.

23. **Didde, R.** 1998. Voor alle zekerheid sabbelen op PVC. Volkskrant, 24-01-1998.

24. **Dimroth, K.** 1983. Thermochemische daten organischer verbindungen, p. 997-1038, D'ans-Lax, Taschenbuch für chemiker und Physiker, vol. 2. Springer-Verlag, Berlin.

25. **Donlon, B. A., E. Razo-Flores, J. A. Field, and G. Lettinga.** 1995. Toxicity of N-substituted aromatics to acetoclastic methanogenic activity in granular sludge. Appl. Environ. Microbiol. **61**:3889-3893.

26. **Duffel, J. v.** 1993. Anaerobe behandeling van organische zuren. Presented at the National Conference on Anaerobic Treatment of Complex Wastewaters, Breda, The Netherlands.

27. **Edwards, E. A., and D. Grbic-Galic.** 1994. Anaerobic degradation of toluene and *o*-xylene by a methanogenic consortium. Appl. Environ. Microbiol. **60**:313-322.

28. **Ejlertsson, J., U. Meyerson, and B. H. Svensson.** 1996. Anaerobic degradation of phthalic acid esters during digestion of municipal solid waste under landfilling conditions. Biodegradation. **7**:345-352.

29. **Elder, D. J. E., and D. J. Kelly.** 1994. The bacterial degradation of benzoic acid and benzenoid compounds under anaerobic conditions: unifying trends and new perspectives. FEMS Microbiol. Rev. **13**:441-468.

30. **Ely, S. R., and G. P. Olsen.** 1989. AMOCO-Process for treatment of PTA-wastewater. USA patent 4,826,600.

31. **Engelhardt, G., and P. R. Wallnöfer.** 1978. Metabolism of di- and mono-n-butyl phthalate by soil bacteria. Appl. Environ. Microbiol. **35**:243-246.

32. **Evans, C. W.** 1977. Biochemistry of the bacterial catabolism of aromatic compounds in anaerobic environments. Nature. **270**:17-22.

33. **Fajardo, C., J. P. Guyot, H. Macarie, and O. Monroy.** 1997. Inhibition of anaerobic digestion by terephthalic acid and its aromatic by products. Water Sci. Technol. **36**:83-90.

34. **Fang, H. H. P., T. Chen, Y. Y. Li, and H. K. Chui.** 1996. Degradation of phenol in wastewater in an upflow sludge blanket reactor. Wat. Res. **30**:1353-1360.

35. **Fang, H. H. P., and G. M. Zhou.** 1997. Anaerobic degradation of benzoate and cresol isomers in sulfate-rich wastewater. Presented at the 8th Int. Conf. on Anaerobic Digestion, Sendai, Japan, May 25-29, 1997.

36. **Field, J. A.** 1989. The effect of tannic compounds on anaerobic wastewater treatment. Ph.D. thesis. Wageningen Agricultural University, Wageningen, The Netherlands.

37. **Franck, H. G., and J. W. Stadelhofer.** 1988. p-Xylene and its derivates: Terephthalic acid, p. 283-290. *In* H. G. Franck (ed.), Industrial aromatic chemistry: raw materials, processes, products. Springer-Verlag, Berlin, Germany.

38. **Frankin, R. J., J. van Gils, and R. J. F. Wermeling.** 1994. Full-scale anaerobic treatment of Shell wastewater containing benzoate with the Biothane UASB process. Presented at the 7th IAWQ Int. Symp. Anaerobic Digestion, Cape Town, South Africa.

39. **Fuchs, G., M. E. S. Mohamed, U. Altenschmidt, J. Koch, A. Lack, R. Brackmann, C. Lochmeyer, and B. Oswald.** 1994. Biochemistry of anaerobic biodegradation of aromatic compounds, p. 513-553. *In* C. Ratledge (ed.), Biochemistry of microbial degradation. Kluwer Academic Publishers, Dordrecht, The Netherlands.

40. **Giam, C. S., E. Atlas, M. A. Powers Jr., and J. E. Leonard.** 1984. Phthalic acid esters, p. 67-140. *In* O. Hutzinger (ed.), Anthropogenic Compounds, vol. 3, Part C. Springer-Verlag, Berlin-Heidelberg-New York-Tokyo.

41. **Goodman, I.** 1988. Polyesters, p. 1-75. *In* H. F. Mark, N. M. Bikales, C. G. Overberg, G. Menges, and J. I. Kroschwitz (ed.), Encyclopedia of of polymer science and engineering., vol. 12. Yohn Wiley and Sons, New York.

42. **Grobicki, A., and D. C. Stuckey.** 1991. Performance of the anaerobic baffled reactor under steady state and shock-loading conditions. Biotechnol. Bioeng. **37:**344-355.

43. **Guiot, S. R., B. Safi, J. C. Frignon, P. Mercier, C. Mullignan, and R. Tremblay.** 1995. Performances of a Full-Scale Novel Multiplate Anaerobic Reactor Treating Cheese Whey Effluent. Biotechnol. Bioeng. **45:**398-405.

44. **Guiot, S. R., and L. van den Berg.** 1985. Performance of an upflow anaerobic reactor combining a sludge blanket and a filter treatinf sugar waste. Biotechnol. Bioeng. **27:**800-806.

45. **Guyot, J. P., H. Macarie, and A. Noyola.** 1990. Anaerobic digestion of a petrochemical waste-water using the UASB process. Appl. Biochem. Biotechnol. **24:**579-89.

46. **Haandel van, A. C., and G. Lettinga.** 1994. Anaerobic sewage treatment; a practicle guide for regions with a hot climate. John Wiley and Sons., Chichester, UK.

47. **Healy, J. B., and Y. Young.** 1979. Anaerobic degradation of eleven aromatic compounds to methane. Appl. Environ. Microbiol. **38:**84-89.

48. **Heider, J., and G. Fuchs.** 1997. Anaerobic metabolism of aromatic compounds. Eur. J. Biochem. **243:**577-596.

49. **Heijnen, J. J., A. Mulder, W. Enger, and F. Hoeks.** 1986. Review on the application of anaerobic fluidized bed reactors in wastewater treatment . Presented at the Water Treatment Conference Aquatech 1986, Amsterdam, The Netherlands.

50. **Heipieper, H. J., F. J. Weber, J. Sikkema, H. Keweloh, and J. A. M. de Bont.** 1994. Mechanisms of resistance of whole cells to toxic organic solvents. Tibtech. **12:**409-415.

51. **Henry, M. P., B. A. Donlon, P. N. Lens, and E. M. Colleran.** 1996. Use of anaerobic hybrid reactors for treatment of synthetic pharmaceutical wastewaters containing organic solvents. J. Chem. Technol. Biotechnol. **66:**251-264.

52. **Henze, M., and P. Harremoes.** 1983. Anaerobic treatment of wastewaters in fixed film reactors - a literature review. Wat. Sci. Technol. **15:**1-101.

53. **Horowitz, A., D. R. Shelton, C. P. Cornell, and J. M. Tiedje.** 1982. Anaerobic degradation of aromatic compounds in sediments and digested sludge. Dev. Ind. Microbiol. **23:**435-444.

54. **Hwang, P. C., and S. S. Cheng.** 1991. The influence of glucose supplement on the degradation of catechol. Wat. Sci. Tech. **23:**1201-1209.

55. **Johnson, B. T., and M. A. Heitkamp.** 1984. Environmental and chemical factors influencing the biodegradation of phthalic acid esters in freshwater sediments. Environ. Pollution. **8:**101-118.

56. **Kato, M. T.** 1994. The anaerobic treatment of low strength soluble wastewaters. Ph.D. thesis. Wageningen Agricultural University, Wageningen, The Netherlands.

57. **Kaufman, A. K., and C. C. Kreuger.** 1993. Applied biotreatment of phthalate-impacted soils: recognizing and overcoming technical challenges., p. 209-219. *In* M. A. Levin and M. A. Gealt (ed.), Biotreatment of industrial and hazardous waste. McGraw-Hill, USA.

58. **Kleerebezem, R., L. W. Hulshoff Pol, and G. Lettinga.** 1996. Anaerobic pre-treatment of petrochemical effluents: terephthalic acid wastewater. Presented at the 2nd Specialized Conference on Pretreatment of Industrial Wastewaters, Athens, Greece, October 16-18.

59. **Koch, J., W. Eisenreich, A. Bacher, and G. Fuchs.** 1993. Products of enzymatic reduction of benzoyl-CoA, a key reaction in anaerobic aromatic metabolism. Eur. J. Biochem. **211:**649-661.

60. **Kuang, X., and J. Wang.** 1994. Anaerobic biodegradability of Terephtalic acid acid and its inhibitory effect on Anaerobic Digestion. J. Environ. Sci. **6:**180-188.

61. **Latkar, M., and T. Chakrabarti.** 1994. Resorcinol, catechol and hydroquinone biodegradation in mono and binary substrate matrices in upflow anaerobic fixed-film fixed-bed reactors. Wat. Res. **28:**599-607.

62. **Lau, C. M.** 1978. Staging aeration for high-efficiency treatment of aromatic acids plant wastewater. Presented at the 32nd Independent Wastewater Conference Purdue University, West Lafayette, Ind.

63. **Leenheer, J. A., R. L. Malcolm, and W. R. White.** 1976. Investigation of the reactivity and fate of certain organic components of an industrial waste after deep-well injection. Environ. Sci. & Technol. **10:**445-451.

64. **Lettinga, G.** 1995. Anaerobic digestion and wastewater treatment systems. Antonie Leeuwenhoek. **67:**3-28.

65. **Lettinga, G., L. W. Hulshoff Pol, I. W. Koster, W. M. Wiegant, W. de Zeeuw, A. Rinzema, P. C. Grin, R. E. Roersma, and S. W. Hobma.** 1984. High-rate anaerobic

wastewater treatment using the UASB reactor under a wide range of temperature conditions. Biotechnol. Gen. Eng. Rev. **2**:253-283.

66. **Lettinga, G., A. F. M. vanVelsen, S. W. Hobma, W. de Zeeuw, and A. Klapwijk.** 1980. Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. Biotechnol. Bioeng. **22**:699-734.

67. **Li, Y. Y., H. H. P. Fang, T. Chen, and H. K. Chui.** 1995. UASB treatment of wastewater containing concentrated benzoate. J. Environ. Eng. **121**:748-751.

68. **Liangming, X., C. Yuxiang, and Z. Xiangdong.** 1991. The anaerobic biological treatment of high strength petrochemical wastewater by a hybrid reactor. Presented at the International Conference on Petroleum Refining and Petrochemical Processing, Beijing, China, September 11-15.

69. **Lier van, J. B.** 1995. Thermophilic anaerobic wastewater treatment; temperature aspects and process stability. Ph.D. Thesis. Wageningen Agricultural University, Wageningen, The Netherlands.

70. **Lier van, J. B., F. Boersma, M. M. W. H. Debets, and G. Lettinga.** 1994. High rate thermophilic anaerobic wastewater treatment in compartmentalized upflow reactors. Wat. Sci. Technol. **30**:251-261.

71. **Long-de Valliere, C. L., S. Petrozzi, D. Zurrer, U. Baier, and I. J. Dunn.** 1989. Methods of anaerobic degradation of toxic compounds in chemical and industrial wastewaters, p. 35-72. *In* A. Mizrahi (ed.), Biological Waste Treatment, vol. 12. Alan R. Liss, Inc., New York.

72. **Macarie, H.** 1996. Anaerobic digestion an adequate technology for the treatment of some chemical and petrochemical wastewaters. Presented at the IV Taller Latinoamericano Sobre Tratiamento Anaerobio de Aguas Residuales, Bucaramanga, Colombia.

73. **Macarie, H.** 1992. Traitement anaerobie des effluents d'une usine petrochimique produisant un compose aromatique l'acide terephtalique. Ph.D. thesis. Universite de Provence, Marseille, France.

74. **Macarie, H., and J. P. Guyot.** 1992. Inhibition of the methanogenic fermentation of p-toluic acid (4-methylbenzoic acid) by acetate. Appl. Microbiol. Biotechnol. **38**:398-402.

75. **Macarie, H., and J. P. Guyot.** 1995. Use of ferrous sulphate to reduce the redox potential and allow the start-up of UASB-reactors treating slowly biodegradable compounds: application to a wastewater containing 4-methylbenzoic acid. Environ. Tech. **16**:1185.

76. **Macarie, H., A. Noyola, and J. P. Guyot.** 1992. Anaerobic treatment of a petrochemical wastewater from a terephtalic acid plant. Water Sci. Technol. **25**:223-235.

77. **Mah, R. A., and M. R. Smith.** 1981. The methanogenic bacteria, p. 948-977. *In* A. Balows, H. G. Trüper, M. Dworkin, W. Hander, and K. H. Schleifer (ed.), The Prokaryotes. Springer-Verlag, New York, USA.

78. **Matsumoto, G.** 1982. Comparative study on organic constituents in polluted and unpolluted inland aquatic environments; phenols and aromatic acids in polluted and unpolluted waters (III). Wat. Res. **16**:551-557.

79. **Mavrovouniotis, M. L.** 1991. Estimation of standard Gibbs Energy Changes of Biotransformations. J. Biological Chemistry. **266:**14440-14445.

80. **Mavrovouniotis, M. L.** 1990. Group Contributions for Estimating Standard Gibbs Energies of formation of biochemical compounds in Acqueous Solution. Biotechnology and Bioengineering. **36:**1070-1082.

81. **Meer, J. v. d.** 1996. De tol van de chemie. Milieudefensie, (4):6-7.

82. **Naumov, A. V., A. B. Gafarov, and A. M. Boronin.** 1996. A novel *ortho*-phthalate utilizer - an acetic acid bacterium *Acetobacter* sp. Microbiology. **65:**182-186.

83. **Noyola, A., H. Macarie, and J. P. Guyot.** 1990. Treatment of terephtalic acid plant wastewater with an anaerobic fixed film reactor. Environ.Technol. **11:**239-248.

84. **Nozawa, T., and Y. Maruyama.** 1988. Anaerobic metabolism of phthalate and other aromatic compounds by a denitrifying bacterium. J. Bacteriol. **170:**5778-5784.

85. **Nozawa, T., and Y. Maruyama.** 1988. Denitrification by a soil bacterium with phthalate and aromatic compounds as substrates. J. Bacteriol. **170:**2501-2505.

86. **O'Connor, O. A., M. D. Rivera, and L. Y. Young.** 1989. Toxicity and biodegradation of phthalic acid esters under methanogenic conditions. Environ. Tox. Chem. **8:**569-576.

87. **Owen, W. F., D. C. Stuckey, J. B. Healy, L. Y. Young, and P. L. McCarty.** 1979. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. Wat. Res. **13:**485-492.

88. **Painter, S. E., and W. J. Jones.** 1990. Anaerobic bioconversion of phthalic acid esters by natural inocula. Environ. Technol. **11:**1015-1026.

89. **Partenheimer, W.** 1990. A chemical model for the Amoco "MC" oxygenation process to produce Terephtalic Acid. Chem. Ind. **40:**321-339.

90. **Pereboom, J. H. F., d. G. Man, and I. T. Su.** 1994. Start-up of full scale UASB-reactor for the treatment of terephtalic acid waste water. Presented at the 7th International Symposium on Anaerobic Digestion, Cape Town, South Africa.

91. **Putcher, R. E.** 1982. Polyamides, p. 328-425. *In* H. F. Mark, D. F. Othmer, C. G. Overberg, G. T. Seaborg, M. Grayson, and D. Eckroth (ed.), Kirk-Othmer Encyclopedia of Chemical Technology, vol. 18. John Wiley and Sons, New York, USA.

92. **Ramakrishna, C., and J. D. Desai.** 1997. High rate anaerobic digestion of a petrochemical wastewater using biomass support particles. World J. Microbiol. Biotechnol. **13:**329-334.

93. **Rebac, S., J. Ruskova, S. Gerbens, J. B. Van Lier, A. J. M. Stams, and G. Lettinga.** 1995. High-rate anaerobic treatment of wastewater under psychrophilic conditions. J. Ferm. Bioeng. **80:**499-506.

94. **Reule, W.** 1990. Methan aus Chemieabwasser. Chemische Industry. **9.**

95. **Ribbons, D. W., P. Keyser, D. A. Kunz, B. F. Taylor, R. W. Eaton, and B. N. Anderson.** 1984. Microbial degradation of phtalates, p. 371-397. *In* D. T. Gibson (ed.), Microbial Degradation of Organic Compounds.

96. **Roffia, P., P. Calini, and L. Motta.** 1984. Byproduct Identification in the Terephthalic Acid Production Process and possible mechanisms of their formation. Ind. Eng. Chem. Prod. Res. Dev. **23:**629-634.

97. **Savostianoff, D.** 1991. Phthalic anhydride: ten years of stagnation. Inf. Chim. **333**:133-146.

98. **Savostianoff, D.** 1990. TPA-DMT, confidence is coming back. Inf. Chim. **317**:193-201.

99. **Savostianoff, D., and S. N. Didier.** 1993. DMT-PTA, Asia advances towards a crushing domination. Inf. Chim. **352**:119-129.

100. **Schink, B.** 1992. Syntrophism among prokaryotes, p. 276-299. *In* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), The prokaryotes, vol. I. Springer-Verlag, New York, N.Y.

101. **Schink, B., A. Brune, and S. Schnell.** 1992. Anaerobic degradation of aromatic compounds, p. 219-242. *In* G. Winkelmann (ed.), Microbial Degradation of Natural Products. VCH, Weinheim, Germany.

102. **Sharma, S., C. Ramakrishna, J. D. Desai, and N. M. Bhatt.** 1994. Anaerobic biodegradation of a petrochemical wastewater using biomass support particles. Appl. Microbiol. Biotechnol. **40**:768-771.

103. **Shelley, S.** 1991. And the winner is... Amoco chemical. Chemical Engineering. **98**:90-93.

104. **Shelton, D. R., S. A. Boyd, and J. M. Tiedje.** 1984. Anaerobic biodegradation of phthalic acid esters in sludge. Environ. Sci. Technol. **18**:93-97.

105. **Shelton, D. R., and J. M. Tiedje.** 1984. General Method for determining anaerobic biodegradation potential. Appl. Environ. Microbiol. **47**:850-857.

106. **Sierra, R., and G. Lettinga.** 1991. The effect of aromatic structure on the inhibition of acetoclastic methanogenesis in granular sludge. Appl. Microbiol. Biotechnol. **34**:544-550.

107. **Sierra-Alvarez, R.** 1990. The role of natural wood constituents on the anaerobic treatability of forest industry wastewaters. Ph.D. thesis. Wageningen Agricultural University, Wageningen, The Netherlands.

108. **Sikkema, J., J. A. M. Bont de, and B. Poolman.** 1994. Interactions of cyclic hydrocarbons with biological membranes. J. Biol. Chem. **269**:8022-8028.

109. **Stams, A. J. M.** 1994. Metabolic interactions between anaerobic bacteria in methanogenic environments. Antonie Leeuwenhoek. **66**:271-294.

110. **Suidan, M. T., J. T. Pfeffer, and G. F. Nakhala.** 1988. Anaerobic expanded -bed GAC reactor for the treatment of biologically inhibitory wastes generated during and petroleum destillation. Presented at the 5th Int. symp. on anaerobic digestion, Bologna, Italy.

111. **Szewzyk, U., and B. Schink.** 1989. Methanogenic degradation of hydroquinone in an anaerobic fixed-bed reactor. Appl. Microbiol. and Biotechnol. **32**:346-349.

112. **Taylor, B. F., and D. W. Ribbons.** 1983. Bacterial decarboxylation of o-phthalic acids. Appl. Environ. Microbiol. **46**:1276-1281.

113. **Thauer, R. K., K. Jungermann, and K. Decker.** 1977. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Rev. **41**:100-179.

114. **Tschech, A., and B. Schink.** 1985. Fermentative degradation of resorcinol and resorcylic acids. Arch. Microbiol. **143**:52-59.

115. **Wang, Y. T., M. T. Suidan, and B. E. Rittman.** 1986. Anaerobic treatment of phenol by an expanded-bed reactor. Res. J. WPCF. **58:**227-233.

116. **Whitman, W. B., T. L. Bowen, and D. R. Boone.** 1992. The methanogenic bacteria, p. 719-767. *In* A. Balows, H. G. Truper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), The prokaryotes, vol. I. Springer-Verlag, New York.

117. **Wolfe, N. L., L. A. Burns, and W. C. Steen.** 1980. Use of linear free energy relationships and an evaluative model to assess the fate and transport of phthalate esters in the aquatic environment. Chemosphere. **9:**393-402.

118. **Wolfe, N. L., W. C. Steen, and L. A. Burns.** 1980. Phthalate ester hydrolysis: linear free energy relationships. Chemosphere. **9:**403-408.

119. **Young, J. C.** 1997. Anaerobic treatability of phthalic acid production wastewaters. Presented at the 8th International Conf. on Anaerobic Digestion, Sendai, Japan, May 25-29, 1997.

120. **Young, J. C.** 1991. Factors affecting the design and performance of upflow anaerobic filters. Wat. Sci. Technol. **24:**133-155.

121. **Young, J. C., and H. W. Young.** 1991. Full-scale treatment of chemical process wastes using anaerobic filters. Res. J. WPCF. **63:**153-159.

122. **Yspeert, P., T. L. F. M. Vereijken, S. Vellenga, and d. A. Vegt.** 1993. The IC-reactor for the anaerobic treatment of industrial wastewater. Presented at the Food Industry Environmental Conference, Atlanta, USA.

123. **Ziogou, K., P. W. W. Kirk, and J. N. Lester.** 1989. Behavior of phthalic acid esters during batch anaerobic digestion of sludge. Wat. Res. **23:**743-748.

124. **Zoutberg, G., K. Heynekamp, and B. I. Versprille.** 1997. Anaerobic treatment of chemical wastewater in Biobed ® EGSB reactors. Presented at the 8th Int. Conf. on Anaerobic Digestion, Sendai, Japan, May 25-29, 1997.

Biodegradability

Abstract

The biodegradability of phthalate isomers and related aromatic compounds was studied using batch-assays. Two types of methanogenic granular sludge and digested sewage sludge were used as inoculum. All phthalate isomers and corresponding methyl esters were found to be degraded after a lag period ranging from 15 to 180 days.

Contents

2.1 Summary

All three phthalic acid isomers (*ortho*, *meta* and *para* benzene dicarboxylic acid) are produced in massive amounts, and used in the chemical industry as plasticizers or for the production of polyester. Wastestreams generated during the production of phthalate isomers generally contain high concentrations of aromatic acids. To study the potential biodegradability of these primarily anthropogenic compounds in anaerobic bioreactors, biodegradability studies were performed. Compounds tested were benzoate, *ortho*-phthalate, isophthalate, terephthalate, dimethyl phthalate, dimethyl terephthalate, *para*-toluate and *para*-xylene. Seed materials tested were two types of granular sludge and digested sewage sludge. It was found that all phthalate isomers and their corresponding dimethyl-esters, could be completely mineralised by all seed materials studied. Lag phases required for 50 % degradation of these compounds, ranged from 17 to 156 days. The observed degradation curves, could be explained by growth of an initially small amount of organisms in the inoculum with the specific ability to degrade one phthalate isomer. The observed order in the length of the lag phases for the phthalate isomers is: phthalate < terephthalate < isophthalate. This order appears to be related to the environmental abundancy of the different phthalate isomers. The initial step in the degradation pathway of both dimethyl phthalate esters was hydrolysis of the ester sidechain, resulting in the formation of the corresponding mono-methyl-phthalate isomer and phthalate isomer. The rate limiting step in mineralization of both dimethyl phthalate and dimethyl terephthalate was found to be fermentation of the phthalate isomer. *Para*-toluate was degraded only by digested sewage sludge after a lag phase of 425 days. The observed degradation rates of this compound were very low. No mineralization of *para*-xylene was observed. In general, the differences in the lag phases between different seed materials were relatively small. These results indicate that the time needed for the start-up of anaerobic bioreactors treating wastewaters containing phthalic acid isomers, depends little on the microbial composition of the seed material applied, but may take several months[a].

[a] A modified version of this chapter has been published in *Biodegradation* (1999), **10:** 63-73.

2.2 Introduction

Besides natural occurrence, human activities have added additional aromatic compounds to nature through manufacture, mining and combustion. In order to assess the fate of these compounds in methanogenic environments, anaerobic degradation of aromatic compounds has been studied extensively in recent years. Anaerobic mineralization of a large range of mono-aromatic compounds has been demonstrated using inocula from various origins. Most mono-aromatic compounds with at least one phenolic or carboxylic functional groups, and even aromatic hydrocarbons like toluene and *ortho*-xylene, were found to be mineralised under methanogenic conditions [1, 5, 7, 11, 12, 18].

The traditional treatment method of the waste generated during production of phthalic acid isomers comprises the activated sludge system, often combined with incineration of the solid waste produced [16]. Anaerobic pre-treatment combined with aerobic post treatment may represent an attractive alternative. However, so far only limited information is available concerning the anaerobic biodegradability of the aromatic compounds in these effluents. Despite this limited information, several full scale anaerobic bioreactors are currently in operation for pre-treatment of terephthalic and isophthalic acid wastewater [6, 22, 28]. A general characteristic of all these anaerobic reactors is a very long start-up time of up to two years.

This work described in this chapter focuses on the ability of different seed-materials to degrade the aromatic compounds in terephthalic acid and isophthalic acid wastewater. Considering the fact that the UASB-process is world-wide the most applied anaerobic treatment process [17, 19], two seed materials originating from full-scale applications of this type of reactor were tested. To date, most anaerobic biodegradability studies have been performed with digested sewage sludge, that we therefore used as a reference seed material. The anaerobic mineralization of phthalic acid and dimethyl phthalate were tested in order to asses whether these environmentally more abundant compounds were degraded more rapidly than their *meta* and *para* isomers.

2.3 Material and methods

Source and characteristics of the anaerobic inocula applied. Biomass from three different full scale reactors was tested for their ability to degrade aromatic compounds present in terephthalic acid wastewater. For this purpose, 25 litre sludge samples were taken from the reactors and stored at 4 °C until use. General characteristics of the different types of biomass applied are summarised in Table 2.1.

Table 2.1: General characteristics of the two types of granular biomass (CAB and EER) and digested sewage sludge (DSR) applied.

	unit	DSR	CAB	EER
waste treated		sewage sludge	starch processing	papermill
reactor-type[1]		CSTR	IC	UASB
conc. volatile solids[2]	g-VS·kg^{-1}	16.2	114	118
ash content[2]	%	37	31	19
methanogenic activity[3]	g-COD·g-VS^{-1}·d^{-1}	0.20	0.59	0.78

[1] CSTR: Continuously Stirred Tank Reactor, IC: Internal Circulation reactor (Yspeert et al., 1993), UASB: Upflow Anaerobic Sludge Bed reactor.

[2] Granular sludge was seeved prior to determination of the concentration volatile solids (VS) and ash content. The concentration volatile solids and the ash content of the digested sewage sludge was determined directly in the mixed liquor.

[3] The specific methanogenic activity of the sludge at 30 °C was measured in serum bottles, using 1 gCOD·l^{-1} sodium acetate as substrate and 1.5 gVS·l^{-1} biomass . The methane concentration in the headspace was used as indicator for acetate conversion in time.

Medium and substrate preparation. The basal medium used in the biodegradability studies contained the following (in mg·l^{-1} liquid volume): $NaHCO_3$ (4000), NH_4Cl (280), K_2HPO_4 (250), $MgSO_4.7H_2O$ (100), $CaCl_2.2H2O$ (10), yeast extract (18) and one millilitre of a trace element stock solution as described by Huser et al. [13]. Stock solutions of di-sodium phthalate, isophthalate and terephthalate, and sodium *para*-toluate were prepared in demineralized water. Dissolution and neutralisation of these poorly soluble acids, particularly terephthalic acid, could be enhanced through addition of a slight overdose of

NaOH resulting in a pH of 11-12 at an elevated temperature of 80-90 °C. After cooling down, the stock solutions were neutralised using 1 M HCl. Dimethyl phthalate, dimethyl terephthalate and *para*-xylene were dosed in the concentrated form. Dimethyl terephthalate is a water insoluble solid and was weighed out and added to the serum bottles as solid. Dimethyl phthalate and *para*-xylene are water insoluble liquids and were dosed to the serum bottles using a 100 µl syringe. *Para*-xylene was dosed after flushing the headspace of the serum bottle to avoid losses of this highly volatile compound. The final concentration of all substrates amounted 500 mg-COD·l^{-1}. Equal COD-concentrations were applied for easy comparison of methane production values.

Preparation of the test bottles. Nutrients, sludge and substrate (except para-xylene) were dosed to the serum bottles. Granular sludge was sieved prior to dosage to the bottles and digested sewage sludge was added directly from a well mixed sample. The initial biomass concentration in all experiments amounted to 5.0 ± 0.5 gVS·l^{-1}. Serum bottles were sealed with 2 cm thick butylrubber septa (Emergo, Schiedam, The Netherlands) and capped with aluminium screw caps. The headspace was replaced by a mixture of N_2/CO_2 in a ratio of 70:30, and 1 ml of a 30 gNa_2S.7-9H_2O.l^{-1} was dosed to the medium to ensure anaerobic conditions in the bottle. All experiments were conducted in duplicate, except for the blanks (no substrate dosed) for which triplicates were applied. Bottles were incubated stationary in the dark at 37 °C. No sterile controls were included in the experimental procedure, because the compounds tested are known to be highly stable in abiotic environments [37, 38]. The method applied in this study is essentially comparable to the method described by Shelton and Tiedje [33].

Experimental. The concentration of methane in the headspace of the bottles was used as a primary indicator for degradation of the substrate. At the beginning of the experiment this measurement was performed on a daily base. In time the frequency of the analysis was gradually decreased to approximately once every two weeks after 500 days. When, based on the methane measurement, mineralization of the substrate could be suspected, liquid samples were withdrawn and substrate concentrations were determined. Determination of the substrate concentration in the liquid was only possible for the water soluble substrates (benzoate, phthalate, isophthalate, terephthalate and *para*-toluate). The bottles incubated with non-soluble substrates were analysed for possibly formed water soluble aromatic intermediates. Once degradation of a substrate manifested, liquid samples were analysed for volatile fatty acids and the gas phase for hydrogen, in order to assess whether non-aromatic

intermediates accumulated. After complete mineralization of the substrate, a second substrate feed was supplied to the bottles.

Analytical procedures. The methane content of the headspace was determined by gas chromatography (Hewlett Packard 438/S). Samples (100 µl) were injected using a gas-lock syringe (Dynatech, Baton Rouge, La.). A stainless steel column (2 m. * 2 mm.) packed with Poropak Q (80-100 mesh) was used. Nitrogen was used as a carrier gas. The temperature of the column, injection port and flame ionisation detector were 60, 200 and 220°C respectively.

The concentration of water soluble aromatic acids was determined by high pressure liquid chromatography (HPLC). Centrifuged liquid samples (3 minutes at 10,000 *g*) were diluted to concentrations smaller than 50 $mg \cdot l^{-1}$ using a Meyvis Dilutor (type no. 401) and a volume of 10 µl was injected by autosampler (Marathon). Separation of the aromatic acids was obtained using a Chromospher 5C18 column (100*3 mm). The solvent used as a carrier was a mixture of methanol and a 1 % acetic acid solution in water, in a 40-60 ratio. The applied flow rate amounted 0.3 $ml \cdot minute^{-1}$. The separated components were detected by UV-detector (Spectroflow 773) at a wavelength of 230 nm. Typical retention times for *ortho*-phthalate terephthalate, isophthalate and benzoate were 2.8, 3.6, 4.1 and 6.8 minutes. Chromatograms were stored and integrated using the software package Minichrom.

The concentration and composition of volatile fatty acids in the medium was determined with a gas chromatograph (Hewlett Packard 5890A). A glass column (2 m. * 4 mm.) packed with Supelcoport (100-200 mesh), coated with 10 % Fluorad FC431, was used. The temperature of the column, injection port and flame ionisation detector were 130, 200 and 280°C respectively. Nitrogen gas saturated with formic acid was used as a carrier gas at a flow rate of 50 $ml \cdot min^{-1}$. Prior to analysis, the samples were diluted and fixed with a formic acid solution (3 % v/v). After formic acid addition aromatic acids precipitate and samples needed to be centrifuged (3 min., 10,000 *g*).

Hydrogen was determined by gas chromatography (Hewlett Packard 5890). The gas chromatograph was equipped with a stainless steel column (1.5 m * 6.4 mm.), packed with molecular sieve 25H (60-80 mesh). The temperature of the column, injection port and thermal conductivity detector were 40, 110 and 125 °C respectively. Argon was used as a carrier gas at a flow rate of 25 $ml \cdot min^{-1}$.

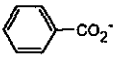
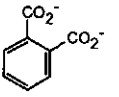
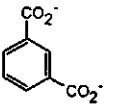
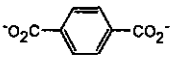
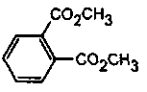
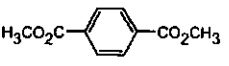
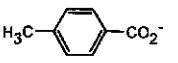
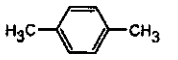
2.4 Results and discussion

Table 2.2 summarises the length of the lag periods prior to anaerobic mineralization of the aromatic substrates by the three different sludges. The length of the lag phase was defined as the time needed to convert approximately 50 % of the substrate into methane. In the following paragraphs the observed lag phases as well as the anaerobic biodegradability of the various compounds tested will be discussed in detail .

Benzoate. Complete mineralization of benzoate was obtained within two weeks for all seed materials tested. Herewith among all compounds tested, benzoate was most rapidly mineralised. This was not surprising because benzoate plays a key role in methanogenic mineralization of a large range of naturally occurring aromatic compounds [31]. Anaerobic degradation of benzoate was first demonstrated by Tarvin and Buswell [36], and in numerous studies since, with seed materials from various origin [1, 12].

***Ortho*-phthalate, isophthalate and terephthalate.** The three phthalate isomers were all degraded by digested sewage sludge and both types of granular sludge. Significant differences were observed in lag-phases between the different phthalate isomers and the different seed materials tested. When after complete degradation of the substrate a second dosage of substrate was supplied, substrate conversion started within one week. The evolution in time of the methane concentration in the headspace and the phthalate isomer concentration in the medium are presented in Figure 2.1 for CAB granular sludge. From this figure can be seen that despite the relatively high methane production in control experiments (800-1500 $mgCH_4$-COD·l^{-1} after 50 days), the methane production related to substrate conversion could easily be identified. In literature the occurrence of anaerobic mineralization of *ortho*-phthalate has been reported repeatedly [1, 12, 26]. Anaerobic terephthalate degradation has been described in technological studies using anaerobic bioreactors [6, 15, 28]. Isophthalate mineralization under methanogenic conditions has only been reported by Van Duffel [6].

Table 2.2: Compounds screened for their anaerobic biodegradability by three different types of inocula.

compound name	structural formula	initial conc [4] (mM)	time required to degrade 50 % of the aromatic substrate (days)[1] DSR	CAB	EER
benzoate		2.1	10 ± 1	9 ± 1	4 ± 0.5
phthalate		2.1	17 ± 1	49 ± 8	16 ± 2
iso-phthalate		2.1	74 ± 4	156 ± 12	87 ± 4
terephthalate		2.1	55 ± 1	61 ± 7	44 ± 4
dimethyl phthalate		1.5	16 ± 2	38 ± 2	17 ± 1
dimethyl terephthalate		1.5	58 ± 2	39 ± 1	48 ± 5
para-toluate		1.7	425 ± 50	>500	>500
para-xylene		1.5	>500	>500	>500

[1] Characteristics of the three types of sludge (DSR, CAB and EER) are described in Table 1.

[2] Concentrations applied were all equivalent to 500 mg-COD·l^{-1}.

Comparing the length of the lag periods required to obtain 50 % mineralization of the phthalate isomers, the following order was found for all sludge types: *ortho*-phthalate < terephthalate < isophthalate. As microbial degradation is the main removal mechanism for phthalate isomers released into the environment, the environmental abbundancy of organisms with the specific ability to degrade one of the phthalate isomers may therefore be directly related to the amounts released into the environment. Although all phthalate isomers are primarily anthropogenic compounds, catabolic pathways have been described that converge at *ortho*-phthalate. This compound for instance was found to be an intermediate in the degradation of polycyclic aromatic hydrocarbons [14]. Besides its natural occurrence, large amounts of *ortho*-phthalate are introduced into the environment anthropogenically in the form of phthalate esters [9]. Considering that the initial step in abiotic and microbiological conversion of phthalate esters is hydrolysis of the ester side chain resulting in the formation of phthalate, it is a plausible assumption that micro-organisms adapted to *ortho*-phthalate are widely distributed in the environment.

Compared to isophthalate, terephthalate is produced in much bigger amounts. Despite the slow hydrolysis of the products in which both compounds are applied, very likely larger amounts of terephthalate than isophthalate are introduced into the environment. This is confirmed by measurement of trace concentrations of terephthalate in surface water, municipal wastewater and air [2, 23], whereas no reports on the environmental occurrence of isophthalate occurrence were found. Based on these considerations it is suggested that the differences observed in the length of the lag periods prior to degradation of the individual phthalate isomers is determined primarily by the corresponding environmental turn over.

The drawn lines in Figure 2.1 were calculated assuming exponential growth of an initially small amount of organisms in the inoculum. Since no significant amounts of intermediate compounds were found (benzoate or volatile fatty acids), and the methane produced corresponds reasonably well to the amounts of substrate degraded, apparently the initial fermentation of the phthalate isomers to methanogenic substrates is the rate limiting step. This means that the growth rate of the mixed culture degrading the phthalate isomers can be described by single species kinetics [29]. Estimated apparent maximum specific growth rates (μ_{max}) ranging from 0.10 to 0.20 day^{-1} resulted in reasonable descriptions of the measured data, as shown in Figure 2.1. These values for the specific growth rate are in the same order of magnitude as those found for other methanogenic syntrophic consortia degrading propionate or butyrate [27]. Accurate estimation of specific growth rates based on the data

presented here is not possible, but it may be speculated that growth of a small amount of specific organisms originally present in the inoculum (with the ability to degrade one specific phthalate isomer) is the main mechanism responsible for the observed increase in the substrate conversion rate. However, time dependent adaptation of specific organisms in the seed material through induction of specific enzyme systems can not be excluded.

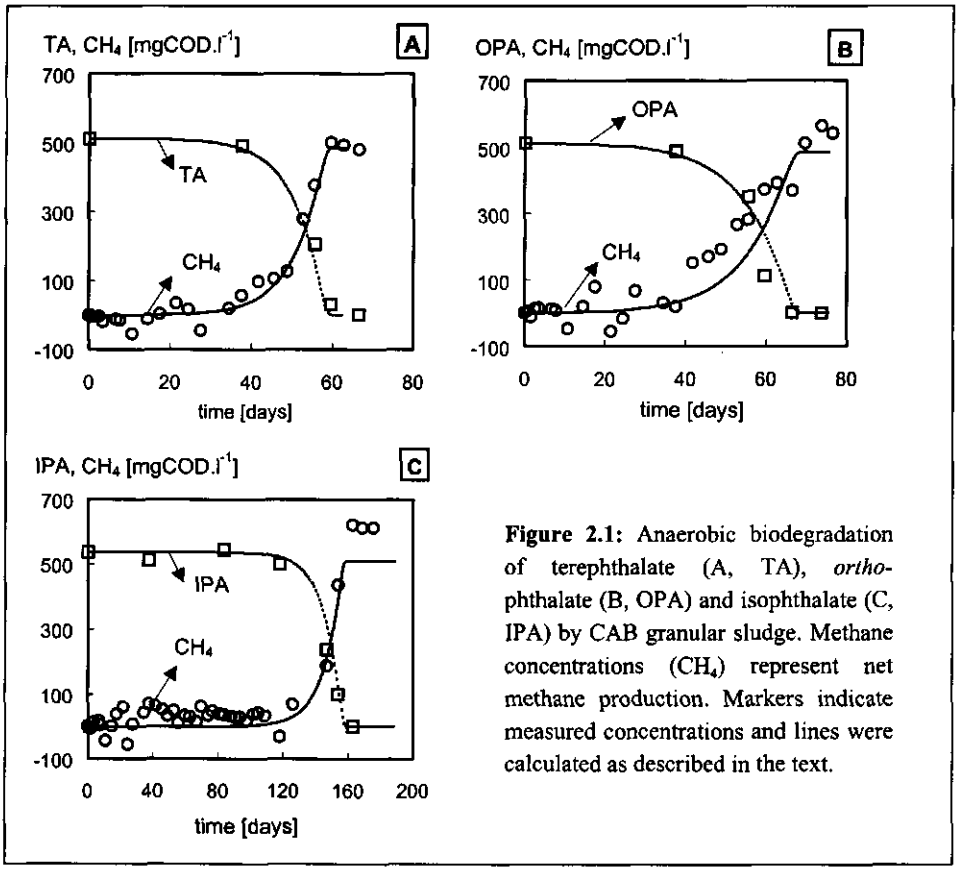


Figure 2.1: Anaerobic biodegradation of terephthalate (A, TA), *ortho*-phthalate (B, OPA) and isophthalate (C, IPA) by CAB granular sludge. Methane concentrations (CH_4) represent net methane production. Markers indicate measured concentrations and lines were calculated as described in the text.

The large differences observed in the length of the lag phases with the different phthalate isomers, indicate that all three phthalate isomers are fermented by different species of organisms. From aerobic environments several strains of bacteria have been isolated with the ability to degrade at least two phthalate isomers [30]. Recently, a soil bacterium has been isolated (*Pseudomonas* sp. strain P136) capable to utilise all three phthalate isomers through nitrate respiration [24, 25]. Higher substrate specifities of organisms in methanogenic

environments, as compared to organisms in aerobic or anoxic environments, are common [31].

Dimethtyl phthalate and dimethyl terephthalate. Both dimethyl phthalate and dimethyl terephthalate were completely mineralised by the three seed materials tested. Adaptation periods required for their complete mineralization were comparable to those found for their phthalate analogues.

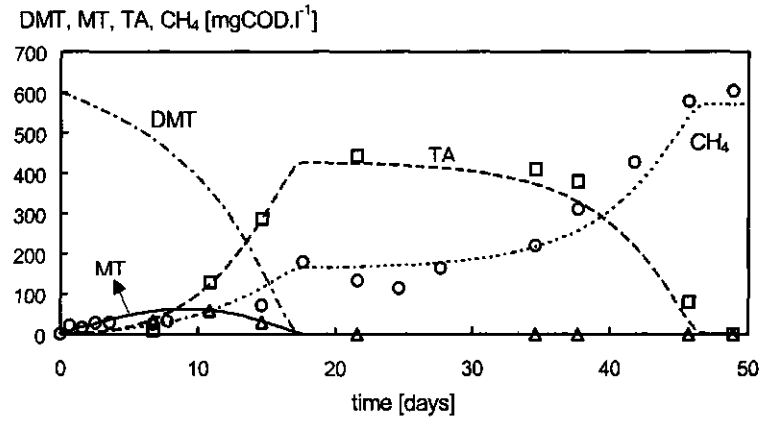


Figure 2.2: Anaerobic biodegradation of dimethyl terephthalate (DMT) by CAB granular sludge. Methyl terephthalate (MT, Δ) and terephthalate (TA, □) are intermediate compounds; methane (CH_4, O) is the endproduct. Markers indicate measured concentrations and lines were calculated with the mathematical model described in the text. DMT was not measured. Methane concentrations represent net methane production.

Liquid samples were analysed by HPLC to assess whether water soluble aromatic compounds accumulated during degradation of dimethyl phthalate and dimethyl terephthalate. Both dimethyl esters could not be detected in the medium, due to their poor solubility and tendency to become adsorbed to the biomass. The results show an accumulation of the mono-methyl ester, as well as the corresponding phthalic acid isomer in all experiments (Figure 2.2). After complete conversion into the phthalate isomers, conversion into methane occurred. Based on these observations the degradation pathway for dimethyl terephthalate is proposed to be as presented in Figure 2.3. An equivalent pathway is proposed for degradation of dimethyl phthalate. The degradation pathway shown in Figure 2.3 is comparable to that proposed for the anoxic mineralization of dibutyl-phthalate by

Pseudomonas pseudoalcaligenes [3] and methanogenic mineralization of butylbenzyl-phthalate by digested sewage sludge [32].

Drawn lines in Figure 2.2 were calculated using a simple model involving two metabolic groups of organisms: (i) organisms responsible for two-step demethylation of dimethyl terephthalate, resulting in formation of methylterephthalate and terephthalate respectively, and (ii, Xta) a mixed culture of organisms converting terephthalate into a mixture of methane (CH_4) and carbon dioxide. It was assumed that the methyl groups removed from DMT and MT were converted into methane. From Figure 2.2 it can be seen that a reasonable description of the experimental data could be obtained by using this approach, confirming the degradation sequence shown in Figure 2.3.

No significant difference in the rate of demethylation of dimethyl phthalate and dimethyl terephthalate was observed, suggesting that demethylation proceeds independently of the location of the methyl-ester group on the aromatic ring. Demethylation by acetogenic bacteria has been observed previously for aromatic compounds containing a metoxy group. These bacteria use the methyl group as a methyl donor in the synthesis of acetyl-CoA [8]. In mixed methanogenic cultures, acetate formed by these bacteria will be converted rapidly into methane and carbon dioxide by acetoclastic methanogens.

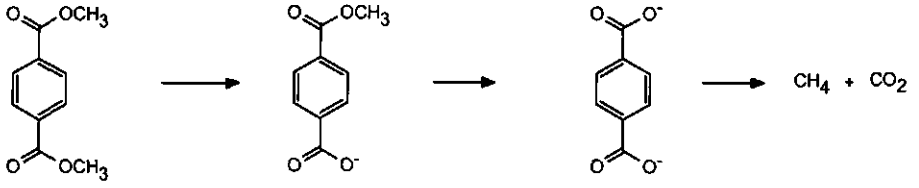


Figure 2.3: Proposed pathway for anaerobic biodegradation of dimethylterephthalate

***Para*-toluate and *para*-xylene.** *Para*-toluate was degraded only by one of the seed materials tested (digested sewage sludge) after an extremely long lag phase of approximately 425 days (Table 2.2). The degradation of *Para*-toluate has been observed previously by Horowitz [12], using fresh water sediments as inoculum. No degradation was observed by this author when two different types of digested sewage sludge were used. Macarie et al [20, 21] observed a slow removal of *para*-toluate in a UASB reactor seeded with digested sewage sludge.

The mineralization of *para*-toluate was found to proceed extremely slowly, even after a second feeding was supplied (Figure 2.4). The growth rate of the *para*-toluate fermenting culture apparently was extremely low. From Figure 2.4 it can be seen that a reasonable

description of the data of the first substrate feeding can be obtained using an overall growth rate of 0.012 day^{-1}. However, as the conversion rate of *para*-toluate did not increase during the second substrate feeding, the growth rate may be even lower than this value.

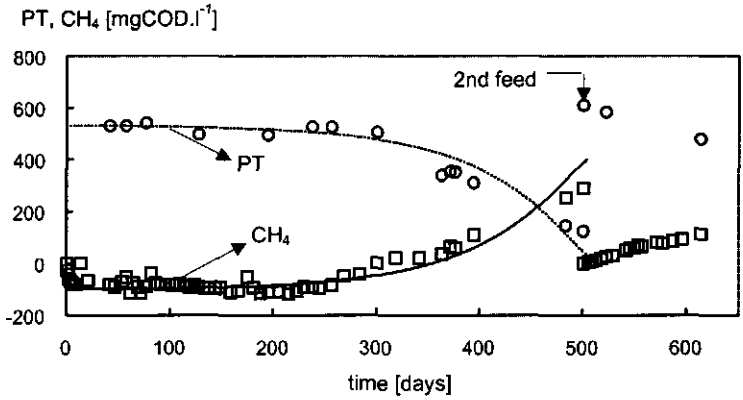


Figure 2.4: Anaerobic biodegradation of *para*-toluate (PT) by digested sewage sludge (DSR). Markers indicate measured concentrations and lines were calculated based on the stoichiometry of the conversion and simple Monod kinetics. Methane concentrations represent net methane production.

No *Para*-xylene degradation was observed in any of the experiments, within the 500 days the experiment lasted. The methane production was even 10-25 % lower compared to reactors where no substrate was dosed (data not shown). This observation suggests that *para*-xylene is toxic to organisms involved in the digestion of the seed material. Blum and Speece [4] found an IC50 value for acetoclastic methanogens of 790 mg-COD·l^{-1}, which is slightly higher than the concentration applied in our experiment (500 mg-COD·l^{-1}). The toxicity of *para*-xylene very likely is the result of its high hydrophobicity (logP is 3.1), which may lead to disturbance of essential membrane functions [34, 35]. In literature no reports were found dealing with the mineralization of *para*-xylene in methanogenic environments. Anoxic mineralization of *para*-xylene has been reported by Häner [10].

Technological implications. The relatively small differences between the different seed materials concerning the lag-phases prior to degradation of the phthalate isomers, suggest that they contain a comparable amount of bacteria with the specific ability to degrade phthalate isomers. Therefore it may be presumed that the source of the inoculum has only a limited impact on the time needed for start-up of anaerobic bioreactors for treatment of

phthalic acids containing wastewaters. However, as the anaerobic degradation of phthalate isomers is dependent on syntrophic consortia consisting of fermentative bacteria and methanogens, granular biomass with a high specific methanogenic activity will be capable to degrade the fermentation products at high rates, herewith creating optimal micro-environments for growth of the fermentative bacteria. Combined with the excellent settling properties of granular biomass, this suggests that granular sludge is the preferred inoculum over digested sewage sludge for start-up of UASB reactors treating phthalic acids containing wastewaters.

Both granular sludges used in the experiments described here, were grown on "rich substrates": paper mill wastewater and potato starch processing wastewater. These substrates may contain a variety of aromatic compounds, ranging from lignin derivatives in papermill wastewater to aromatic amino acids in potato starch processing wastewater. Methanogenic granular sludge grown on substrates not containing any aromatic compounds, may not be able to degrade the substrates tested here, or only after significantly longer lag periods.

With respect to the application of anaerobic treatment for terephthalic acid wastewater, the long lag periods prior to terephthalate degradation, combined with the observed inhibition of terephthalate degradation by the wastewater constituents acetate and benzoate [15], suggests that the start-up of anaerobic reactors for treatment of terephthalic acid wastewater may take a long time. Start-up times of more than 1 year are indeed more the rule than the exception [6, 15, 28]. Despite such a long start-up period, anaerobic pre-treatment of wastewaters generated during terephthalic acid production may represent an attractive contribution to conventionally applied aerobic treatment methods, provided that sufficiently high and stable volumetric removal capacities can be achieved after successful start-up. In this respect it should be noted that in anaerobic bioreactors, acetate and benzoate can normally removed to a large extent within a short period of time. Taken into account that these two compounds are important wastewater constituents, treatment efficiencies of around 50 % can normally be obtained within weeks of operation. It should furthermore be emphasised that the length of the lag-period prior to terephthalate degradation in anaerobic bioreactors may significantly be reduced by pre-removal of acetate and benzoate in a staged bioreactor concept. Whether the lag period prior to terephthalate degradation through pre-removal of acetate and benzoate is indeed decreased from at least one year in full-scale reactors to approximately 2 months, as observed during this study, will be studied in the near future in our lab.

Mineralization of *para*-toluate can normally not be expected in anaerobic bioreactors for pre-treatment of terephthalic acid or dimethyl terephthalate wastewater. Due to the low growth rate of the specific *para*-toluate degrading biomass, a very long solid retention time needs to be maintained to enable degradation. In general, this means that *para*-toluate needs to be removed during aerobic post-treatment of the anaerobic effluent.

2.5 References

1. **Battersby, N. S., and V. Wilson.** 1989. Survey of the anaerobic biodegradation potential of organic chemicals in digesting sludge. Appl. Environ. Microbiol. **55**:433-39.
2. **Behret, H.** 1991. Terephthalic acid. *In* E. Bayer (ed.), BUA Reports, vol. 68-70. S. Hirzel, Wissenschaftliche Verlagsgesellschaft, Stuttgart, Germany.
3. **Benckiser, G., and J. C. G. Ottow.** 1982. Metabolism of the plasticizer di-n-butylphthalate by *Pseudomonas pseudoalcaligenes* under anaerobic conditions with nitrate as the only electron acceptor. Appl. Environ. Microbiol. **44**:576-578.
4. **Blum, D. J. W., and R. E. Speece.** 1991. A database of chemical toxicity to environmental bacteria and its use in interspecies comparisons and correlations. Res. J. WPCF. **63**:198-207.
5. **Boyd, S. A., D. R. Shelton, D. Berry, and J. M. Tiedje.** 1983. Anaerobic biodegradation of phenolic compounds in digested sludge. Appl. Environ. Microbiol. **46**:50-54.
6. **Duffel, J. v.** 1993. Anaerobe behandeling van organische zuren. Presented at the National Conference on Anaerobic Treatment of Complex Wastewaters, Breda, The Netherlands.
7. **Edwards, E. A., and D. Grbic-Galic.** 1994. Anaerobic degradation of toluene and *o*-xylene by a methanogenic consortium. Appl. Environ. Microbiol. **60**:313-322.
8. **Fuchs, G., M. E. S. Mohamed, U. Altenschmidt, J. Koch, A. Lack, R. Brackmann, C. Lochmeyer, and B. Oswald.** 1994. Biochemistry of anaerobic biodegradation of aromatic compounds, p. 513-553. *In* C. Ratledge (ed.), Biochemistry of microbial degradation. Kluwer Academic Publishers, Dordrecht, The Netherlands.
9. **Giam, C. S., E. Atlas, M. A. Powers Jr., and J. E. Leonard.** 1984. Phthalic acid esters, p. 67-140. *In* O. Hutzinger (ed.), Anthropogenic Compounds, vol. 3, Part C. Springer-Verlag, Berlin-Heidelberg-New York-Tokyo.
10. **Häner, A., P. Höhener, and J. Zeyer.** 1995. Degradation of *p*-xylene by a denitrifying enrichment culture. Appl. Environ. Microbiol. **61**:3185-3188.
11. **Healy, J. B., and Y. Young.** 1979. Anaerobic degradation of eleven aromatic compounds to methane. Appl. Environ. Microbiol. **38**:84-89.
12. **Horowitz, A., D. R. Shelton, C. P. Cornell, and J. M. Tiedje.** 1982. Anaerobic degradation of aromatic compounds in sediments and digested sludge. Dev. Ind. Microbiol. **23**:435-444.

13. **Huser, B. A., K. Wuhrmann, and A. B. J. Zehnder.** 1980. *Methanothrix soehngenii* gen. nov. sp. nov., a new acetotrophic non hydrogen oxidizing methane bacterium. Arch. Microbiol. **132:**1-9.

14. **Kiyohara, H., and K. Nagao.** 1978. The catabolism of phenanthrene and naphthalene by bacteria. J. Gen. Microbiol. **105:**69-75.

15. **Kleerebezem, R., J. Mortier, L. W. Hulshoff Pol, and G. Lettinga.** 1997. Anaerobic pre-treatment of petrochemical effluents: terephthalic acid wastewater. Wat. Sci. Tech. **36:**237-248.

16. **Lau, C. M.** 1978. Staging aeration for high-efficiency treatment of aromatic acids plant wastewater. Presented at the 32nd Independent Wastewater Conference Purdue University, West Lafayette, Ind.

17. **Lettinga, G.** 1995. Anaerobic digestion and wastewater treatment systems. Antonie Leeuwenhoek. **67:**3-28.

18. **Long-de Valliere, C. L., S. Petrozzi, D. Zurrer, U. Baier, and I. J. Dunn.** 1989. Methods of anaerobic degradation of toxic compounds in chemical and industrial wastewaters, p. 35-72. *In* A. Mizrahi (ed.), Biological Waste Treatment, vol. 12. Alan R. Liss, Inc., New York.

19. **Macarie, H.** 1996. Anaerobic digestion an adequate technology for the treatment of some chemical and petrochemical wastewaters. Presented at the IV Taller Latinoamericano Sobre Tratiamento Anaerobio de Aguas Residuales, Bucaramanga, Colombia.

20. **Macarie, H., and J. P. Guyot.** 1992. Inhibition of the methanogenic fermentation of p-toluic acid (4-methylbenzoic acid) by acetate. Appl. Microbiol. Biotechnol. **38:**398-402.

21. **Macarie, H., and J. P. Guyot.** 1995. Use of ferrous sulphate to reduce the redox potential and allow the start-up of UASB-reactors treating slowly biodegradable compounds: application to a wastewater containing 4-methylbenzoic acid. Environ. Tech. **16:**1185.

22. **Macarie, H., A. Noyola, and J. P. Guyot.** 1992. Anaerobic treatment of a petrochemical wastewater from a terephtalic acid plant. Water Sci. Technol. **25:**223-235.

23. **Matsumoto, G.** 1982. Comparative study on organic constituents in polluted and unpolluted inland aquatic environments; phenols and aromatic acids in polluted and unpolluted waters (III). Wat. Res. **16:**551-557.

24. **Nozawa, T., and Y. Maruyama.** 1988. Anaerobic metabolism of phthalate and other aromatic compounds by a denitrifying bacterium. J. Bacteriol. **170:**5778-5784.

25. **Nozawa, T., and Y. Maruyama.** 1988. Denitrification by a soil bacterium with phthalate and aromatic compounds as substrates. J. Bacteriol. **170:**2501-2505.

26. **O'Connor, O. A., M. D. Rivera, and L. Y. Young.** 1989. Toxicity and biodegradation of phthalic acid esters under methanogenic conditions. Environ. Tox. Chem. **8:**569-576.

27. **Pavlostathis, S. G., and E. Giraldo-Gomez.** 1991. Kinetics of anaerobic treatment: a critical review. Critical Rev. Environ. Control. **21:**411-490.

28. **Pereboom, J. H. F., d. G. Man, and I. T. Su.** 1994. Start-up of full scale UASB-reactor for the treatment of terephtalic acid waste water. Presented at the 7th International Symposium on Anaerobic Digestion, Cape Town, South Africa.

29. **Powell, G. E.** 1984. Equalisation of specific growth rates for syntrophic associations in batch cultures. J. Chem. Tech. Biotechnol. **34B:**97-100.

30. **Ribbons, D. W., P. Keyser, D. A. Kunz, B. F. Taylor, R. W. Eaton, and B. N. Anderson.** 1984. Microbial degradation of phtalates, p. 371-397. *In* D. T. Gibson (ed.), Microbial Degradation of Organic Compounds.

31. **Schink, B., A. Brune, and S. Schnell.** 1992. Anaerobic degradation of aromatic compounds, p. 219-242. *In* G. Winkelmann (ed.), Microbial Degradation of Natural Products. VCH, Weinheim, Germany.

32. **Shelton, D. R., S. A. Boyd, and J. M. Tiedje.** 1984. Anaerobic biodegradation of phthalic acid esters in sludge. Environ. Sci. Technol. **18:**93-97.

33. **Shelton, D. R., and J. M. Tiedje.** 1984. General Method for determining anaerobic biodegradation potential. Appl. Environ. Microbiol. **47:**850-857.

34. **Sierra, R., and G. Lettinga.** 1991. The effect of aromatic structure on the inhibition of acetoclastic methanogenesis in granular sludge. Appl. Microbiol. Biotechnol. **34:**544-550.

35. **Sikkema, J., J. A. M. Bont de, and B. Poolman.** 1994. Interactions of cyclic hydrocarbons with biological membranes. J. Biol. Chem. **269:**8022-8028.

36. **Tarvin, D., and A. M. Buswell.** 1934. The methane fermentation of organic acids and carbohydrates. J. Amer. Chem. Soc. **56:**1751-1755.

37. **Wolfe, N. L., L. A. Burns, and W. C. Steen.** 1980. Use of linear free energy relationships and an evaluative model to assess the fate and transport of phthalate esters in the aquatic environment. Chemosphere. **9:**393-402.

38. **Wolfe, N. L., W. C. Steen, and L. A. Burns.** 1980. Phthalate ester hydrolysis: linear free energy relationships. Chemosphere. **9:**403-408.

Microbiology I: Kinetics

Abstract

Maximum specific growth rates and biomass yields were determined for methanogenic enrichment cultures grown on *ortho*-phthalate, isophthalate or terephthalate. Implementation of measured and estimated parameter values in a mathematical model enabled the description of intermediate accumulation and end product formation during degradation of the phthalate isomers.

Contents

3.1 Summary

Three methanogenic enrichment cultures, grown on *ortho*-phthalate, isophthalate and terephthalate were obtained from digested sewage sludge or methanogenic granular sludge. Cultures grown on one of the phthalate isomers were not capable of degrading the other phthalate isomers. All three cultures had the ability to degrade benzoate. Maximum specific growth rates (μ_S^{max}) and biomass yields ($Y_{X_{tot}S}$) of the mixed cultures were determined by using both the phthalate isomers and benzoate as substrates. Comparable values for these parameters were found for all three cultures. Values for μ_S^{max} and $Y_{X_{tot}S}$ were higher for growth on benzoate compared to the phthalate isomers. Based on measured and estimated values for the microbial yield of the methanogens in the mixed culture, specific yields for the phthalate and benzoate fermenting organisms were calculated. A kinetic model, involving three microbial species, was developed to predict intermediate acetate and hydrogen accumulation and the final production of methane. Values for the ratio of the concentrations of methanogenic organisms, versus the phthalate isomer and benzoate fermenting organisms, and apparent half saturation constants (K_S) for the methanogens were calculated. By using this combination of measured and estimated parameter values, a reasonable description of intermediate accumulation and methane formation was obtained, with the initial concentration of phthalate fermenting organisms being the only variable. The energetic efficiency for growth of the fermenting organisms on the phthalate isomers was calculated to be significantly smaller than for growth on benzoate[a].

3.2 Introduction

All three phthalic acid isomers (*ortho*-, *meta*- and *para*-benzene dicarboxylic acid) are produced in massive amounts around the world and are primarily anthropogenic compounds. They are used in the chemical production of a wide range of plastics. Diesters of *ortho*-phthalic acid are mainly used as plasticizer in the production of polyvinyl chloride [11]. *para*-Phthalic acid (terephthalic acid) and the corresponding dimethyl ester are used in the production of polyester fibres and polyethylene terephthalate (PET). This latter compound is well known for its application in bottles for carbonated drinks. *meta*-Benzene dicarboxylic acid (isophthalic acid) is produced in smaller amounts than its *ortho*- and *para*-oriented isomers, and is used in the production of specialty chemicals.

[a] A modified version of this chapter has been published in *Appl. Environ. Microbiol.* (1999), **65**: 1152-1160

Introduction of phthalic acid isomers into the environment may occur through leaching from plastics [11]. In addition to this diffuse source of environmental pollution with phthalic acids, large point sources are generated during production of phthalate isomers from their corresponding xylenes [2]. Both liquid and solid wastestreams, generated during production of the phthalate isomers contain high concentrations of these aromatic acids. Furthermore, heavily contaminated soil with high concentrations of phthalic acid isomers can be found around chemical factories [19].

Microbial activity is the principal method for the removal of phthalic acid isomers and their corresponding esters from the environment. The aerobic and anoxic degradation of phthalic acid isomers has been studied extensively as described in Chapter 1, but hardly any information is available about the anaerobic mineralization of phthalate isomers under sulphate reducing or methanogenic conditions. It has been suggested that methanogenic degradation of *ortho*-phthalate proceeds analogously to degradation under denitrifying conditions because methanogenic enrichment cultures grown on *ortho*-phthalate had the ability to degrade benzoate [26].

In methanogenic environments complex organic matter (including aromatic compounds) is converted into a mixture of methane and carbon dioxide in a complex network of various metabolic groups of bacteria. Bacteria in these consortia depend entirely on each other to perform the metabolic conversions observed [25, 30] and are therefore referred to as syntrophic consortia. In these syntrophic consortia, fermentative bacteria convert complex organic matter into a mixture of acetate and hydrogen or formate, which are substrates for methanogenic bacteria. Fermentative conversions of substrates are often energetically unfavourable under standard conditions ($\Delta G^{0'} > 0$), implying the need for continuous removal of the fermentative products by methanogens.

In this chapter three methanogenic enrichment cultures degrading either *ortho*-phthalate, isophthalate, or terephthalate are described. Emphasis is put on the kinetics of mineralization of the phthalate isomers. A method is described to calculate the kinetic parameters of the individual trophic groups involved in mineralization of the aromatic substrates. The specific role of benzoate in the anaerobic degradation of terephthalate is presented in Chapter 4.

3.3 Materials and methods

Source of the biomass and enrichment procedure. Biodegradability experiments were performed with three different types of biomass and a set of aromatic compounds that are present in phthalic acid isomer production wastewaters (Chapter 2). Sludge types used for

these experiments were digested sewage sludge and two types of granular sludge from full-scale upflow anaerobic sludge bed (UASB) reactors. The initial concentrations of the phthalate isomers were 2.1 mM. When, after a lag phase ranging from 15 to 150 days, mineralization of the phthalate isomers was observed, the substrate was replenished to a concentration of 6 mM. Substrate dosage was repeated until a phthalate isomer degradation rate of approximately 1 $mM \cdot day^{-1}$ was reached. At this point 20% of the culture liquid was transferred into prereduced fresh medium containing 6 to 12 mM phthalate isomer. Cultivation was performed in 300-ml serum bottles sealed with butyl rubber septa, using a liquid volume of 70 ml. Serum bottles were incubated statically at 37°C in the dark. To monitor degradation of the phthalate isomers, the methane concentration in the headspace of the serum bottles was measured at least twice a week. This procedure was used for more than 18 months in order to obtain stable and highly enriched cultures. Best growth on *ortho*-phthalate was obtained with the culture initially seeded with granular sludge from a UASB reactor treating wastewater from a paper factory, while cultivation on isophthalate proceeded most rapidly with cultures initially seeded with digested sewage sludge. These two enrichment cultures were therefore used for the experiments described here.

The inoculum of the terephthalate degrading enrichment culture was obtained from a laboratory-scale anaerobic hybrid reactor (Chapter 6). Hybrid reactors are upflow reactors equipped with carrier material in the top of the reactor instead of the three phase separator used in UASB reactors. The hybrid reactor had been in continuous operation for a period of 12 months with terephthalate as the sole carbon and energy source. The specific terephthalate conversion rate of the biomass grown in this reactor was approximately 1.7 $mmol \cdot g^{-1} \cdot day^{-1}$. Similar cultivation methods were used as described for the *ortho*-phthalate and isophthalate degrading cultures for more than 1 year before the experiments described here were carried out.

Medium and substrate preparation. The composition of the basal medium used in the experiments has been described in Chapter 2. Stock solutions of disodium *ortho*-phthalate, isophthalate, and terephthalate were prepared in demineralized water.

Experimental procedure. Experiments were performed in 300- or 120-ml serum bottles with liquid volumes of 70 or 25 ml respectively. Medium and substrate were added to the serum bottles. The bottles were sealed with butyl rubber septa and aluminium screw or crimp caps, and the headspace was flushed with a mixture of N_2 and CO_2 (70:30 [vol/vol]). After flushing, $Na_2S \cdot 7\text{-}9H_2O$ was added from a concentrated stock solution to obtain a final concentration of 150 $mg \cdot l^{-1}$. Serum bottles were preincubated at 37 ± 1 °C in an orbital-motion shaker prior to inoculation by syringe. Liquid samples (1 ml) were withdrawn from

the serum bottles for component analyses. Measured methane concentrations in the headspace of the bottles were corrected for the volumes of samples removed.

Analytical procedures. The concentration of aromatic acids was determined by high pressure liquid chromatography (HPLC). The methane and hydrogen concentration in the headspace of the serum bottles, and the concentration and composition of volatile fatty acids in the liquid were determined by gas chromatography (GC). A detailed description of these methods can be found in Chapter 2.

Determination of kinetic parameters. The total biomass yield of the mixed cultures growing on the phthalate isomers and benzoate was determined in batch experiments. A known amount of biomass was transferred into fresh medium containing a high concentration of substrate (10 to 12 mM for benzoate and the phthalate isomers). After complete mineralization of the substrate, the concentration of volatile solids was determined and the biomass yield was calculated by relating the increase of the biomass concentration to the amount of substrate degraded. In case of degradation of a complex substrate by a syntrophic culture, the sum of the yield factors of all the species participating in the degradation is measured. Consequently, the measured yield is referred to as $Y_{X_{tot}S}$ ($g \cdot mol\text{-}S^{-1}$). For determination of the microbial yield on acetate ($Y_{X_{AcM}C2}$), several portions of 5 mM acetate were supplied to avoid substrate inhibition.

The maximum specific growth rate (μ_S^{max}, day^{-1}) of the cultures was calculated from the exponential part of substrate depletion and/or product formation curves. In case of exponential growth of the culture ($C_S >> K_S$), Monod-based equations for substrate depletion and product formation can be integrated and the following equations apply (neglecting maintenance and/or decay):

$$C_S(t) = C_S(0) + \frac{C_X(0)}{Y_{X_{tot}S}} \cdot \left(1 - e^{\mu_S^{max} \cdot t}\right) \tag{3.1}$$

$$C_P(t) = C_P(0) - f_{SP} \cdot \left(1 - \eta_{X_{tot}S}\right) \cdot \frac{C_X(0)}{Y_{X_{tot}S}} \cdot \left(1 - e^{\mu_S^{max} \cdot t}\right) \tag{3.2}$$

where f_{SP} stands for the number of moles of product, produced per mol of substrate ($mol\text{-}P \cdot mol\text{-}S^{-1}$) according to the stoichiometry of the reaction (Table 3.1), and $\eta_{X_{tot}S}$ is the electron yield as can be calculated from the measured yield according to Equation A-4 (see Appendix). Using measured values for the microbial yield and measured substrate concentrations (C_S, $mol \cdot liter^{-1}$) and/or product concentrations (C_P, $mol \cdot liter^{-1}$) as a function of

time, the initial biomass concentration ($C_X(0)$, g·liter^{-1}) and μ_S^{max} can be estimated with an optimisation procedure as available in most spreadsheet programs. Optimisation was based on minimising the absolute error between measured and calculated values for C_S and C_P.

Calculation of kinetic parameters. In order to describe intermediate accumulation and final methane production during growth of the mixed cultures on the phthalate isomers and benzoate, a mathematical model was derived (see Appendix). Nonmeasured parameter values that were required as input for the model were estimated by using the procedures described below.

The biomass yield of the organisms responsible for fermentation of the phthalate isomers and benzoate ($Y_{X_{Ferm}S}$) was calculated from the (measured) total biomass yields ($Y_{X_{tot}S}$) and the biomass yields of the acetoclastic and hydrogenotrophic methanogens ($Y_{X_{AcM}}$ and $Y_{X_{HyM}}$) according to the following equation:

$$Y_{X_{Ferm}PA} = Y_{X_{tot}PA} - f_{PAC2} \cdot \left(1 - \eta_{X_{Ferm}PA}\right) \cdot Y_{X_{AcM}C2} - f_{PAH2} \cdot \left(1 - \eta_{X_{Ferm}PA}\right) \cdot Y_{X_{HyM}H2} \quad (3.3)$$

where f_{PAC2} and f_{PAH2} are respectively the number of moles of acetate and of hydrogen formed per mole of phthalate isomer (mol-C2/H2·mol-PA^{-1}) according to the chemical reaction equation for phthalate fermentation (Table 3.1, reaction 1), and $\eta_{X_{Ferm}PA}$ is the electron yield of the phthalate isomer fermenting culture (e-mol-X_{Ferm}·e-mol-PA^{-1}). Substitution of Equation A-4 (Appendix) into Equation 1 allows for direct calculation of the biomass yield for the fermenting bacteria.

Since during growth on the phthalate isomers the intermediate concentrations of acetate and hydrogen were found to be relatively constant (dC_{C2}/dt and dC_{H_2}/dt equal 0, Table A-1), the actual specific growth rates of the different species in the mixed culture must be equal [23]. Definition of this boundary condition allows for calculation of the concentration ratio of the acetoclastic methanogens relative to the fermenting organisms according to the following equation:

$$\frac{C_{X_{Ferm}}}{C_{X_{AcM}}} = \frac{Y_{X_{Ferm}PA}}{f_{PAC2} \cdot (1 - \eta_{X_{Ferm}PA}) \cdot Y_{X_{AcM}C2}} \quad (3.4)$$

From the average concentrations of acetate and hydrogen measured during exponential growth on the phthalate isomers (see Table 3.3) and the observation that these concentrations remained constant during growth on the phthalate isomers, the apparent half-saturation

constant for acetate and hydrogen (K_{C2} and K_{H2}) can be estimated. At phthalate concentrations significantly higher than the half-saturation constant for phthalate fermentation (K_{PA}), the following equation enables calculation of K_{C2}:

$$K_{C2} = \frac{C_{C2}^{avg}}{\frac{q_{X_{Ferm}PA}^{max} \cdot f_{PAC2} \cdot (1 - \eta_{X_{Ferm}PA})}{q_{X_{AcM}C2}^{max}} \cdot \frac{X_{Ferm}}{X_{AcM}}} - C_{C2}^{avg} \qquad (3.5)$$

Expressions equivalent to Equation 4 and 5 were used for calculation of $C_{X_{Ferm}}/C_{X_{HyM}}$ and K_{H2}.

Table 3.1: Chemical reaction equations for the individual steps in mineralisation of phthalate isomers and benzoate, and standard Gibbs free-energy changes during the conversions, corrected for a temperature of 37 °C.

	reaction	equation	$\Delta G^{0'}$ (37 °C) (kJ·reaction^{-1})
1	phthalate fermentation	$C_8H_4O_4^{2-} + 8H_2O \rightarrow 3C_2H_3O_2^- + 3H^+ + 3H_2 + 2HCO_3^-$	38.9[1]
2	benzoate fermentation	$C_7H_5O_2^- + 7H_2O \rightarrow 3C_2H_3O_2^- + 3H^+ + 3H_2 + HCO_3^-$	59.6
3	hydrogenotrophic methanogenesis	$0.75HCO_3^- + 0.75H^+ + 3H_2 \rightarrow 0.75CH_4 + 2.25H_2O$	-98.4
4	acetoclastic methanogenesis	$3C_2H_3O_2^- + 3H^+ \rightarrow 3CH_4 + 3CO_2$	-97.8
5	mineralization of phthalate	$C_8H_4O_4^{2-} + 6.5H_2O \rightarrow 3.75CH_4 + 2HCO_3^- + 2.25CO_2$	-157.3
6	mineralization of benzoate	$C_7H_5O_2^- + 5.5H_2O \rightarrow 3.75CH_4 + HCO_3^- + 2.25CO_2$	-136.6

[1] The value represents the calculated $\Delta G^{0'}$ for isophthalate and terephthalate fermentation, the value for *ortho*-phthalate fermentation is estimated to be 34.9 kJ·reaction^{-1}. Applied ΔG_f^0 values for *ortho*-phthalate, isophthalate and terephthalate were -548.6, -552.6 and -552.7 kJ·mol^{-1} respectively (5).

Gibbs free energy changes. Standard Gibbs free-energy changes for the individual steps in anaerobic mineralisation of the phthalate isomers and benzoate were calculated according to Thauer et al. [33] (Table 3.1). ΔG_f^0 values for the phthalate isomers were calculated from benzoate by using the group contribution method described by Dimroth [5]. ΔG_f^0 values were corrected for a temperature of 37°C by using the Van't Hoff equation [4].

Microscopical observation. The cultures were routinely observed by using a phase contrast microscope. Cultures grown on *ortho*-phthalate and isophthalate were prepared for scanning electron microscopy. Samples (10 ml) were filtered into 1 ml observation chambers. The observation chambers were closed and fixed with 2.5% gluteraldehyde. After fixation, samples were stored overnight in a 0.5% osmium tetraoxide solution in a buffer of sodium cacodylate (0.1 M, pH 7.1). After three rinses with demineralized water, samples were dehydrated stepwise with ethanol. Samples were mounted on stubs with carbon cement, critical point dried with CO_2, and sputter coated with 3 nm of platinum. The coated specimen were observed in a Jeol JSM 6300F scanning microscope at 5 to 8 kV.

3.4 Results

Enrichment cultures. Three stable enrichment cultures with the ability to degrade either *ortho*-phthalate, isophthalate, or terephthalate were obtained through numerous transfers into fresh medium throughout a period of more than one year. Multiple doses of substrate (6 to 12 mM phthalate isomer) were necessary to obtain high conversion rates. Once the conversion rate amounted to approximately 1 $mM \cdot day^{-1}$, 20% of the culture was transferred into fresh medium. No stable growth was obtained when (i) smaller amounts of the cultures were transferred or when (ii) the cultures were transferred when rates were significantly lower. Moreover, when transferring the cultures after complete depletion of the substrate, long lag phases and nonexponential growth were observed. These observations were studied in more detail by using the terephthalate grown enrichment culture, and the results obtained are described in Chapter 4. Due to their low growth rate (see Table 3.2), the cultures could be transferred approximately once a month. A typical example of the methane production profile found during several transfers of the isophthalate grown culture into fresh medium is shown in Figure 3.1.

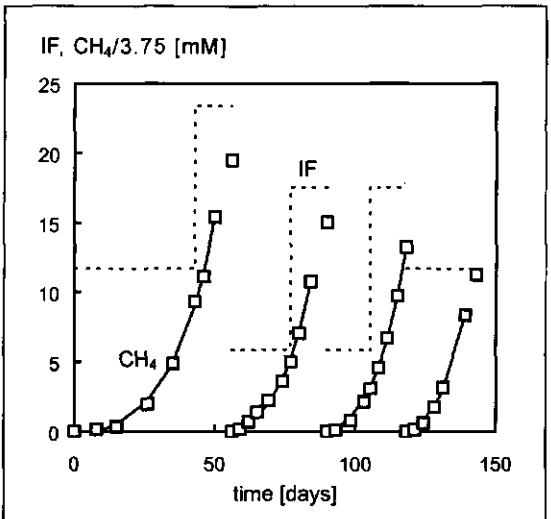


Figure 3.1: Calculated (solid lines) and measured (□) methane concentrations (CH_4) and substrate levels (dashed lines) during several transfers of the isophthalate (IF)-grown culture.

Cultures grown on one of the phthalate isomers were not able to degrade either of the other two phthalate isomers, suggesting that specific organisms are responsible for the degradation of each of the phthalate isomers. All three cultures were able to degrade benzoate without a lag period at rates comparable to the phthalate isomers degradation rates.

Table 3.2: Maximum specific growth rate (μ_S^{max}) and total biomass yield ($Y_{X_{tot}S}$) for three phthalate isomers-degrading enrichment cultures, grown on the phthalate isomers, benzoate or acetate.

culture	substrate	$Y_{X_{tot}S}$ (g.d.w.·mol^{-1})		μ_S^{max} (day^{-1})	
isophthalate	iso-phthalate	8.5	(0.8)[1]	0.092	(0.012)
	benzoate	11.5	(0.6)	0.130	(0.020)
	acetate	0.97	(0.09)	0.131	(0.014)
***ortho*-phthalate**	*ortho*-phthalate	8.2	(1.2)	0.087	(0.007)
	benzoate	12.8	(0.5)	0.216	(0.009)
	acetate	1.01	(0.15)	0.100	(0.013)
terephthalate	terephthalate	8.6	(0.5)	0.094	(0.007)
	benzoate	12.9	(1.2)	0.180	(0.011)
	acetate	1.23	(0.11)	0.120	(0.009)

Values are the average of at least three seperate measurements. Values in parentheses represent standard deviations in the measured values.

During exponential growth on the phthalate isomers only small amounts of acetate and hydrogen were found (approximately 1 to 4 mM and 3 to 5 Pa respectively), indicating tight syntrophic coupling between the fermenting organisms and the methanogens (Figure 3.2). Intermediate accumulation of benzoate was only observed in the *ortho*-phthalate-grown culture, but the measured concentrations were very low (1 to 3 μM). No other intermediates were detected during exponential growth on the phthalate isomers.

Addition of Na_2SO_4 as an exogenous electron-acceptor (final concentration, 5 mM) did not affect the phthalate isomer conversion rate in any of the three cultures. The addition of $NaNO_3$ (final concentration, 5 mM) resulted in complete inhibition of the degradation of the phthalate isomers.

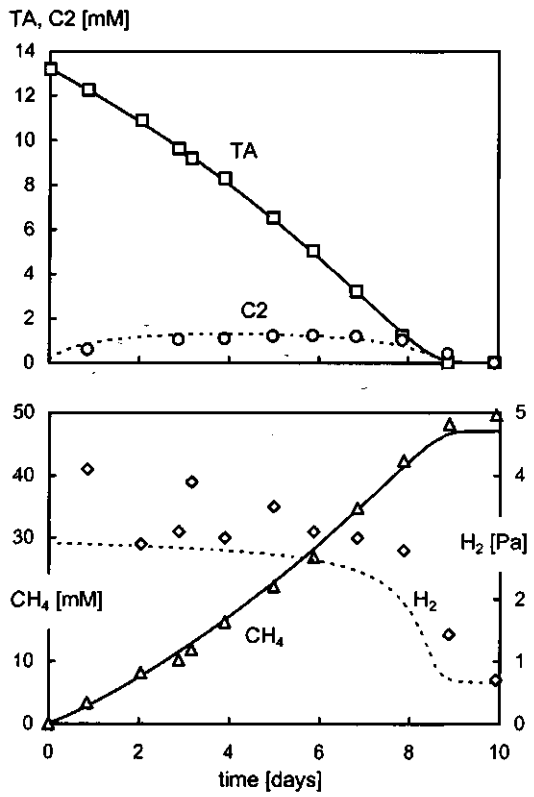


Figure 3.2: Terephthalate (TA, □) degradation and intermediate accumulation of acetate (C2, O) and hydrogen (H_2, ◊) and final production of methane (CH_4, Δ). Markers correspond to measured concentrations and lines were obtained by using the mathematical model described in the Appendix.

Kinetic parameters. The calculated values for the maximum specific growth rate (μ_S^{max}) and the total yield ($Y_{X_{tot}S}$) of the three cultures are summarised in Table 3.2. Values for these parameters were determined for the three phthalate isomers, benzoate and acetate as substrates. Both parameters were measured in exponentially growing batch cultures.

From the data presented in Table 3.2 it can be seen that the differences in μ_S^{max} and $Y_{X_{tot}S}$ between the three cultures grown on either phthalate, isophthalate, or terephthalate are small. For all enrichment cultures, μ_S^{max} and $Y_{X_{tot}S}$ are significantly smaller for the phthalate isomer than for benzoate.

When the maximum specific growth rates on acetate and the phthalate isomers are compared, it can be seen that higher values were found for growth on acetate. These data suggest that only limited amounts of acetate will accumulate during degradation of the phthalate isomers. This was confirmed by the low concentrations of acetate, as measured during exponential growth on the phthalate isomers (1-4 mM) and shown in Figure 3.2 for growth on terephthalate.

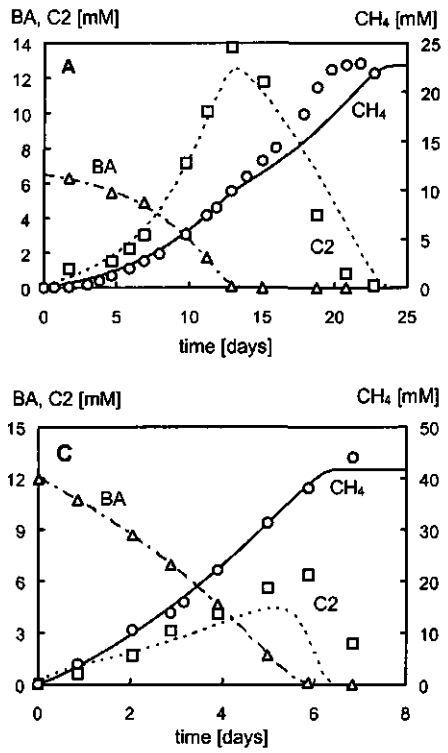


Figure 3.3: Benzoate (BA, Δ) degradation and concomitant acetate (C2, □) accumulation and methane (CH_4, O) formation in *ortho*-phthalate (graph A), isophthalate (graph B), and terephthalate (graph C) grown cultures. Markers correspond to measured concentrations, and lines were calculated using the mathematical model described in the Appendix.

The situation is different for growth on benzoate. The calculated maximum specific growth rates of the cultures grown on benzoate is either comparable (isophthalate and terephthalate) or significantly higher (*ortho*-phthalate) than the maximum specific growth rates determined with acetate. These parameter values indicate that during incubation of the *ortho*-phthalate grown culture with benzoate, high concentrations of acetate will accumulate. Lower concentrations of acetate are predicted to accumulate in the isophthalate- and terephthalate-grown cultures when incubated with benzoate. These indications were confirmed by measured acetate concentrations during benzoate degradation, as shown in Figure 3.3.

Product formation during degradation of the phthalate isomers when incubated with BES. In order to study product formation of the fermenting organisms, the cultures were incubated with the phthalate isomers and 10 mM bromoethanosulfonate BES, a specific inhibitor of methanogenesis. From the results shown in Figure 3.4 it can be seen that trace amounts of benzoate accumulated during incubation with BES of a terephthalate grown culture. No benzoate was detected in exponentially growing cultures, except for the *ortho*-phthalate grown culture where upto 3 μM benzoate was found.

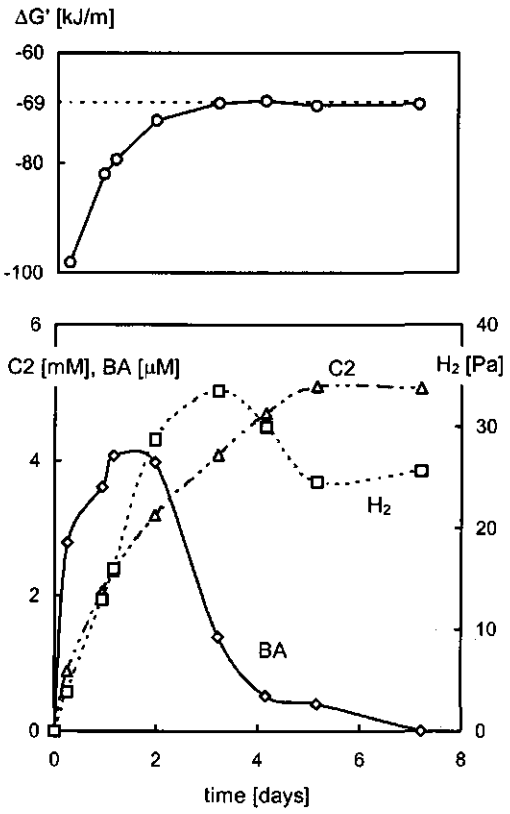


Figure 3.4: Accumulation of acetate (C2, Δ), benzoate (BA, ◊), and molecular hydrogen (H2, □) during incubation of the terephthalate grown culture with terephthalate and 20 mM BES (bottom graph). The actual Gibbs free energy change for terephthalate fermentation (Reaction 1, Table 3.1) is indicated in the top graph)

The methanogenic substrates acetate and hydrogen accumulated to concentrations significantly higher that those observed in exponentially growing cultures. According to the stoichiometry of the fermentation of the phthalate isomers (Table 3.1, reaction 1), equimolar amounts of acetate and hydrogen should accumulate during incubation with BES. However, the results show that much higher concentrations of acetate accumulate. Two reasons can be distinguished to explain this observation: (i) small amounts of methane were formed during the initial 2 days of incubation, suggesting incomplete initial inhibition of methanogenesis from hydrogen by BES, and (ii) the formation of another reduced product besides molecular hydrogen was observed. This product was identified as cyclohexanecarboxylate by gas chromatography.

Based on the measured concentrations of the phthalate isomer, acetate, and hydrogen, the actual Gibbs free energy (ΔG') for fermentation of the phthalate isomers (Table 3.1, reaction 1) can be calculated. From Figure 3.4 it can be seen that fermentation of terephthalate stopped at ΔG'-values exceeding -69 kJ·mol-terephthalate^{-1}. Like for the fermentation of terephthalate, the degradation of *ortho*-phthalate and isophthalate stopped when ΔG' exceeded approximately -65 kJ·mol-phthalate-isomer^{-1}.

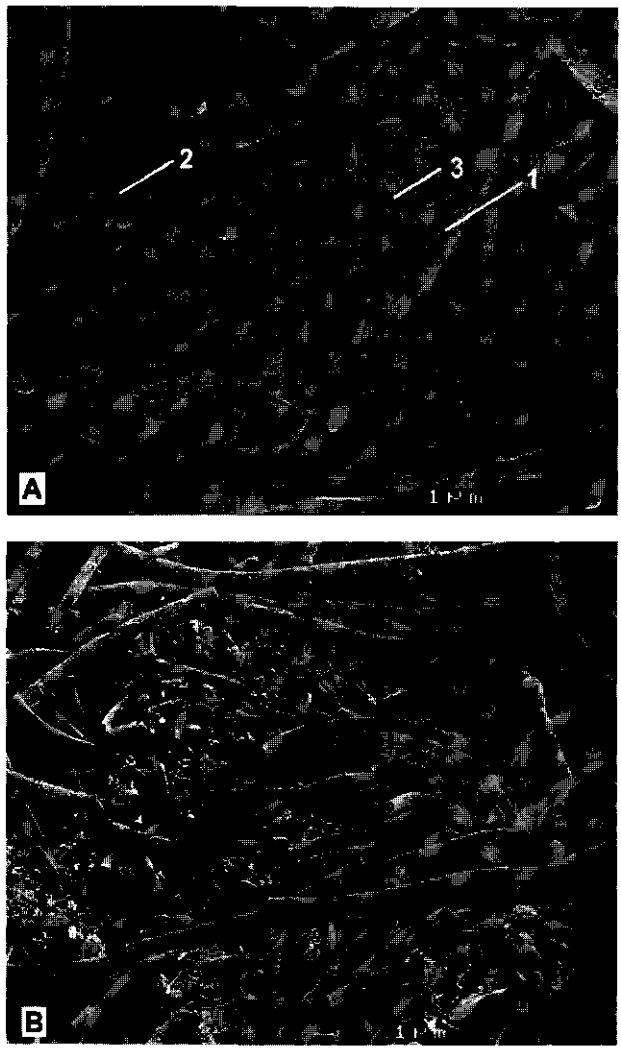


Figure 3.5: Scanning electron micrographs of the methanogenic enrichment cultures grown on *ortho*-phthalate (A) and isophthalate (B). The numbers are explained in the text.

Microscopical observation. All three phthalate isomer-grown cultures formed dense flocs of 0.5 to 2 mm in statically incubated serum bottles. *Methanosaeta*-like organisms were identified as the predominant acetoclastic methanogen in all three cultures.

In the *ortho*-phthalate grown culture (Figure 3.5A), two dominant types of organisms were observed other than the *Methanosaeta*-like organisms (arrow 1): short fat rods (0.6 to 0.8 by 1 to 2 μm) with rounded ends (arrow 2) and very small rods (0.3 by 1.0 to 1.2 μm) (arrow 3). The fat rods were found in large amounts and are presumed to be responsible for the fermentation of *ortho*-phthalate. The very small rods were embedded in extracellular material and always close to the short fat rods and may be hydrogenotrophic methanogens belonging to the genus *Methanobacterium*.

The isophthalate-grown culture (Figure 3.5B) was less well defined than the *ortho*-phthalate-grown culture, which may partly be the result of the low conversion rates observed in this culture at the moment samples were drawn for electron microscopy. Dense clusters of different types of organisms, embedded in extracellular material, were observed in a loose matrix of *Methanosaeta*-like organisms.

The terephthalate grown culture was only examined with a light microscope (not shown) and sporeforming rods of 3 to 4 μm were observed besides *Methanosaeta*- and *Methanospirillum*-like organisms. The spore-forming rods were probably involved in the fermentation of terephthalate.

3.5 Discussion

General observations. At least three different species of bacteria are involved in the methanogenic degradation of the phthalate isomers: fermentative organisms that convert the phthalates isomers to a mixture of acetate and hydrogen (Table 3.1, reaction 1), acetoclastic methanogens that convert acetate into a mixture of methane and bicarbonate (Table 3.1, reaction 4), and hydrogenotrophic methanogens that reduce bicarbonate with hydrogen under formation of methane (Table 3.1, reaction 3). Conversion of the phthalate isomers into acetate and hydrogen is energetically unfavourable under standard conditions ($\Delta G^{0'}$ = 38.6 kJ·mol-phthalate^{-1}), suggesting that the phthalate isomers fermenting cultures strictly depend on the presence of acetoclastic and hydrogenotrophic methanogens in the mixed culture to maintain sufficiently low intermediate concentrations acetate and hydrogen. Methanogens depend on the phthalate isomers fermenting bacteria for generation of their substrates. Due to their mutual dependency, the mixed cultures can be designated as syntrophic cultures [25].

As proposed for denitrifying bacteria [20, 21, 32], the initial step in the degradation of phthalate isomers is suggested to be decarboxylation to benzoate because (i) all the phthalate isomer-grown cultures were capable of benzoate degradation without a lag phase, and (ii) small amounts of benzoate accumulated in phthalate isomer degrading cultures, incubated with the methanogenic inhibitor BES.

Modelling degradation of the phthalate isomers and benzoate. In order to describe the dynamic formation and consumption of acetate and hydrogen during degradation of the phthalate isomers and benzoate, a mathematical model was developed (see Appendix). The model was based on the reaction stoichiometries shown in Table 3.1 and assumed that one organism was responsible for the fermentation of the phthalate isomers and benzoate. Additional kinetic parameter values that were required as input for the model were calculated using Equations 3 to 5.

Table 3.3: Specific biomass yields, specific phthalate- and benzoate-fermenting activities, and the specific biomass yield values normalised to an energy quantum of 70 kJ·mol^{-1} of the fermentative organisms in the three mixed cultures grown on benzoate or the phthalate isomers.

Cult.	sub.[1]	$Y_{X_{Ferm}S}$	$q^{max}_{X_{Ferm}S}$ [2]	acetate[3]	H_2[3]	$\Delta G'$ [4]	$Y_{X_{Ferm}\Delta G}$ [5]
		g·mol^{-1}	mol-S·g^{-1}·day^{-1}	mM	Pa	kJ·mol-S^{-1}	g·70-kJ^{-1}
i-PA	*i*-PA	4.7	0.019	1.6	3.5	- 92.4	3.6
	BA	7.8	0.017	2.5	6.0	-56.0	9.7
o-PA	*o*-PA	4.3	0.020	4.0	4.0	-88.5	3.4
	BA	9.0	0.024	10.0	6.0	-45.2	13.9
TA	TA	4.0	0.023	1.3	3.0	-95.4	3.0
	BA	8.4	0.021	2.5	4.0	-59.1	10.0

[1] The abbreviations o-PA, i-PA, TA and BA stand for *ortho*-phthalate, isophthalate, terephthalate and benzoate respectively,

[2] the maximum specific conversion rate was calculated according to $q^{max}_{X_{Ferm}S} = \mu_S^{max} / Y_{X_{Ferm}S}$

[3] acetate and hydrogen concentrations represent average concentrations as measured during exponential growth of the cultures,

[4] values for the actual Gibbs free-energy change for fermentation of the phthalate isomers (Table 1, reaction 1) and benzoate (Table 1, reaction 2), calculated with a concentration of 4 mM for the phthalate isomers and benzoate, and a bicarbonate concentration of 40 mM,

[5] measured biomass yields (see Table 2) normalised to an energy quantum of 70 kJ·(mol S)$^{-1}$.

The calculated biomass yields of the phthalate- and benzoate-fermenting organisms (Equation 3) are presented in Table 3.3. For the biomass yield of the acetoclastic methanogens in the mixed culture, the measured values as reported in Table 3.2 were used. For the biomass yield of the hydrogenotrophic methanogens we estimated a value of 0.33 g·mol-H_2^{-1} from the energetic efficiency for growth under hydrogen limiting conditions of *Methanobacterium bryantii* [28]. With this estimated value, the maximum contribution of the biomass yield of the hydrogenotrophic methanogens to the total biomass yield during growth on the phthalate isomers or benzoate amounts only 12%, suggesting that the error introduced into the calculations of the biomass yield of the fermenting organisms are small.

Intermezzo

Hydrogentrophic methanogenesis according to reaction equation 3 in Table 3.1 is a key process in syntrophic degradation of non-methanogenic substrates. Only when molecular hydrogen is continuously removed, the fermentative reactions are sufficiently exergonic to sustain microbial activity in syntrophic cultures

Experimentally, the determination of the biomass yield of hydrogenotrophic methanogens is seriously hampered by the ability of these organisms to uncouple substrate conversion and microbial growth [8, 28, 29]. Herewith the biomass yield becomes strongly dependent on the applied hydrogen concentration, and yield values determined at high hydrogen concentrations may be distinctly different from the values obtained at low hydrogen partial pressures, as observed in the syntrophic mixed cultures described here. Measurement of the biomass yield of hydrogenotrophic methanogens in syntrophic mixed cultures should therefore be based on cell counts combined with values for the specific cell mass as described by Seitz et al. [27, 28] These authors described the energetic efficiency for growth ($Y_{X_{HyM}\Delta G}$) under hydrogen limiting conditions of *Methanobacterium bryantii*, and we used this value for estimation of the biomass yield of the hydrogenotrophic methanogens in our cultures.

The values we calculated for the half saturation constant (K_{H_2}, Table 3.4) of the hydrogenotrophic methanogens in our cultures (4.2 ± 0.7 Pa) are significantly lower than those reported in literature (> 100 Pa, [12, 22]). Literature values, however, have been obtained using exogenously supplied molecular hydrogen, which probably led to strong mass transfer limitations from the gas to the liquid phase, resulting in overestimation of the half saturation constant. To avoid mass transfer limitations, measurement of K_{H_2} should therefore be based on steady state measurements in chemostat cultures with endogenous supply of hydrogen, or on concentration measurements in the liquid phase.

Based on measured values for the maximum specific growth rate on the phthalate isomers and benzoate (Table 3.2) and the corresponding biomass yield values of the fermenting bacteria (Table 3.3), the maximum specific conversion rates for benzoate and the phthalate isomers ($q^{max}_{X_{Ferm}S}$) were calculated (Table 3.3). The results indicate that the maximum specific conversion rates for benzoate and the phthalate isomers are in the same order of magnitude, which confirms the observation that the initial rate of degradation of benzoate and the phthalate isomers are comparable, as described above.

Table 3.4: Calculated specific biomass ratios and apparent half- saturation constants for acetate and hydrogen for the methanogens in the phthalate-isomers-degrading mixed cultures.

culture	$\frac{X_{Ferm}}{X_{AcM}}$	$\frac{X_{Ferm}}{X_{HyM}}$	K_{C2}	K_{H2}
	[g·g^{-1}]	[g·g^{-1}]	[mM]	[Pa]
iso-phthalate	1.7	4.9	0.7	4.0
ortho-phthalate	1.5	4.4	0.6	5.1
terephthalate	1.1	4.2	0.4	3.4

The observation that acetate and hydrogen concentrations remained constant during degradation of the phthalate isomers allowed for calculation of the biomass concentration ratios $C_{X_{Ferm}}/C_{X_{AcM}}$ and $C_{X_{Ferm}}/C_{X_{HyM}}$ according to Equation 4, and the half-saturation constants for the methanogenic substrates acetate and hydrogen (K_{C2} and K_{H_2}) according to Equation 5. To enable calculation of K_{H_2}, we estimated from the data reported by Seitz *et al.* (31) a value of 0.60 mol-H_2·g^{-1}·day^{-1} for $q^{max}_{H_2}$. Calculated values for these parameters are shown in Table 3.4.

By using the measured and estimated parameter values (shown in Tables 3.2 to 3.4), the degradation of the phthalate isomers and benzoate can be described with the initial concentration of fermenting bacteria ($X_{ferm}[0]$) as only variable (and estimated values for K_{BA} and K_{PA}). When the boundary conditions are sufficiently fulfilled, the derived model accurately describes the accumulation of acetate and hydrogen during the degradation of the phthalate isomers, as shown for terephthalate degradation in Figure 3.2.

The assumptions made to calculate the biomass ratios and the half-saturation constants for the methanogens (Table 3.4) were based on measurements with the phthalate isomers as substrate. To validate the model, intermediate accumulation and product formation during benzoate degradation were calculated. From the results shown in Figure 3.4 it can be seen that the intermediate formation of acetate and final production of methane are reasonably well predicted for benzoate degradation. If it is taken into account that minor errors in, for

example, the measured values for $Y_{X_{AcM}C2}$ lead to large differences in the estimated biomass ratio, the results are satisfactory.

In summary, we suggest that the developed model adequately describes the intermediate accumulation of acetate and hydrogen and final production of methane during degradation of the phthalate isomers and benzoate. Description of the conversions observed as a function of the kinetic properties of the individual trophic groups in the mixed culture provides additional insight in the metabolic properties of the mixed culture.

Kinetic parameter values. Measured and estimated values of the kinetic parameters for benzoate and acetate degradation correspond well with previously reported values. A growth yield on benzoate of 8.5 g·mol-benzoate^{-1} has been reported for strain BZ-2 cocultured with *Methanospirillum* sp. strain PM-1 (assuming a protein content of 60%) [6], and of 6.2 and 8.2 g·mol-benzoate^{-1} for *Syntrophus buswelli* GA cocultured with *Methanospirillum hungatei* or *Desulfovibrio* sp., respectively [1]. From our data we calculated a similar average value of 9.3 ± 0.5 g·mol-benzoate^{-1}, if the growth of acetoclastic methanogens was omitted from the calculation.

Maximum specific growth rates reported for two different *Syntrophus buswelli* strains, cocultured with either *M. hungatei* or *Desulfovibrio* sp., are 0.10 to 0.29 and 0.17 to 0.37 day^{-1} respectively [18, 34]. The average value of 0.17 ± 0.04 day^{-1} we obtained for the maximum growth rate on benzoate in our cultures is in the same order of magnitude.

Methanosaeta-like organisms were observed in all three phthalate isomers degrading mixed cultures by microscopical observation. The biomass yield, half-saturation constant and maximum growth rate reported in the literature for *Methanosaeta soehngenii* grown on acetate at 37°C are 1.47 g·mol-acetate^{-1}, 0.47 mM and 0.11 day^{-1} respectively [35]. These values are in the same order of magnitude as the average values we measured or calculated for our cultures (Y_{XC2} = 1.15 ± 0.28 g·mol-acetate^{-1}, K_{C2} 0.55 ± 0.13 mM and μ_{C2}^{max} = 0.12 ± 0.02 day^{-1}).

In the literature no kinetic parameter-values for methanogenic cultures degrading one of the phthalate isomers were found. The maximum growth rates we calculated for the phthalate isomers (μ_{PA}^{max}) were low: 0.091 ± 0.003 day^{-1}. Comparable values have been found for methanogenic enrichment cultures degrading the poorly degradable substrates toluene and *ortho*-xylene (0.11 and 0.07 day^{-1} respectively [7]).

Energetic efficiency for growth on the phthalate isomers and benzoate. Correlations between thermodynamics and biomass yields have been developed by several researchers [13, 16, 31]. Stouthamer [31] stated that the biomass yield of anaerobic bacteria is in the

range of 5 to 12 g·mol-ATP^{-1}, with an average value of 10.5 g·mol-ATP^{-1}. Under physiological conditions, an average amount of 70 kJ·mol-ATP^{-1} was estimated to be needed for irreversible ATP-synthesis [25]. Using average values for the substrate and product concentrations for fermentation of the phthalate isomers and benzoate, the Gibbs free energy change of the fermentations and the biomass yield normalised to an energy quantum of 70 kJ·mol^{-1} ($Y_{X_{Ferm}\Delta G}$) can be calculated from the measured specific biomass yields ($Y_{X_{Ferm}S}$), as shown in Table 3.3.

The average value we calculated for $Y_{X_{Ferm}\Delta G}$ for benzoate fermentation is 11.2 ± 1.9 g·70-kJ^{-1}, which is close to the average value of 10.5 g·mol-ATP^{-1} reported by Stouthamer [31]. Values for $Y_{X_{Ferm}\Delta G}$ for fermentation of the phthalate isomers are much lower (3.3 ± 0.3 g·70-kJ^{-1}), suggesting that the energetic efficiency for growth on the phthalate isomers is much lower compared to benzoate. The observation that no degradation of the phthalate isomers was observed at Gibbs free-energy changes exceeding -65 kJ·mol^{-1} confirms that an energetic inefficiency in degradation of the phthalate isomers may exist. Assuming that (i) the phthalate isomers and benzoate are degraded by the same organism and (ii) benzoate is the first intermediate in the degradation of the phthalate isomers, the postulated energetic inefficiency during growth on the phthalate isomers should manifest within the initial steps of the phthalate isomers degradation.

A possible reason for the postulated energetic inefficiency in fermentation of the phthalate isomers can be found in the mechanism of substrate transport across the microbial membrane. The $pK_{a1,2}$ values for the phthalate isomers ($pK_{a1,2}$ for *ortho*-, iso- and terephthalate are 3.0, 3.6, and 3.5, respectively) are considerably lower than the pK_a value of 4.2 for benzoate [9]. To enable comparable uptake rates for the phthalate isomers and benzoate as suggested by the comparable values for $q^{max}_{X_{Ferm}PA}$ and $q^{max}_{X_{Ferm}BA}$ (Table 3.3), active uptake under expense of Gibbs free energy may be required for the phthalate isomers, whereas no (or less) energy is required for the uptake of benzoate.

Another explanation for the postulated energetic inefficiency can be found in the decarboxylation of the phthalates to benzoate (or their CoA analogues). Taylor and Ribbons [32] suggested that decarboxylation of phthalate may proceed after the initial partial reduction of the aromatic ring (in a one- or two-electron mechanism), followed by oxidative decarboxylation. The initial reduction of the aromatic ring is endergonic and requires the investment of energy in the form of ATP [3]. If this amount of energy cannot (or can only partially) be regained during oxidative decarboxylation, this may explain the net energy consumption during decarboxylation of the phthalate isomers.

In summary, it is postulated that the calculated low energetic efficiency for growth on the phthalate isomers can be due to the need for energy consumption for (i) active uptake of the phthalate isomers across the microbial membrane or (ii) to initiate the decarboxylation of the phthalate isomers to benzoate.

Appendix: Modelling intermediate accumulation and end-product formation.

A mathematical model was developed for predicting the intermediate accumulation of acetate and hydrogen, and production of methane during degradation of the phthalate isomers and benzoate. The model is based on Monod kinetics [17] and, consequently, the volumetric rate of substrate consumption (A-1), biomass growth (A-2) and product formation (A-3) in a batch-reactor can be described by using the following equations (neglecting maintenance and/or decay):

$$R_S = -\frac{\mu_S^{max}}{Y_{XS}} \cdot \frac{C_S}{K_S + C_S} \cdot C_X \qquad \text{(A-1)}$$

$$R_X = -Y_{XS} \cdot R_S \qquad \text{(A-2)}$$

$$R_P = -f_{SP} \cdot (1 - \eta_{XS}) \cdot R_S \qquad \text{(A-3)}$$

where f_{SP} stands for the number of moles of product formed per mole of substrate according to the chemical reaction equation (mol-S·mol-P^{-1}) and where η_{XS} is the biomass yield for growth of biomass X on substrate S, expressed as electron yield (e-mol-X·e-mol-S^{-1}). This parameter can be calculated from the measured (or calculated) biomass yield according to the following equation:

$$\eta_{XS} = \frac{\gamma_X \cdot Clength_X}{\gamma_S \cdot Clength_S \cdot MW_X} \cdot Y_{XS} \qquad \text{(A-4)}$$

where γ is the degree of reduction (e-mol·C-mol^{-1}) as defined by Heijnen et al. [13], Clength is the carbon chain length (C-mol·mol^{-1}) and MW is the molecular weight (g·mol^{-1}). We used an average biomass composition of $C_4H_{7.2}O_2N_{0.8}$ [13, 15] and NH_4^+ as the nitrogen source. By using these kinetic equations and mass balances based on the stoichiometry of the conversion reactions shown in Table 3.1, differential equations for all substrates, products, and different types of biomass were derived (Table A-1). During the derivation of these equations the following assumptions were made: (i) the phthalate isomers and benzoate are

fermented by the same organism (X_{Ferm}), (ii) kinetic parameters for fermentation of the phthalate isomers and benzoate may be different, and (iii) the ratio of fermentation products equals the ratio predicted by the chemical conversion-reactions described in Table 3.1, and is therefore independent of biomass-growth.

Table A-1: Differential equations describing terephthalate and benzoate degradation with concomitant production and consumption of acetate and hydrogen, and production of methane.

$\frac{dC_{PA}}{dt} =$	R_{PA}	A-5
$\frac{dC_{BA}}{dt} =$	R_{BA}	A-6
$\frac{dC_{C2}}{dt} =$	$-f_{BAC2}\cdot\left(1-\eta_{X_{Ferm}BA}\right)\cdot R_{BA} - f_{PAC2}\cdot\left(1-\eta_{X_{Ferm}PA}\right)\cdot R_{PA} + R_{C2}$	A-7
$\frac{dC_{H2}}{dt} =$	$\left\{\begin{array}{l}-f_{BAH2}\cdot\left(1-\eta_{X_{Ferm}BA}\right)\cdot R_{BA} - f_{PAH2}\cdot \\ \left(1-\eta_{X_{Ferm}PA}\right)\cdot R_{PA} + R_{H2}\cdot\left(1-\exp(\Delta G_{H2ox}/R\cdot T)\right)\end{array}\right\}\cdot\frac{V_g}{V_l}\cdot V_{mg}$	A-8
$\frac{dC_{CH4}}{dt} =$	$-f_{H2CH4}\cdot\left(1-\eta_{X_{HyM}H2}\right)\cdot R_{H2} - f_{C2CH4}\left(1-\eta_{X_{AcM}C2}\right)\cdot R_{C2}$	A-9
$\frac{dC_{X_{Ferm}}}{dt} =$	$-Y_{X_{Ferm}BA}\cdot R_{BA} - Y_{X_{Ferm}PA}\cdot R_{PA}$	A-10
$\frac{dC_{X_{AcM}}}{dt} =$	$-Y_{X_{AcM}C2}\cdot R_{C2}$	A-11
$\frac{dC_{X_{HyM}}}{dt} =$	$-Y_{X_{HyM}H2}\cdot R_{H2}$	A-12

The differential equation describing the change in hydrogen concentration over time is expressed in Atm·(liter·day)$^{-1}$ by correction of the molar rates for the liquid volume (V_l, liter) and the headspace volume (V_g, liter) of the serum bottle, and the volume of 1 mole gas (V_{mg}, liter·mol^{-1}). To include the observed threshold concentrations hydrogen in the model, an additional term was included in the differential equation describing hydrogen consumption due to hydrogenotrophic methanogenesis. This term is based on reversible enzyme kinetics as described by Labib et al. [14] and avoids hydrogen consumption if hydrogenotrophic methanogenesis is endergonic ($\Delta G_{H2ox} > 0$). No product inhibition terms for the fermentation of the phthalate isomers and benzoate were included in the model, because in general only small amounts of acetate and hydrogen accumulated during our experiments.

The differential equations A-5 to A-12 (Table A-1) were integrated by using a fourth order Runge-Kutta algorithm with adaptive step-size control [24]. Optimisation of parameter values or initial conditions was performed by using the downhill simplex method in multidimensions as described by Press *et al.* [24]. Optimisation was based on minimising the absolute error between measured and calculated values for the individual concentrations. The software program incorporating the model was written in Turbo Pascal 6.0 on a personal computer (80486-DX33).

An example of the model is shown in Figure A-1 for degradation of terephthalate and benzoate. This figure clearly demonstrates the differences in intermediate accumulation during terephthalate and benzoate degradation. The parameter values used for calculating Figure 3.6 are presented in Tables 3.2 to 3.4.

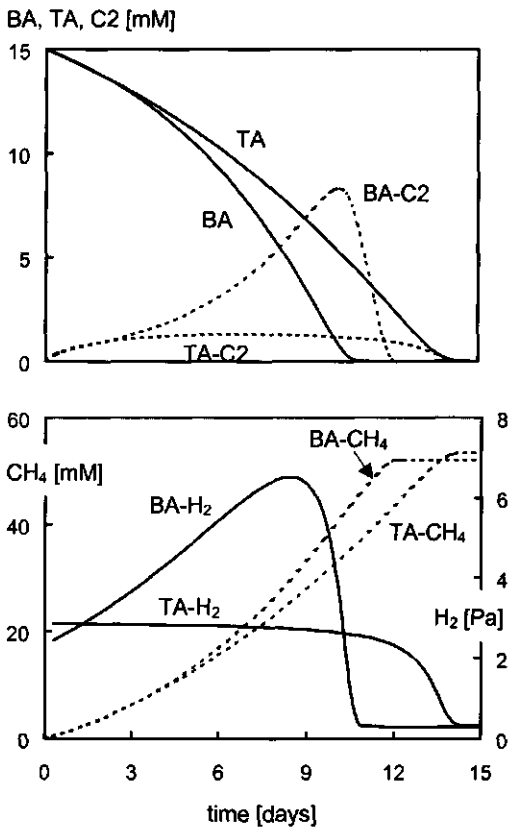


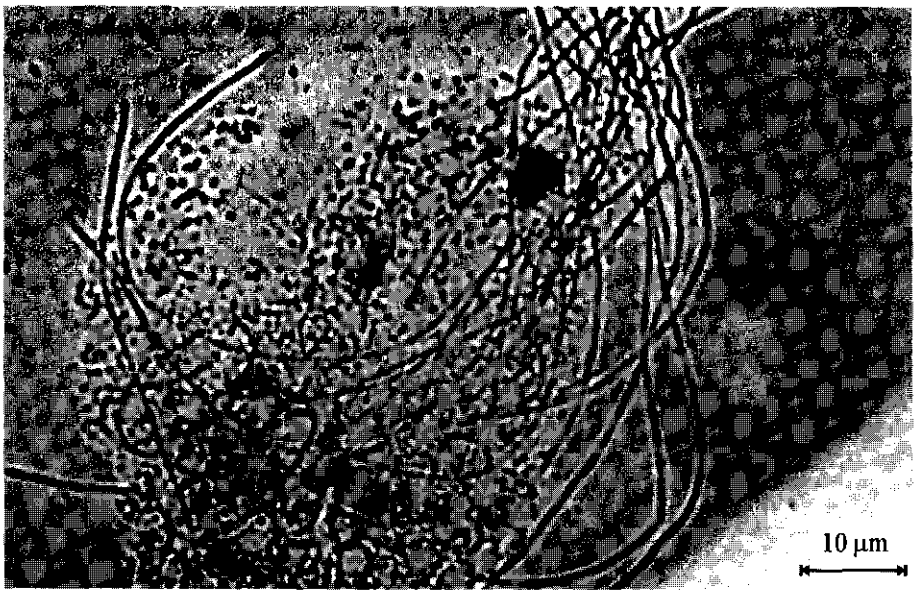
Figure A-1: Terephthalate (TA) and benzoate (BA) degradation and concomitant intermediate accumulation of acetate (C2) and hydrogen (H_2) and final production of methane (CH_4) as predicted by the model.

Nomenclature

C_S	substrate concentration	$mol \cdot l^{-1}$
C_P	product concentration	$mol \cdot l^{-1}$
C_X	biomass concentration	$g \cdot l^{-1}$
μ_S^{max}	maximum specific growth rate on substrate S	day^{-1}
K_S	Monod constant	$mol \cdot l^{-1}$
Y_{XS}	biomass yield of X grown on S	$g \cdot mol^{-1}$
q_{XS}^{max}	maximum specific conversion rate of X on S	$mol \cdot (g \cdot day)^{-1}$
η_{XS}	electron biomass yield of X grown on S	$e\text{-}mol\text{-}X \cdot e\text{-}mol\text{-}S^{-1}$
Cl	carbon chain length	$C\text{-}mol \cdot mol^{-1}$
γ	degree of reduction	$e\text{-}mol \cdot C\text{-}mol^{-1}$
MW	molecular weight	$g \cdot mol^{-1}$
f_{SP}	number of moles P, produced per mol S	$mol\text{-}P \cdot mol\text{-}S^{-1}$
T	temperature	K
R	gas constant	$J \cdot (mol \cdot K)^{-1}$

subscripts:

PA	phthalate isomer
BA	benzoate
C2	acetate
H2	molecular hydrogen
CH4	methane
X_{tot}	sum of the individual organisms involved in degradation of the phthalate isomers and benzoate
X_{Ferm}	phthalate isomers and benzoate fermenting organisms
X_{AcM}	acetoclastic methanogens
X_{HyM}	hydrogenotrophic methanogens



Floc-formation in statically incubated serum bottles with the *ortho*-phthalate degrading enrichment culture

References

1. **Auburger, G., and J. Winter.** 1995. Isolation and physiological characterization of *Syntrophus buswellii* strain GA from a syntrophic benzoate-degrading, strictly anaerobic coculture. Appl. Microbiol. Biotechnol. **44:**241-248.
2. **Bemis, A. G., J. A. Dindorf, H. B., and S. C.** 1982. Phthalic acids and other benzenepolycarboxylic acids, p. 732-777. *In* H. F. Mark, D. F. Othmer, C. G. Overberg, G. T. Seaborg, M. Grayson, and D. Eckroth (ed.), Kirk Othmer Encyclopedia of Chemical Technology, vol. 17. John Wiley and Sons, New York, USA.
3. **Boll, M., and G. Fuchs.** 1995. Benzoyl-coenzyme A reductase (dearomatising), a key enzyme of anaerobic aromatic metabolism. ATP dependance of the reaction, purification and some properties of the enzyme from *Thauera aromatica* strain K172. Eur. J. Biochem. **234:**921-933.
4. **Conrad, R., and B. Wetter.** 1990. Influence of temperature on energetics of hydrogen metabolism in homoacetogenic, methanogenic, and other bacteria. Arch. Microbiol. **155:**94-98.
5. **Dimroth, K.** 1983. Thermochemische daten organischer verbindungen, p. 997-1038, D'ans-Lax, Taschenbuch fur chemiker und Physiker, vol. 2. Springer-Verlag, Berlin.
6. **Dolfing, J., and J. M. Tiedje.** 1988. Acetate inhibition of methanogenic, syntrophic benzoate degradation. Appl. Environ. Microbiol. **54**(7):1871-1873.

7. **Edwards, E. A., and D. Grbic-Galic.** 1994. Anaerobic degradation of toluene and *o*-xylene by a methanogenic consortium. Appl. Environ. Microbiol. **60**(1):313-322.

8. **Fardeau, M.-L., and Y.-P. Belaich.** 1986. Energetics of the growth of *Methanococcus thermolothotrophicus*. Arch. Microbiol. **144**:381-385.

9. **Fieser, L. F., and M. Fieser.** 1956. Organic chemistry, third ed. Reinhold Publishing Corporation, New York, USA.

10. **Fuchs, G., M. E. S. Mohamed, U. Altenschmidt, J. Koch, A. Lack, R. Brackmann, C. Lochmeyer, and B. Oswald.** 1994. Biochemistry of anaerobic biodegradation of aromatic compounds, p. 513-553. *In* C. Ratledge (ed.), Biochemistry of Microbial Degradation. Kluwer Academic Publishers.

11. **Giam, C. S., E. Atlas, M. A. Powers Jr., and J. E. Leonard.** 1984. Phthalic acid esters, p. 67-140. *In* O. Hutzinger (ed.), Anthropogenic Compounds, vol. 3, Part C. Springer-Verlag, Berlin-Heidelberg-New York-Tokyo.

12. **Giraldo-Gomez, E., S. Goodwin, and M. S. Switzenbaum.** 1992. Influence of mass transfer limitations on determination of the half saturation constant for hydrogen uptake in a mixed-culture CH4-producing enrichment. Biotechnol. Bioeng. **40**:768-776.

13. **Heijnen, J. J., M. C. M. Loosdrecht van, and L. Tijhuis.** 1992. A black box mathematical model to calculate auto- and heterotrophic biomass yields based on Gibbs energy dissipation. Biotechnol. Bioeng. **40**:1139-1154.

14. **Labib, F., J. F. Ferguson, M. M. Benjamin, M. Merigh, and N. L. Ricker.** 1993. Mathematical modelling of an anaerobic butyrate degrading consortium: predicting their response to organic overloading. Environ. Sci. Technol. **27**(13):2673-2683.

15. **McCarty, P. L.** 1971. Energetics and bacterial growth, p. 495-512. *In* S. D. Faust and J. V. Hunter (ed.), Organic compounds in aquatic environments. Marcel Dekker, Inc., New York.

16. **McCarty, P. L.** 1972. Energetics of organic matter degradation, p. 91-118. *In* R. Mitchell (ed.), Water Pollution Microbiology. John Wiley & Sons, New York.

17. **Monod, J.** 1949. The growth of bacterial cultures. Ann. Rev. Microbiol. **3**:371-394.

18. **Mountfort, D. O., W. J. Brulla, L. R. Krumholz, and M. P. Bryant.** 1984. Syntrophus buswellii gen. nov., sp. nov.: a benzoate catabolizer from methanogenic ecosystems. Int. J. Syst. Bacteriol. **34**:216-217.

19. **Naumov, A. V., A. B. Gafarov, and A. M. Boronin.** 1996. A novel *ortho*-phthalate utilizer - an acetic acid bacterium *Acetobacter* sp. Microbiology. **65**(2):182-186.

20. **Nozawa, T., and Y. Maruyama.** 1988. Anaerobic metabolism of phthalate and other aromatic compounds by a denitrying bacterium. J. Bacteriol. **170**(12):5778-5784.

21. **Nozawa, T., and Y. Maruyama.** 1988. Denitrification by a soil bacterium with phthalate and aromatic compounds as substrates. J. Bacteriol. **170**(6):2501-2505.

22. **Pavlostathis, S. G., and E. Giraldo-Gomez.** 1991. Kinetics of anaerobic treatment: a critical review. Critical Rev. Environ. Control. **21**(5,6):411-490.

23. **Powell, G. E.** 1984. Equalisation of specific growth rates for syntrophic associations in batch cultures. J. Chem. Tech. Biotechnol. **34B**:97-100.

24. **Press, W. H., B. P. Flannery, S. A. Teukolsky, and W. T. Vetterling.** 1989. Numerical recipes in Pascal: The art of scientific computing. Cambrige University Press, Cambridge, UK.

25. **Schink, B.** 1992. Syntrophism among prokaryotes, p. 276-299. *In* A. Balows, H. G. Truper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), The prokaryotes, vol. I. Springer-Verlag, New York.

26. **Schink, B., A. Brune, and S. Schnell.** 1992. Anaerobic degradation of aromatic compounds, p. 219-242. *In* G. Winkelmann (ed.), Microbial Degradation of Natural Products. VCH, Weinheim-New York-Basel-Cambridge.

27. **Seitz, H. J., B. Schink, N. Pfennig, and R. Conrad.** 1990. Energetics of syntrophic ethanol oxidation in defined chemostat cocultures; energy requirements for H_2 production and H_2 oxidation (1). Arch. Microbiol. **155:**82-88.

28. **Seitz, H. J., B. Schink, N. Pfennig, and R. Conrad.** 1990. Energetics of syntrophic ethanol oxidation in defined chemostat cocultures; energy sharing in biomass production (2). Arch. Microbiol. **155:**89-93.

29. **Shonheit, P., J. Moll, and R. K. Thauer.** 1980. Growth Parameters(Ks, μ^{max}, Ys) of *Methanobacterium thermoautotrophicum*. Arch. Microbiol. **127:**59-65.

30. **Stams, A. J. M.** 1994. Metabolic interactions between anaerobic bacteria in methanogenic environments. Antonie van Leeuwenhoek. **66:**271-294.

31. **Stouthamer, A. H.** 1979. The search for correlation between theoretical and experimental growth yields, p. 1-47. *In* J. R. Quayle (ed.), International review of biochemistry, vol. #21. University Park Press, Baltimore.

32. **Taylor, B. F., and D. W. Ribbons.** 1983. Bacterial decarboxylation of o-phthalic acids. Appl. Environ. Microbiol. **46:**1276-1281.

33. **Thauer, R. K., K. Jungermann, and K. Decker.** 1977. Energy Conservation in Chemotrophic Anaerobic bacteria. Bacteriol. Rev. **41:**100-179.

34. **Wallrabenstein, C., and B. Schink.** 1994. Evidence of reversed electron transport in syntrophic butyrate or benzoate oxidation by *Syntrophomonas wolfei* and *Syntrophus buswellii*. Arch. Microbiol. **162:**136-142.

35. **Zehnder, J. B., B. A. Huser, T. D. Brock, and K. Wuhrmann.** 1980. Characterization of an acetate-decarboxylating, non-hydrogen-oxidizing methane bacterium. Arch. Microbiol. **124:**1-11.

Microbiology II: Specific Properties

Abstract

The terephthalate grown enrichment culture, described in chapter 3, was used to determine the impact of short periods of starvation (hours), or co-incubation of terephthalate with benzoate or acetate. The culture was found to be highly sensitive to either of these measures, resulting in a dramatic loss of terephthalate degrading capacity.

Contents

4.1 Summary

The effects of acetate, benzoate, and periods without substrate, on the anaerobic degradation of terephthalate (1,4-benzene-dicarboxylate) by a syntrophic methanogenic culture were studied. The culture had been enriched on terephthalate and was capable of benzoate degradation without a lag phase. When incubated with a mixture of benzoate and terephthalate, subsequent degradation with preference for benzoate was observed. Both benzoate and acetate inhibited the anaerobic degradation of terephthalate. The observed inhibition is partially irreversible, resulting in a decrease (or even a complete loss) of the terephthalate-degrading activity after complete degradation of benzoate or acetate. Irreversible inhibition was characteristic for terephthalate degradation only, because the inhibition of benzoate degradation by acetate could well be described by reversible noncompetitive product inhibition. Terephthalate degradation was furthermore irreversibly inhibited by periods without substrate of only a few hours. The inhibition of terephthalate degradation due to periods without substrate could be overcome through incubation of the culture with a mixture of benzoate and terephthalate. In this case no influence of a period without substrate was observed. Based on these observations it is postulated that decarboxylation of terephthalate under formation of benzoate is strictly dependent on the concomitant fermentation of benzoate. In the presence of higher concentrations of benzoate, however, benzoate is the favoured substrate over terephthalate, and the culture loses its capacity to degrade terephthalate. In order to overcome the inhibition of terephthalate degradation by benzoate and acetate, a two-stage reactor system is suggested for the treatment of wastewater generated during terephthalic acid production[■].

4.2 Introduction

During both steps of Purified Terephthalic Acid (PTA) production, wastestreams are generated with a high level of organic contamination. Main components in these wastestreams are in decreasing order of concentration: terephthalic acid, acetic acid, benzoic acid and *para*-toluic acid (Chapter 1). Several technological studies have been conducted to assess the feasibility of anaerobic pre-treatment of terephthalic acid wastewater [7, 10, 13] and approximately 10 full scale treatment systems are currently in operation or under construction [2, 11, 16]. Results obtained during these studies indicate that most wastewater

[■] A modified version of this chapter has been published in *Appl. Environ. Microbiol.* (1999), **65:** 1161-1167

constituents are biodegradable under methanogenic conditions (Chapter 2) and are hardly toxic to methanogenic organisms [4, 7]. However, the degradation rates found in anaerobic bioreactors are low [2, 7, 13], and lag phases prior to degradation of the phthalic acid isomers are long, ranging from 1 to 3 months in batch studies (Chapter 2), to more than 1 year in full scale reactors [2, 16].

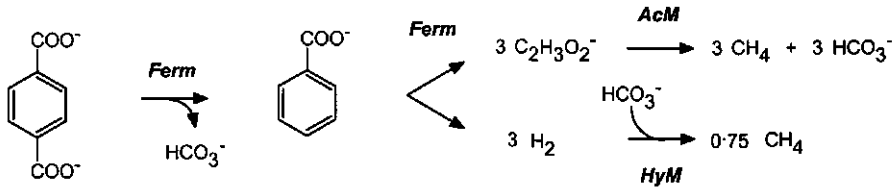


Figure 4.1: Proposed degradation scheme for anaerobic degradation of terephthalate by a syntrophic culture consisting of terephthalate decarboxylating and benzoate fermenting organisms (Ferm), acetoclastic methanogens (AcM) and hydrogenotrophic methanogens (HyM).

In anaerobic bioreactors, organic compounds are converted into a mixture of methane and carbon dioxide in a complex network of several types of bacteria. These metabolic networks have been studied extensively for the anaerobic degradation of important agroindustrial wastewater constituents, such as volatile fatty acids and alcohols [18, 20]. Combined with the development of high-rate anaerobic bioreactors, which have the ability to uncouple the solid retention time and the hydraulic retention time [8, 9], this knowledge contributed to the successful implementation of high-rate anaerobic bioreactors for the treatment of concentrated, non-complex wastewaters. Contrary to these relatively noncomplex substrates, hardly any information is currently available about the kinetic properties of the methanogenic degradation of aromatic pollutants. Furthermore, the influence of rapidly degradable substrates on the anaerobic degradation of aromatic substrates is poorly documented. This lack of information seriously hampers the successful introduction and application of anaerobic treatment methods for the more complex wastewaters, such as those generated in the petrochemical industries. With respect to the anaerobic treatment of PTA-wastewater, it has been shown that terephthalate and *para*-toluate are the rate limiting substrates [2, 7, 12, 16]. Taking into account that terephthalate is the main polluting compound in PTA-wastewater, we focused our work on the anaerobic degradation of this compound. In Chapter 3 we described the kinetic properties of the different types of bacteria involved in the methanogenic degradation of terephthalate, as well as its *ortho*- and *meta*-oriented isomers. These experiments were conducted with enrichment cultures obtained from methanogenic granular sludge or digested sewage sludge. We postulated in this Chapter that

the anaerobic degradation of the phthalate isomers and benzoate proceeds according to the reaction scheme shown in Figure 4.1.

This Chapter focuses on the following characteristics of a terephthalate grown, methanogenic enrichment culture: (i) the influence of acetate and benzoate on terephthalate degradation, as well as the influence of acetate on benzoate degradation, and (ii) the influence of periods without substrate on benzoate and terephthalate degradation. Based on the results obtained, the specific role of benzoate in the anaerobic degradation of terephthalate is discussed, and the practical implications for anaerobic treatment of PTA-wastewater are presented.

4.3 Materials and methods

Biomass. The terephthalate grown enrichment culture used in the experiments was obtained from granular biomass from a laboratory scale anaerobic hybrid reactor as described in Chapter 3. In order to cultivate a large amount of biomass, a continuously stirred 5 litre batch reactor was operated with the enrichment culture. The temperature of the polyacrylate reactor was controlled at 37 ± 1°C by a thermostat-bath-circulator (Haake D1-L) connected to the double wall of the reactor. Prior to inoculation of the 5 litre cultivation reactor, cultures were transferred into serum bottles with increasing volume (upto 2 litres, liquid volume of 500 ml) in order to obtain a sufficient amount of biomass for inoculation. The cultivation reactor was operated in a fed-batch mode: Approximately once a week, 1 litre of the culture was removed, and the reactor was replenished with a mixture of substrate and nutrients. By using this approach it was possible to grow a large amount of terephthalate degrading biomass with a relatively constant volumetric conversion rate of 1 to 2 mM·day^{-1}. Due to the low growth rate of the terephthalate-degrading enrichment culture, it took approximately 6 months to obtain a stable culture in the 5 litre reactor.

Kinetic analyses. Experimental data were analysed using the previously described mathematical model, with the same set of experimentally determined or estimated parameter values for the different trophic groups in the terephthalate-degrading mixed culture (Chapter 3).

Noncompetitive product inhibition of benzoate degradation by acetate was modelled by using the following rate equation for benzoate degradation:

$$R_{BA} = -\frac{\mu_{BA}^{max}}{Y_{X_{Ferm}BA}} \cdot \frac{C_{BA}}{K_{BA} + C_{BA}} \cdot \frac{Ki_{C2}}{C_{C2} + Ki_{C2}} \cdot C_{X_{Ferm}} \qquad (1)$$

where R, μ, Y, and C stand for volumetric conversion rate ($mol \cdot liter^{-1} \cdot day^{-1}$), maximum specific growth rate (day^{-1}), biomass yield ($g \cdot mol^{-1}$), and concentration ($mol \cdot liter^{-1}$) respectively. Subscripts X_{Ferm}, BA, and C2 stand for benzoate fermenting biomass, benzoate, and acetate respectively. Ki_{C_2} is a noncompetitive inhibition coefficient.

On-line measurement of the CH_4-production rate. Methane production in the 5 litre cultivation reactor was measured through liquid displacement with a mariotte bottle. Prior to the mariotte bottle, carbon dioxide was washed from the biogas by leading it over a 20% NaOH solution and a column filled with soda lime pellets for removal of water vapour and traces of carbon dioxide. The liquid displaced was collected in a container placed on a pressure sensor (DS-Europe, model QB745) to detect the weight increase of the container. The pressure sensor was connected to a data logger (Campbell CR10), and weights were recorded every 30 minutes. The data logger was connected to a personal computer for continuous monitoring of the methane production.

Degradation of mixed substrates. In order to study the degradation of mixtures of two substrates (acetate, benzoate and/or terephthalate), batch experiments were performed in 300 ml serum bottles. Media were prepared with mixtures of substrates at the desired concentration as described in Chapter 3. All organic substrates were dosed as sodium salts from stock-solutions, and if necessary, sodium concentrations were corrected through dosage of NaCl. Serum bottles were sealed with butyl rubber stoppers and aluminium screw caps. The headspace was flushed with a mixture of N_2 and CO_2 (70:30 [vol:vol]), and $Na_2S.7to9H_2O$ was dosed from a concentrated stock solution to obtain a final concentration of 150 $mg \cdot l^{-1}$. Serum bottles were preincubated at 37 ± 1°C in an orbital motion shaker and, after temperature equalisation inoculated by syringe with the terephthalate-grown enrichment culture. Samples for inoculation (10 to 20 ml) were taken from the cultivation reactor at the end of the exponential-growth phase. The total liquid volume in the serumbottles amounted 50 to 70 ml. Throughout the experimental period, serum bottles were sampled at least once a day for analyses of the concentration terephthalate and benzoate by high pressure liquid chromatography (HPLC), and volatile fatty acids, molecular hydrogen, and methane were analysed by gas chromatography. A detailed description of the analytical methods applied can be found in Chapter 2. Measured concentrations in the headspace were corrected for the reduction in liquid volume due to sampling. All experiments were performed in duplicate.

Influence of periods without substrate on benzoate and terephthalate degradation. To study the influence of short periods without substrate on the terephthalate-degrading activity of the culture, 20 ml samples were regularly taken from the cultivation reactor. The samples were incubated with terephthalate (5 mM) in 117 ml serum bottles with a N_2-CO_2 gas-phase

mixture for determination of the specific terephthalate-degrading activity of the biomass. The experimental procedure was basically the same as described above, except no additional nutrients were dosed. Terephthalate degradation in the serum bottles was monitored by repeated measurement of the methane concentration in the headspace for one to two days. The volumetric terephthalate conversion rate (mol-terephthalate·liter-inoculum^{-1}·day^{-1}) was calculated from the measured methane production by using linear regression techniques. Using this approach, the specific terephthalate degrading activity, as measured at high terephthalate concentrations in the serum bottles, could be related to the terephthalate concentration and consequently the terephthalate conversion rate in the cultivation reactor.

In order to compare the influence of a short period of a few hours without substrate on the degradation of terephthalate and a mixture of benzoate and terephthalate, a slightly different procedure was used. Serum bottles (117 ml) with a N_2-CO_2 gas-phase mixture were inoculated by syringe with a mixture of biomass and terephthalate from the cultivation reactor. Terephthalate, or a mixture of benzoate and terephthalate (final concentration of both substrates, 5 mM) were dosed to four bottles (in duplicate), while no substrate was dosed to four other bottles. After 1 day, benzoate or a benzoate-terephthalate mixture was dosed to the bottles that had not received any substrate at the moment of sampling. With this approach, the latter bottles were exposed to a period without substrate, due to the depletion of the terephthalate in the inoculum. In time the concentrations of benzoate and terephthalate, volatile fatty acids, and the methane content of the headspace were measured.

4.4 Results

Mutual influence of acetate, benzoate, and terephthalate. Terephthalate grown cultures had the ability to degrade benzoate without a lag period, and specific conversion rates obtained with either benzoate or terephthalate as substrate were comparable (Figure 4.2). When terephthalate grown biomass was incubated with a mixture of terephthalate and benzoate, a sequential conversion of both substrates was obtained (Figure 4.3). From this figure it can be seen that benzoate is the preferred substrate over terephthalate. Since hardly any terephthalate is degraded in the presence of benzoate, the degradation of a mixture of benzoate and terephthalate approaches diauxic degradation.

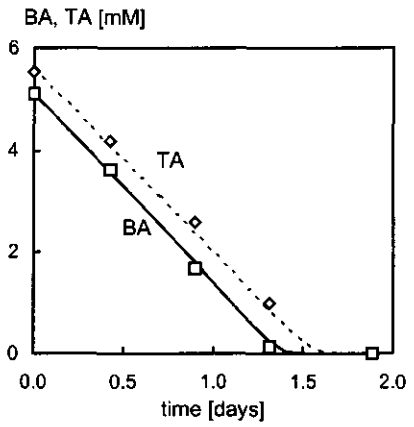


Figure 4.2: Degradation of terephthalate (TA, ◊) or benzoate (BA, □) by the terephthalate grown enrichment culture.

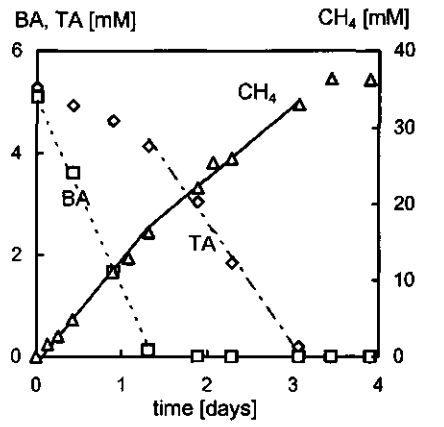


Figure 4.3 Degradation of a mixture of terephthalate (TA, ◊) and benzoate (BA, □), and concomitant methane production (CH_4, Δ) by the terephthalate grown enrichment culture. Calculated lines indicate that the terephthalate degradation rate after complete removal of benzoate is approximately 31 % lower than the initial benzoate degradation rate.

From Figure 4.3 it can be seen that the calculated terephthalate conversion rate after complete removal of benzoate is 31% lower than the initial benzoate conversion rate, suggesting that part of the terephthalate degrading capacity is lost during the degradation of benzoate. At higher initial concentrations of benzoate and lower biomass concentrations, this loss in terephthalate degrading activity is more pronounced, as shown in Figure 4.4. Even though all of the benzoate is degraded within 6 days, a lag phase prior to terephthalate degradation of approximately 20 days is observed. Terephthalate conversion rates, after complete conversion of benzoate, are significantly lower compared to the experiment with terephthalate as the sole carbon and energy source.

A similar effect is observed in cultures incubated with a mixture of acetate and terephthalate (Figure 4.5). Despite complete degradation of acetate within 8 days, no degradation of terephthalate is observed in a culture incubated with a mixture of terephthalate and acetate within 38 days.

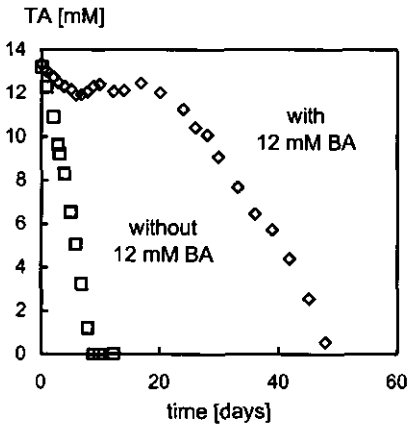


Figure 4.5: Terephthalate (TA) degradation by the terephthalate grown enrichment culture incubated with (◊) and without (□) 12 mM benzoate (BA). In the experiment incubated with a mixture of BA and TA, BA was completely degraded after 6 days.

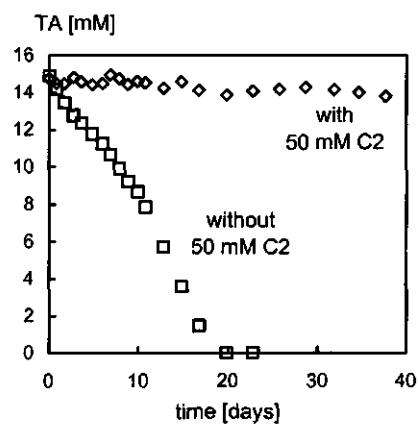


Figure 4.4: Terephthalate (TA) degradation by the terephthalate grown enrichment culture incubated with (◊) and without (□) 50 mM acetate (C2). In the experiment incubated with a mixture of TA and C2, C2 was completely degraded after 8 days.

The loss in degrading capacity is characteristic for terephthalate degradation because no loss in activity was observed when the terephthalate-grown culture was incubated with a mixture of benzoate and acetate (Figure 4.6). Fully reversible product-inhibition of benzoate degradation by acetate could accurately be described by a simple non-competitive inhibition model as described in the material and methods section (Equation 1). For calculating the drawn lines in Figure 4.6, a non-competitive inhibition coefficient of acetate (Ki_{C2}) of 33 mM was used. It can furthermore be seen that at equal acetate concentrations, terephthalate degradation was completely inhibited, while benzoate conversion still proceeded.

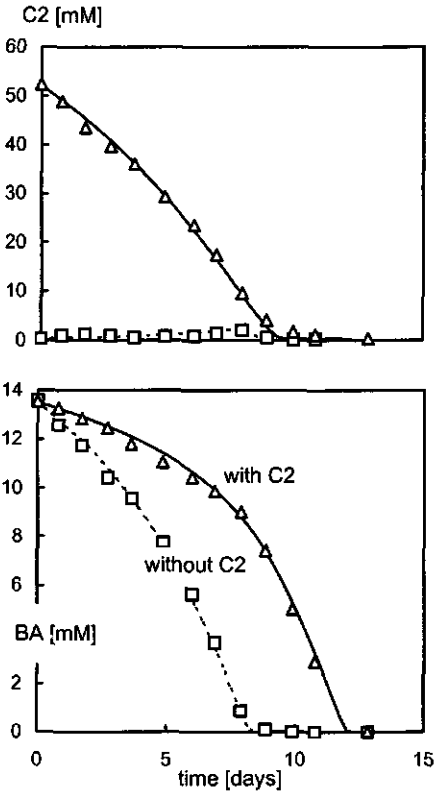


Figure 4.6: Inhibition by acetate (C2, upper graph) of benzoate (BA, bottom graph) degradation by the terephthalate grown enrichment culture. Markers correspond to measured concentrations and drawn lines were calculated using a non-competitive inhibition model (Eq. 3) with a value for Ki_{C2} of 33 mM.

Influence of substrate depletion on benzoate and terephthalate degradation. During cultivation of the terephthalate-grown enrichment culture, it was observed that if substrate was dosed after complete conversion of terephthalate, a long lag-phase (of up to more than 1 month) occurred prior to terephthalate degradation. In order to quantify this inactivation due to substrate depletion, samples were regularly taken from the cultivation reactor and incubated with 5 mM terephthalate in 117-ml serum bottles. The specific terephthalate conversion rate was measured in the serum bottles for a period of 1 to 2 days. By this approach, the specific terephthalate-degrading activity of the culture was measured shortly before and after depletion of terephthalate in the cultivation reactor. From Figure 4.7 it can be seen that the volumetric terephthalate conversion rate in the first sample, taken after approximately 2 days, is highly comparable to the rate in the 5-litre cultivation reactor. This result shows that no loss in activity occurred due to transfer of the culture.

In time the rate of methane production in the cultivation reactor decreases due to substrate depletion. Kinetically, this decrease in the methane production rate is expressed with the apparent half saturation constant (K_{TA}), which was estimated to be 0.8 mM for terephthalate fermentation. From Figure 4.7 it can be seen that the volumetric conversion rate of terephthalate degradation in the serum bottles (where sufficient substrate is present) proceeds parallel with the decrease in the conversion rate in the cultivation reactor. This observation evidently shows that the terephthalate-degrading culture almost completely loses its capacity to degrade terephthalate during short periods of starvation.

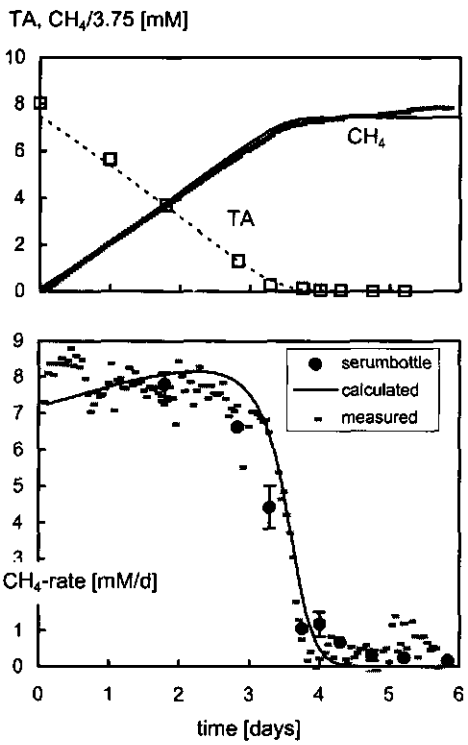


Figure 4.7: Terephthalate (TA, □) degradation and cumulative methane production (CH_4 +) in the cultivation reactor (top graph), and the volumetric rates of methane production in the cultivation reactor (-), compared to the volumetric terephthalate conversion rate of biomass sampled from the cultivation reactor, and incubated with 5 mM terephthalate in serum bottles (●) (bottom graph). Drawn lines describing terephthalate degradation and methane production in the cultivation reactor were calculated using a half saturation constant for terephthalate fermentation (K_{TA}) of 0.8 mM.

A similar experiment was performed with both terephthalate and a mixture of benzoate and terephthalate as substrates. The specific objective of this experiment was to determine if the culture only lost its ability to degrade terephthalate during short periods without substrate, or if the benzoate degrading activity was lost as well.

The data shown in Figure 4.8 confirm that due to short periods without substrate, the terephthalate degradation rate is negligible during the first 2 days after terephthalate was dosed. Partial recovery of the terephthalate-degrading activity is obtained during the following days, but the terephthalate degradation rate remains distinctly lower than in experiments without a period of starvation. The initial increase in the methane concentration observed in the serum bottles that received no substrate at time zero is due to presence of terephthalate in the inoculum. From this initial increase in the methane concentration, the length of the period without substrate was estimated to be approximately 4 hours.

The results of the experiments with a terephthalate-benzoate mixture were clearly different (Figure 4.9). First off all, it can clearly be seen that the period without substrate hardly affects the conversion of benzoate. It is furthermore evident that terephthalate degradation is not affected by the period without substrate if a mixture of benzoate and terephthalate is used. These observations were confirmed by the highly parallel methane production curves.

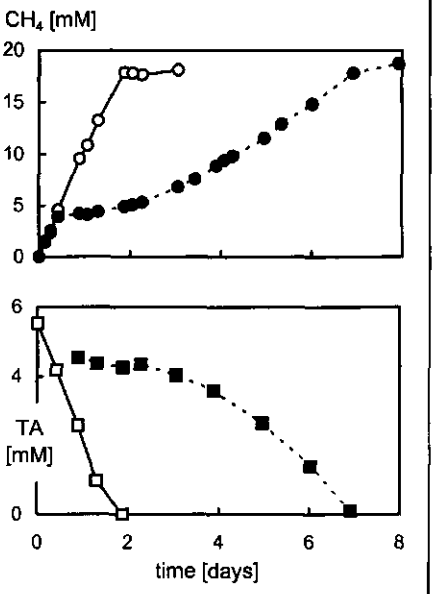


Figure 4.8: Terephthalate degradation (TA, bottom graph) and concomitant methane production (CH_4, top graph) with (dashed lines, solid markers) and without (solid lines, open markers) a 4 hour period without substrate.

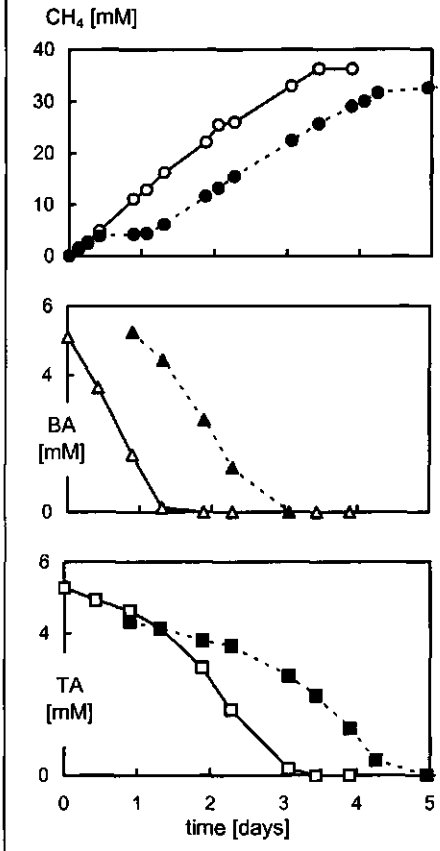


Figure 4.9: Terephthalate (TA, bottom graph) and benzoate (BA, middle graph) degradation and concomitant production of methane (CH_4, top graph) by the terephthalate grown enrichment culture incubated with a mixture of BA and TA, with (dashed lines, solid markers) and without (solid lines, open markers) a 4 hour period without substrate.

4.5 Discussion

Substrate competition during degradation of mixtures of benzoate and terephthalate. If benzoate and terephthalate are fermented by the same organism, the observed preference for benzoate degradation can be attributed to substrate competition. Fermentation of terephthalate is energetically more favourable than benzoate fermentation because decarboxylation of terephthalate is an exergonic process ($\Delta G^{0'} \approx -20$ kJ·mol^{-1}). Despite this energetic advantage of terephthalate fermentation, benzoate is the preferred substrate.

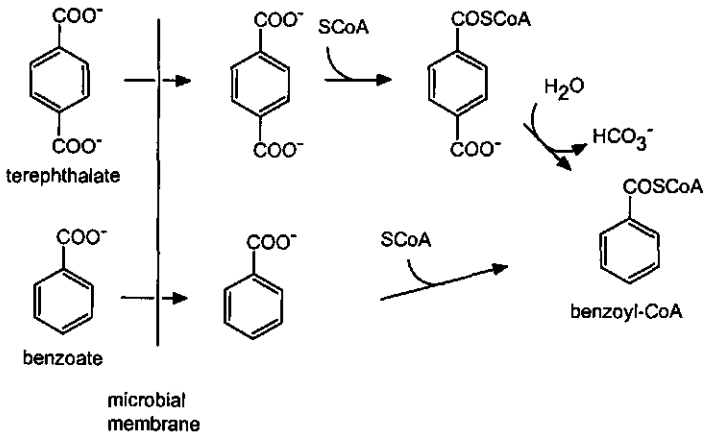


Figure 4.10: Schematic representation of the proposed initial steps in terephthalate and benzoate degradation, both converging at benzoyl-CoA [14, 19].

Based on the literature information [5, 6, 14, 15, 19], the initial steps in the degradation of terephthalate and benzoate likely proceed according to the pathway shown in Figure 4.10. From this figure it can be seen that both terephthalate and benzoate degradation converge at benzoyl-coenzyme-A (benzoyl-CoA), a central intermediate in the anaerobic degradation of aromatic compounds. We suggest that kinetic differences between these limited number of steps in the formation of benzoyl-CoA have to determine the preference for benzoate conversion because the preference for benzoate degradation over terephthalate is observed immediately after benzoate addition.

From the conversion steps shown in Figure 4.10, the rate of substrate uptake across the microbial membrane may represent the rate-determining step in benzoyl-CoA formation. Since the pK_a-value for terephthalate ($pK_{a1,2} = 3.5$) is lower than for benzoate ($pK_a = 4.2$) the flux of terephthalate across the cytoplasmic membrane will be lower compared to benzoate,

if both terephthalate and benzoate are activated at comparable rates. In presence of both benzoate and terephthalate, this may result in higher concentrations of benzoyl-CoA from benzoate compared to terephthalate and, consequently, benzoate conversion will proceed faster. The activation rate may not be the rate-determining step in the overall conversion of benzoate and terephthalate, and therefore comparable maximum specific conversion rates for terephthalate and benzoate can still be obtained (Figure 4.2). Aromatic ring reduction steps were proposed to be rate limiting in anaerobic benzoate degradation by *Rhodopseudomonas palustris* [17].

Product inhibition by acetate of benzoate and terephthalate degradation. Benzoate and terephthalate degradation are both inhibited by acetate. Benzoate inhibition by acetate could be well described with a noncompetitive inhibition model, with an inhibition constant (Ki_{C2}) of 33 mM. This value for Ki_{C2} is in the same order of magnitude as the value of 40 mM determined in a defined coculture consisting of the benzoate degrader BZ-2 and *Methanospirillum* sp. strain PM-1 [1]. In a coculture consisting of the benzoate degrader SB with *Desulfovibrio* sp. strain G-11, 50% inhibition of the benzoate degradation rate was obtained at an acetate concentration of approximately 10 mM [21].

Contrary to the inhibition of benzoate degradation by acetate, terephthalate degradation was found to be irreversibly inhibited by acetate, resulting in long lag phases prior to terephthalate degradation after complete degradation of acetate (Figure 4.5). The reasons for the apparent loss in terephthalate degrading capacity are discussed below.

Deactivation of the terephthalate degrading enrichment culture. The terephthalate-degrading enrichment culture lost a large part of its terephthalate degrading capacity when (i) the culture had been incubated with a mixture of acetate and terephthalate, (ii) the culture had been degrading benzoate for a prolonged period of time (several days), or (iii) when the culture had been exposed to a short period (hours) of starvation.

The extent of deactivation due to incubation with a benzoate-terephthalate mixture, appears to be related to the time needed for complete degradation of benzoate. At low biomass concentrations and/or high benzoate concentrations, long lag phases prior to terephthalate degradation were observed after complete removal of benzoate. At higher biomass and/or lower benzoate concentrations, the inhibition of terephthalate degradation by benzoate appeared to be partially reversible. Partially reversible inhibition of terephthalate degradation by benzoate has previously been observed with biomass from anaerobic bioreactors treating terephthalate containing wastewater [4, 7]. Irreversible inhibition of terephthalate degradation was observed in experiments incubated with a mixture of terephthalate and

glucose [4].The inhibition in the latter case can probably be attributed to the accumulation of intermediates of glucose degradation (acetate and hydrogen).

Short periods without substrate resulted in an almost complete loss of the terephthalate-degrading capacity. As long as the periods without substrate were short (a few hours), part of the terephthalate-degrading activity could be recovered within a few days. However, when cultures were kept unfed for a period of several days, recovery of the terephthalate degrading activity took more than 1 month (data not shown). The time periods without substrate leading to deactivation of the culture were too short to be explained by bacterial decay. Lag phases prior to growth due to periods without substrate have previously been reported for butyrate degrading syntrophic cocultures [3].

The observation that the degradation of a mixture of benzoate and terephthalate is unaffected by a short period without substrate (Figure 4.9), suggests that (one of) the first steps in the degradation pathway of terephthalate as shown in Figure 4.10, are highly dependent on the latter steps (fermentation of benzoyl-CoA) in a coupled "chain reaction". It may be speculated that the organism needs the energy generated during fermentation of benzoyl-CoA (approximately 60 kJ/mol) to initiate the decarboxylation of terephthalate or to maintain gradients across the bacterial membrane, as may be needed for the active uptake of terephthalate. If the conversion of benzoyl-CoA is interrupted due to a feedless period, the chain is broken and one of the initial steps in terephthalate degradation may not be possible anymore.

In summary it is suggested that benzoate plays a peculiar double role in the degradation of terephthalate: benzoate (i) stimulates the degradation of terephthalate when supplied in a low concentration after a short period of starvation, (ii) inhibits terephthalate degradation when both substrates are present, and (iii) may cause a loss in terephthalate-degrading capacity after benzoate degradation for a prolonged period of time.

Practical implications. The results described here have clear implications for anaerobic reactor technology for PTA-wastewater treatment. Due to the presence of both acetate and benzoate in the wastewater, the anaerobic degradation of terephthalate will be strongly inhibited in well-mixed reactors. Only if the reactor concentrations of acetate and benzoate can be kept low, growth on terephthalate can be expected. Taking this into account, as well as the measured low growth rates on terephthalate of the methanogenic enrichment culture (Chapter 3), we suggest that this type of wastewater should preferably be treated in a staged bioreactor fashion. In the first stage of such a system, acetate and benzoate can be removed at high rates, while in the later stages terephthalate can be removed at lower volumetric

conversion rates, and maximised solid retention times. As a result of pre-removal of acetate and benzoate, anaerobic mineralisation of terephthalate in the latter stages can be optimised.

It should furthermore be emphasised that wastewater needs to be fed to the anaerobic bioreactors continuously in order to avoid inactivation of the terephthalate-degrading biomass. Since the industrial production of terephthalic acid is accomplished in a continuous process, continuous operation of the anaerobic reactors will normally not represent a problem. However, terephthalic acid production plants are normally stopped once or twice a year for a period of 1 to 2 weeks for maintenance purposes. It is clear that during these periods, measures should be taken to prevent a dramatic loss of the terephthalate-degrading capacity of the system due to feed interruption. If no sufficient measures are taken, a renewed start-up procedure of several months may be required to regain the terephthalate-degrading capacity. If the terephthalate-degrading biomass in the latter stages of a staged anaerobic bioreactor is deactivated due to periods without substrate, it may be beneficial to direct a part of the benzoate-containing raw wastewater to the later stages of the process to enhance the recovery of the terephthalate-degrading activity.

4.6 References

1. **Dolfing, J., and J. M. Tiedje.** 1988. Acetate inhibition of methanogenic, syntrophic benzoate degradation. Appl. Environ. Microbiol. **54:**1871-1873.
2. **Duffel, J. v.** 1993. Anaerobe behandeling van organische zuren. Presented at the National Conference on Anaerobic Treatment of Complex Wastewaters, Breda, The Netherlands.
3. **Dwyer, D. F., E. Weeg-Aerssens, D. R. Shelton, and J. M. Tiedje.** 1988. Bioenergetic conditions of butyrate metabolism by a syntrophic anaerobic bacterium in coculture with hydrogen-oxidizing methanogenic and sulfidogenic bacteria. Appl. Environ. Microbiol. **54:**1354-1359.
4. **Fajardo, C., J. P. Guyot, H. Macarie, and O. Monroy.** 1997. Inhibition of anaerobic digestion by terephthalic acid and its aromatic by products. Water Sci. Technol. **36:**83-90.
5. **Fuchs, G., M. E. S. Mohamed, U. Altenschmidt, J. Koch, A. Lack, R. Brackmann, C. Lochmeyer, and B. Oswald.** 1994. Biochemistry of anaerobic biodegradation of aromatic compounds, p. 513-553. *In* C. Ratledge (ed.), Biochemistry of microbial degradation. Kluwer Academic Publishers, Dordrecht, The Netherlands.
6. **Heider, J., and G. Fuchs.** 1997. Anaerobic metabolism of aromatic compounds. Eur. J. Biochem. **243:**577-596.
7. **Kleerebezem, R., J. Mortier, L. W. Hulshoff Pol, and G. Lettinga.** 1997. Anaerobic pre-treatment of petrochemical effluents: terephthalic acid wastewater. Wat. Sci. Tech. **36:**237-248.

8. **Lettinga, G.** 1995. Anaerobic digestion and wastewater treatment systems. Antonie Leeuwenhoek. **67**:3-28.

9. **Lettinga, G., L. W. Hulshoff Pol, I. W. Koster, W. M. Wiegant, W. de Zeeuw, A. Rinzema, P. C. Grin, R. E. Roersma, and S. W. Hobma.** 1984. High-rate anaerobic wastewater treatment using the UASB reactor under a wide range of temperature conditions. Biotechnol. Gen. Eng. Rev. **2**:253-283.

10. **Liangming, X., C. Yuxiang, and Z. Xiangdong.** 1991. The anaerobic biological treatment of high strength petrochemical wastewater by a hybrid reactor. Presented at the International Conference on Petroleum Refining and Petrochemical Processing, Beijing, China, September 11-15.

11. **Macarie, H.** 1996. Anaerobic digestion an adequate technology for the treatment of some chemical and petrochemical wastewaters. Presented at the IV Taller Latinoamericano Sobre Tratiamento Anaerobio de Aguas Residuales, Bucaramanga, Colombia.

12. **Macarie, H., and J. P. Guyot.** 1992. Inhibition of the methanogenic fermentation of p-toluic acid (4-methylbenzoic acid) by acetate. Appl. Microbiol. Biotechnol. **38**:398-402.

13. **Macarie, H., A. Noyola, and J. P. Guyot.** 1992. Anaerobic treatment of a petrochemical wastewater from a terephtalic acid plant. Water Sci. Technol. **25**:223-235.

14. **Nozawa, T., and Y. Maruyama.** 1988. Anaerobic metabolism of phthalate and other aromatic compounds by a denitrifying bacterium. J. Bacteriol. **170**:5778-5784.

15. **Nozawa, T., and Y. Maruyama.** 1988. Denitrification by a soil bacterium with phthalate and aromatic compounds as substrates. J. Bacteriol. **170**:2501-2505.

16. **Pereboom, J. H. F., d. G. Man, and I. T. Su.** 1994. Start-up of full scale UASB-reactor for the treatment of terephtalic acid waste water. Presented at the 7th International Symposium on Anaerobic Digestion, Cape Town, South Africa.

17. **Perrotta, J. A., and C. S. Harwood.** 1994. Anaerobic metabolism of cyclohex-1-ene-1-carboxylate, a proposed intermediate of benzoate degradation, by *Rhodopseudomonas palustaris*. Appl. Environ. Microbiol. **60**:1775-1782.

18. **Schink, B.** 1992. Syntrophism among prokaryotes, p. 276-299. *In* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), The prokaryotes, vol. I. Springer-Verlag, New York, N.Y.

19. **Schink, B., A. Brune, and S. Schnell.** 1992. Anaerobic degradation of aromatic compounds, p. 219-242. *In* G. Winkelmann (ed.), Microbial Degradation of Natural Products. VCH, Weinheim, Germany.

20. **Stams, A. J. M.** 1994. Metabolic interactions between anaerobic bacteria in methanogenic environments. Antonie Leeuwenhoek. **66**:271-294.

21. **Warikoo, V., M. J. McInerney, J. A. Robinson, and J. M. Suflita.** 1996. Interspecies acetate transfer influences the extent of anaerobic benzoate degradation by syntrophic consortia. Appl. Environ. Microbiol. **62**:26-32.

Microbiology III: Energetics

Abstract

The phthalate isomers grown enrichment cultures, described in chapter 3, were incubated with benzoate and the phthalate isomers and bromoethanosulfonate to study product formation during fermentation of the aromatic substrates. Gibbs free energy calculations were used for interpretation of the experimental results.

Contents

5.1 Summary

Methanogenic enrichment cultures grown on phthalate, isophthalate and terephthalate were incubated with the corresponding phthalate isomer on which they were grown, and a mixture of benzoate and the phthalate isomer. All cultures were incubated with bromoethanosulfonate (BES) to inactivate the methanogens in the mixed culture. Herewith, product formation during fermentation of the aromatic substrates could be studied. It was found that reduction equivalents generated during oxidation of the aromatic substrates to acetate were incorporated in benzoate under formation of carboxycyclohexane. During fermentation of the phthalate isomers, small amounts of benzoate were detected, suggesting that the initial step in the anaerobic degradation of the phthalate isomers is decarboxylation to benzoate. Gibbs free energy analyses indicated that during degradation of the phthalate isomers, benzoate, carboxycyclohexane, acetate and molecular hydrogen accumulated in such amounts that both the reduction and oxidation of benzoate yielded a constant and comparable amount of energy of approximately 30 kJ·mol^{-1}. Based on these observations it is suggested that within narrow energetic limits, oxidation and reduction of benzoate may proceed simultaneously. Whether this is controlled by the Gibbs free energy change for carboxycyclohexane oxidation remains unclear[a].

5.2 Introduction

The application of thermodynamic laws to biochemical processes provides a theoretical basis for analysis of experimental results. The second law of thermodynamics states that microbial conversions can only sustain growth if the reaction is exergonic. It furthermore states that the amount of energy generated during a biochemical conversion equals the Gibbs free energy change of the chemical reaction. The most well known application of thermodynamic principles for biotechnological processes is the observed correlation between the microbial yield and Gibbs free energy changes of microbial conversions [5, 8, 19]. Furthermore, the mechanisms of formation of intermediate compounds can be analysed using thermodynamic considerations [12, 16, 17, 26]. A principle limitation of thermodynamic laws is that they provide no information about the rate of electron transport in complex microbial conversion reactions. It has been suggested, however, that Gibbs free energy dissipation values can be correlated to reaction rates in complex microbial conversions [14, 15, 25].

[a] A modified version of this chapter has been published in *FEMS Microbiology Ecology* (1999) **29**: 273-282.

Microbial conversions in methanogenic environments proceed close to thermodynamical equilibrium. Several anaerobic fermentation reactions are endergonic under standard conditions. Examples of these reactions are the fermentation of ethanol, propionate, butyrate and benzoate to a mixture of acetate, carbon dioxide and hydrogen (or formate). Combined with the fact that methanogenic organisms only utilise a limited range of simple substrates (e.g. CO_2/H_2, formate, methanol or acetate), the degradation of substrates in methanogenic environments becomes dependent on a mixture of fermenting and methanogenic organisms. Only if the methanogenic organisms keep the concentration of the fermentation products low, the fermentation is exergonic and the reaction can be pulled to the product side. Due to their mutual dependency these mixed cultures are referred to as syntrophic cultures [11].

Concerning the minimum amount of Gibbs free energy needed to sustain growth and/or conversion of a substrate, some controversy exists. Values for critical Gibbs free energy changes for the fermentation of e.g. ethanol, propionate, butyrate, lactate or benzoate range from -2 to -30 kJ/mol [4, 7, 13, 14, 16-18, 23, 24]. Schink [11] postulated that the minimum amount of Gibbs free energy should equal approximately -20 to -30 kJ/mol, corresponding to 1/3 to 1/4 ATP. ATP can be synthesised in these small quanta via ion translocation across the cytoplasmic membrane. Considering, however, that anaerobic fermentations have been observed at Gibbs free energy changes much closer to 0 kJ/mol, it may be suggested that electron transport across the cytoplasmic membrane and ATP formation should be variable, implying that any exergonic reaction may sustain growth [21].

In Chapter 3 and 4 we described the kinetic properties and related specific characteristics of three methanogenic enrichment cultures grown on phthalate, isophthalate and terephthalate. It was postulated in these chapters that the phthalate isomers are fermented to a mixture of acetate and hydrogen with benzoate (or benzoyl-CoA) as the central intermediate, analogous to the denitrifying organism *Pseudomonas* K136 [9, 10]. All three cultures were found to have the ability to degrade benzoate without a lag-phase, and they were only able to degrade the phthalate isomer on which they were cultivated. This chapter describes product formation of the three cultures incubated with the phthalate isomer, or a mixture of benzoate and the phthalate isomer, in presence of bromoethanosulfonate (BES), a specific inhibitor of methanogenesis. The Gibbs free energy changes of the conversions observed were calculated, and the implications for the energetic limits of the conversions are discussed.

5.3 Material and methods

Biomass. Three enrichment cultures were used with the ability to degrade either phthalate, isophthalate or terephthalate. The cultures were obtained from digested sewage sludge or granular sludge and were enriched on one of the three phthalate isomers as described in Chapter 3. The phthalate isomer grown cultures had the ability to degrade benzoate without a lag phase, at rates comparable to the phthalate isomers' degradation rates.

Experimental procedure. Experiments were performed in 117-ml serum bottles using a liquid volume of 25 or 40 ml. Medium, substrate and bromoethanosulfonate (BES) were added to the bottles from concentrated stock-solutions. The composition of the medium has been described in Chapter 2. Either the phthalate isomer, or a mixture of the phthalate isomer and benzoate were used as substrate at concentrations between 2 and 5 mM. Serum bottles were sealed with butyl rubber stoppers and the headspace was flushed with a N_2/CO_2-mixture (70/30 v/v). Sulphide was dosed from a concentrated stock (final concentration 0.7 mM) to ensure anaerobic conditions. Serum bottles were pre-incubated at 37 °C in an orbital shaker prior to inoculation with the phthalate isomer grown cultures by syringe. Liquid samples (2 ml) were withdrawn from the bottles for component analyses. All data shown are average values from duplicate experiments.

Analytical procedures. The concentration of aromatic acids was determined by high pressure liquid chromatography (HPLC). Intermediate formation of low concentrations of benzoate could be detected by injection of undiluted samples down-to concentrations of 1 μM. The concentration of acetate and carboxycyclohexane were measured by gas chromatography. The concentration of hydrogen and methane in the headspace of the serumbottles were determined by gas chromatography as well. A full account of these analytical methods can be found in Chapter 2.

Energetic analyses. Standard Gibbs free energy changes for the observed conversions during degradation of benzoate and the phthalate isomers were calculated according to Thauer et al. [20] Table 5.1. ΔG_f^0 values for the phthalate isomers were calculated from benzoate, using the group contribution method described by Dimroth [3]. The Gibbs free energy change for reduction of benzoate to carboxycyclohexane was calculated from the reduction of benzene to cyclohexane as suggested by Schink [11]. ΔG_f^0 values were corrected for a temperature of 37 °C using the Van 't Hoff equation [2].

Table 5.1: Chemical reaction equations for the individual steps in mineralization of phthalate isomers and benzoate, and standard Gibbs free energy changes during the conversions, corrected for a temperature of 37 °C.

	reaction	equation	ΔG0' (37 °C) [kJ·reaction]
1	phthalate oxidation	$C_8H_4O_4^{2-} + 8H_2O \rightarrow 3C_2H_3O_2^- + 3H^+ + 3H_2 + 2HCO_3^-$	38.9[1]
2	phthalate decarboxylation	$C_8H_4O_4^{2-} + H_2O \rightarrow C_7H_5O_2^- + HCO_3^-$	-20.7
3	benzoate oxidation	$C_7H_5O_2^- + 7H_2O \rightarrow 3C_2H_3O_2^- + 3H^+ + 3H_2 + HCO_3^-$	59.6
4	benzoate reduction	$C_7H_5O_2^- + 3H_2 \rightarrow C_7H_{11}O_2^-$	-93.5
5	carboxycyclohexane oxidation	$C_7H_{11}O_2^- + 7H_2O \rightarrow 3C_2H_3O_2^- + 3H^+ + 6H_2 + HCO_3^-$	153.1

[1] value represents calculated $\Delta G^{0'}$ for isophthalate and terephthalate fermentation, the value for *ortho*-phthalate fermentation is estimated to be 34.9 kJ·reaction^{-1}. Applied $\Delta G_f^{0'}$ values for *ortho*-phthalate, isophthalate and terephthalate were -548.6, -552.6 and -552.7 respectively [3].

Mass and electron balances. Based on measured concentrations of all organic substrates and products, balances were derived to study if non-identified products accumulated or analytical errors had occurred during the experiments. These balances are based on the concept of degree of reduction (γ, e-mol·C-mol^{-1} or e-mol·mol^{-1} for inorganic products) and the carbon-chain-length (Cl, C-mol·mol^{-1}) of all relevant compounds. The degree of reduction of a compound is defined as the number of electrons liberated upon complete oxidation of 1 C-mol of organic material (or 1 mol of inorganic material) to CO_2 and H_2O [5]. Herewith these balances are equivalent to Chemical Oxygen Demand (COD) based balances. Mathematically these balances can be described using the following equation:

$$\sum \gamma_S \cdot Cl_S \cdot C_S(t) + \sum \gamma_P \cdot Cl_P \cdot C_P(t) = constant \qquad (5.1)$$

In this equation, C(t) stands for time dependent concentration (M), and subscripts S and P stand for substrate(s) and product(s) respectively.

Another approach is based on the fact that during anaerobic fermentations, all electrons liberated during oxidation of a substrate should be incorporated in reduced products. Using the degree of reduction of the substrate as a frame of reference, the following electron balance can be derived:

$$\sum(\gamma_P - \gamma_S)\cdot Cl_P \cdot C_P(t) = 0 \qquad (5.2)$$

Using this equation, oxidation is defined as negative and reduction positive. If the product concentrations do not equal zero at the beginning of the experiment, the initial concentrations should be subtracted from the measured concentrations. From the stoichiometry of the fermentations shown in Table 5.1, it can be seen that inorganic carbon (HCO_3^-) is formed during the oxidation of benzoate and the phthalate isomers. Oxidation equivalents formed due to HCO_3^- formation, were calculated based on the measured acetate concentrations according to reaction stoichiometries shown in Table 5.1. Taken into account all conversions with benzoate shown in Table 5.1, the equation for benzoate fermentation becomes the following:

$$(\gamma_{C2} - \gamma_{BA})\cdot Cl_{C2}\cdot C_{C2} + (\gamma_{HCO_3} - \gamma_{BA})\cdot Cl_{HCO_3}\cdot\frac{C_{C2}}{3} + (\gamma_{CCH} - \gamma_{BA})\cdot Cl_{CCH}\cdot C_{CCH} + \gamma_{H_2}\cdot C_{H_2} = 0 \qquad (5.3)$$

Subscripts C2, BA, HCO3, CCH, and H_2, stand for acetate, benzoate, bicarbonate, carboxycyclohexane and molecular hydrogen respectively. Because decarboxylation of terephthalate to benzoate does not involve net-oxidation or reduction, the same equation can be used for analyses of products formed during conversion of the phthalate isomers. Incorporation of electrons into biomass is neglected during these calculations.

5.4 Results

Phthalate, isophthalate and terephthalate grown methanogenic cultures were incubated with the corresponding phthalate isomers, or a mixture of benzoate and the phthalate isomers in the presence of bromoethanosulfonate (BES, 20 mM). BES is a specific inhibitor for methanogenesis. During the incubation, substrate depletion and product formation were studied for analysis of product formation from the phthalate isomers and benzoate fermenting cultures.

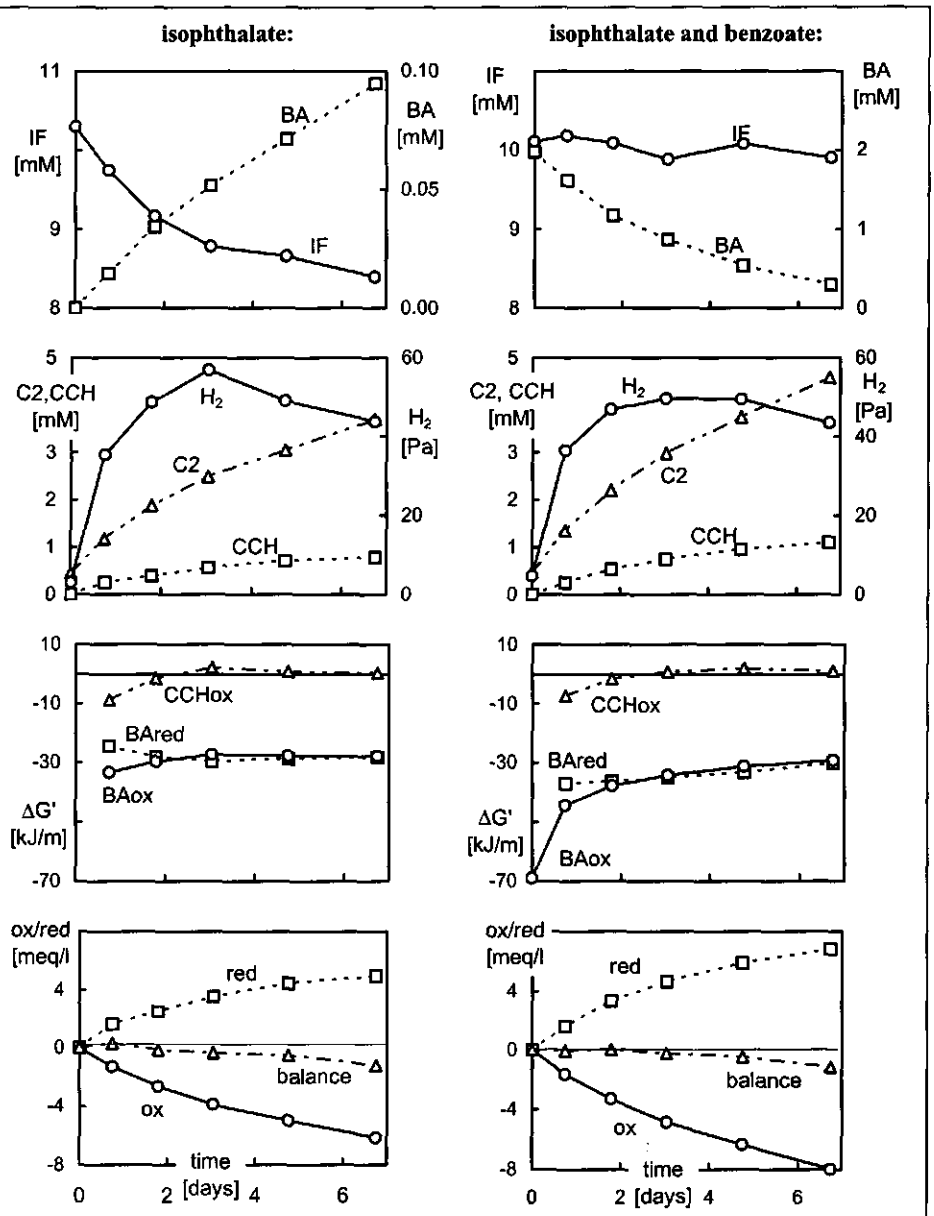


Figure 5.1: Degradation of isophthalate (left graphs) and a mixture of isophthalate and benzoate (right graphs) by the isophthalate grown enrichment culture in presence of 20 mM BES. From top to bottom the graphs show (i) the concentration isophthalate (IF) and benzoate (BA), (ii) the concentration acetate (C2), carboxycyclohexane (CCH) and molecular hydrogen (H_2), (iii) the actual Gibbs free energy change for benzoate oxidation (BAox, reaction 3, Table 5.1), benzoate reduction (BAred, reaction 4, Table 5.1) and carboxycyclohexane oxidation (CCHox, reaction 5, Table 5.1), and (iv) the electron-balance according to Equation 5.2.

It was found that during incubation of the isophthalate grown culture with isophthalate as the sole carbon and energy source, benzoate, acetate, carboxycyclohexane and molecular hydrogen accumulated (Figure 5.1). When the culture was incubated with a mixture of benzoate and isophthalate, benzoate was the preferred substrate over isophthalate, as was previously described for the terephthalate grown enrichment culture (Chapter 4). As in the culture incubated with isophthalate, acetate, hydrogen and carboxycyclohexane accumulated during incubation with a benzoate/isophthalate mixture. In both experiments methane formation was negligible. Molecular hydrogen accumulated to comparable concentrations in both experiments, whereas acetate and carboxycyclohexane concentrations were slightly higher in the experiment incubated with a mixture of benzoate and isophthalate. Hydrogen concentrations were approximately ten times higher than under exponential growth conditions (Chapter 2).

It is evident from these data that part of the reducing equivalents generated during benzoate and isophthalate oxidation (reaction 1 and 3, Table 5.1) were incorporated into benzoate resulting in the formation of carboxycyclohexane (reaction 4, Table 5.1). From the electron-balance, calculated according to Equation 5.5.3 and shown in Figure 1, it can be seen that the amount of reduction equivalents formed during oxidation of the aromatic substrates corresponds reasonably well to the amount of reduction equivalents incorporated in the reduced products. Herewith it should be noted that molecular hydrogen corresponds to less than 1% of the accumulating concentration reduced products and therefore plays a minor role in the electron-balance.

The actual Gibbs free energy changes of the different conversions observed were calculated from the measured substrate and product concentrations. Decarboxylation of isophthalate ($\Delta G' \cong -31$ kJ/mole, reaction 2 Table 5.1) and oxidation of isophthalate to acetate and hydrogen ($\Delta G' \cong -59$ kJ/mol, reaction 1, Table 5.1), remained exergonic throughout the period of the elevated hydrogen concentration. Gibbs free energy changes for oxidation and reduction of benzoate (reaction 3 and 4, Table 5.1) are shown in Figure 5.1. In the experiment with isophthalate as sole substrate, both reduction and oxidation of benzoate remained exergonic throughout the experiment, and the Gibbs free energy change of approximately -30 kJ/mol for both conversions are highly comparable. Consequently, the Gibbs free energy change for oxidation of carboxycyclohexane remained around 0 kJ/mol, because the Gibbs free energy change for this conversion equals the value for benzoate oxidation, minus the value for benzoate reduction. Also during the experiment with a mixture of isophthalate and benzoate, the Gibbs free energy change for carboxycyclohexane oxidation was relatively constant and close to 0 kJ/m. The initial values for benzoate

oxidation and reduction were, however, more negative compared to the experiment with isophthalate as sole carbon source, due to the higher benzoate concentrations.

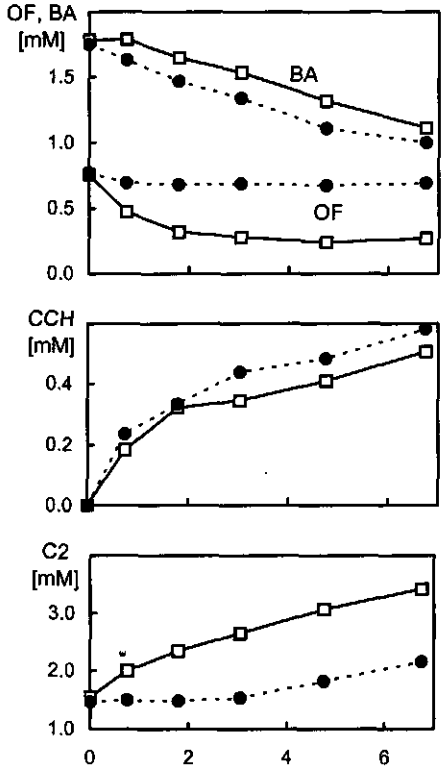


Figure 5.2: Degradation of benzoate (BA, □/■) and phthalate (OF, ○/●, top graph) by the phthalate grown enrichment culture, and the accumulation of carboxycyclohexane (CCH, middle graph) and acetate (C2, bottom graph) in absence (open markers, solid lines) and presence (solid markers, dashed lines) of hydrogen in the headspace.

The phthalate grown culture was incubated with phthalate, and a mixture of phthalate and benzoate as well. In this case, experiments were also performed in the absence or presence of approximately 2.5 % molecular hydrogen in the headspace of the serum bottles. Results of the experiments with a mixture of benzoate and phthalate are shown in Figure 5.2, and the Gibbs free energy changes for the different conversions involving benzoate are shown in Figure 5.3. These figures show that in the absence of molecular hydrogen, benzoate and phthalate were initially converted to carboxycyclohexane and acetate. After approximately three days, phthalate conversion stopped and only benzoate was degraded further. Like in the isophthalate experiment, the Gibbs free energy change for carboxycyclohexane oxidation was approximately 0 $kJ \cdot mol^{-1}$. In the presence of hydrogen in the headspace, no conversion of phthalate was observed, but benzoate was degraded at a comparable rate compared to the experiment without hydrogen. Initially, however, no acetate was produced, despite the observation that benzoate oxidation is exergonic, and all benzoate converted was reduced to carboxycyclohexane with molecular hydrogen. Acetate was only formed once the hydrogen concentration in the headspace has reached comparable values to those observed in experiments without hydrogen dosage. As with the bottles incubated with the benzoate/phthalate-mixture, an initial reduction of phthalate to carboxycyclohexane was observed in serum bottles incubated with phthalate and molecular hydrogen (data not

shown). Trace amounts of benzoate (app. 50 μM) accumulated in these bottles. Once the Gibbs free energy change for carboxycyclohexane oxidation became exergonic due to hydrogen uptake, acetate formation was observed.

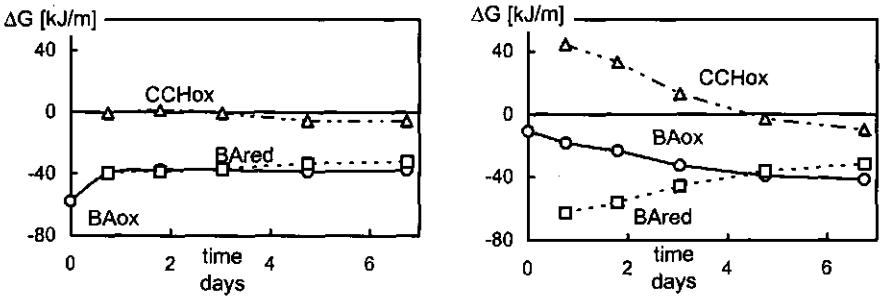


Figure 5.3: Gibbs free energy change for benzoate oxidation (BAox, O), benzoate reduction (BAred, □) and carboxycyclohexane oxidation (CCHox, Δ) in absence (left graph) and presence (right graph) of molecular hydrogen in the headspace.

Product formation during degradation of phthalate and terephthalate by their corresponding enrichment cultures (without hydrogen dosage) showed a similar pattern as described for isophthalate. The average values for the Gibbs free energy changes of the various conversions (Table 5.1) are presented in Table 5.2. It is evident from this table that the Gibbs free energy changes of the different conversions were highly comparable for the different cultures utilised. A minor difference between the three cultures was that isophthalate degradation was completely inhibited by benzoate (Figure 5.1), while limited conversion of phthalate and terephthalate was observed during incubations with benzoate (not shown). However, conversion of phthalate and terephthalate stopped within a few days when incubated with benzoate, even though benzoate conversion continued. This observation indicates that the cultures rapidly lost their capacity to convert the phthalate isomers in the presence of the preferred substrate benzoate, as described previously for the terephthalate grown enrichment culture (Chapter 4).

Because during our experiments carboxycyclohexane was formed, we checked the cultures for their ability to degrade carboxycyclohexane in absence of BES. All three phthalate isomers grown enrichment cultures were found to be capable of carboxycyclohexane degradation (initial concentration 5 mM) at rates ranging from 5 to 25% of the degradation rates of the phthalate isomers and without a lag-phase (data not shown).

Table 5.2: Actual Gibbs free energy change (ΔG') for the conversions shown in Table 1 in experiments incubated with the phthalate isomers as sole carbon and energy sources in presence of BES. Calculations were performed during the time-period that values were more or less constant and an equilibrium appeared to exist. Reaction numbers correspond to the chemical reaction equations shown in Table 5.1. Values between brackets represent standard deviations in the calculated ΔG' values.

	phthalate oxidation (reaction 1)	benzoate oxidation (reaction 3)	benzoate reduction (reaction 4)	carboxycyclo-hexane ox. (reaction 5)
culture	ΔG' (kJ·reaction^{-1})			
phthalate	-60.5 (1.3)	-32.5 (1.5)	-31.0 (1.5)	-1.5 (2.1)
isophthalate	-59.6 (1.8)	-28.2 (1.1)	-28.7 (0.6)	0.5 (1.5)
terephthalate	-62.2 (2.3)	-29.7 (2.8)	-27.4 (2.6)	-2.3 (1.6)

5.5 Discussion

The patterns of product formation during incubation with either the phthalate isomer, or with a mixture of phthalate and benzoate were found to be highly comparable for all three phthalate isomer-grown methanogenic enrichment cultures. In all experiments part of the phthalate isomer or benzoate was oxidised to acetate, and reduction equivalents generated during the oxidation were incorporated in benzoate and carboxycyclohexane was formed. Small amounts of benzoate accumulated in the experiments incubated with the phthalate isomers as sole carbon and energy source. A schematic representation of the conversions observed is presented in Figure 5.4.

Reduced product formation at elevated hydrogen partial pressures, comparable to the carboxycyclohexane formation in our experiments, has been observed before. Smith and McCarty demonstrated that ethanol perturbation of an ethanol and propionate fed chemostat led to accumulation of hydrogen in the biogas and consequently the formation of n-propanol and 4 to 7 n-carboxylic acids [16, 17]. Hickey and Switzenbaum suggested that hydrogen accumulated only to slightly higher concentrations during organic overloads of an anaerobic digester, due to reduced product formation [6, 7].

The electron balances showed that 85 to 95 % of the reduction equivalents generated in the oxidation of the aromatic substrates were incorporated into carboxycyclohexane. The observation that in all our experiments the concentration of reduced products could not fully account for the oxidised products detected, suggests that an unidentified reduced product

may have accumulated. A possible reduced product is formate. Formate has previously been identified as an alternative electron carrier for molecular hydrogen in methanogenic systems [1, 11, 22]. Mainly at elevated bicarbonate concentrations, as applied during our experiments, formate may play an important role in electron transfer among species. However, formate concentrations calculated, considering a thermodynamical equilibrium between formate and hydrogen/bicarbonate, could not account for the observed gap in the electron balance (calculations not shown).

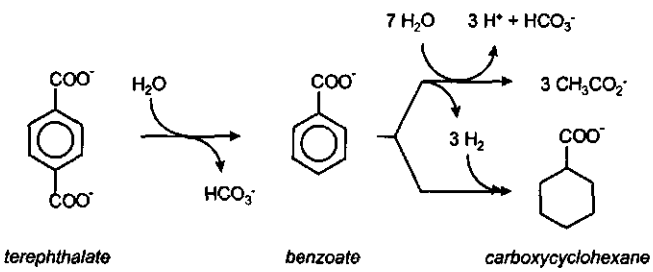


Figure 5.4: Schematic representation of the conversions observed in the BES-amended phthalate isomers grown cultures.

Energetics of the conversions observed. From the data in Table 2 it appears that all phthalate isomer grown cultures gave comparable Gibbs free energy changes for all conversions observed. The energetics of the different conversions will therefore be discussed independent of the phthalate isomer grown culture utilised.

The Gibbs free energy change for oxidation of the phthalate isomers reached a stable value of approximately -60 kJ·mol^{-1} in all cultures incubated with the phthalate isomer as sole carbon source. Even though initially some conversion of the phthalate isomers was observed in cultures incubated with a mixture of the phthalate isomers and benzoate, benzoate was the preferred substrate in all cases.

Both in cultures incubated with the phthalate isomers or the phthalate/benzoate mixture, the Gibbs free energy change for oxidation and reduction of benzoate were found to be around -30 kJ·mol^{-1}. No benzoate conversion was observed at Gibbs free energy changes for oxidation or reduction of benzoate exceeding approximately -28 kJ·mol^{-1}, suggesting that this value may represent a minimum amount of energy required to sustain the conversion of benzoate. This critical Gibbs free energy change for benzoate conversion is remarkably close to the value of -30 kJ·mol^{-1}, reported by Warikoo et al. [24]. These authors determined the threshold concentrations of benzoate and the corresponding critical Gibbs free energy change

as a function of the acetate concentration in a defined coculture consisting of the syntrophic benzoate degrader, strain SB, and the *Desulfovibrio* sp. strain G-11.

The observation that the Gibbs free energy change for oxidation and reduction of benzoate are highly comparable in all our experiments, suggests that the Gibbs free energy change for carboxycyclohexane oxidation is close to 0 $kJ \cdot mol^{-1}$ (see Table 5.2). Two lines of reasoning can be used to describe the mechanistic basis for this observation: carboxycyclohexane may either be a true intermediate in the oxidation of benzoate (i), or a side product originating from a different non-aromatic intermediate (ii). The first option suggests that oxidation of benzoate only proceeds as long as the Gibbs free energy change for carboxycyclohexane oxidation is negative. The second option suggests that an equilibrium may develop if both oxidation and reduction of benzoate proceed at a comparable efficiency. In this case intermediates may accumulate to such concentrations that the Gibbs free energy change for both oxidation and reduction of benzoate remain sufficiently negative to sustain growth and/or conversion of the aromatic substrate.

In summary we suggest that within narrow energetic limits, simultaneous oxidation and reduction of benzoate may proceed. However, our experiments provide no answer to the question if carboxycyclohexane (or its' CoA-derivative) is an intermediate in the oxidation of benzoate, or a side-product originating from a different non-aromatic intermediate. The fact that we worked with mixed cultures furthermore keeps the question open whether one or more organisms were involved in the conversions observed.

References

1. **Boone, D. R., R. L. Johnson, and Y. Liu.** 1989. Diffusion of the interspecies electron carriers hydrogen and formate in methanogenic ecosystems and its implication in the measurement of Km for hydrogen or formate uptake. Appl. Environ. Microbiol. **55:**1735-1741.
2. **Conrad, R., and B. Wetter.** 1990. Influence of temperature on energetics of hydrogen metabolism in homoacetogenic, methanogenic, and other bacteria. Arch. Microbiol. **155:**94-98.
3. **Dimroth, K.** 1983. Thermochemische daten organischer verbindungen, p. 997-1038, D'ans-Lax, Taschenbuch für chemiker und Physiker, vol. 2. Springer-Verlag, Berlin.
4. **Dwyer, D. F., E. Weeg-Aerssens, D. R. Shelton, and J. M. Tiedje.** 1988. Bioenergetic conditions of butyrate metabolism by a syntrophic anaerobic bacterium in coculture with hydrogen-oxidizing methanogenic and sulfidogenic bacteria. Appl. Environ. Microbiol. **54:**1354-1359.

5. **Heijnen, J. J., M. C. M. Loosdrecht van, and L. Tijhuis.** 1992. A black box mathematical model to calculate auto- and heterotrophic biomass yields based on Gibbs energy dissipation. Biotechnol. Bioeng. **40:**1139-1154.

6. **Hickey, R. F., and M. S. Switzenbaum.** 1991. The response and utility of hydrogen and carbon monoxide as process indicators of anaerobic digestors subject to organic and hydraulic overloads. Res. J. WPCF. **63:**129-40.

7. **Hickey, R. F., and M. S. Switzenbaum.** 1991. Thermodynamics of volatile fatty acid accumulation in anaerobic digesters subject to increases in hydraulic and organic loading. Res. J. WPCF. **63:**141-144.

8. **McCarty, P. L.** 1971. Energetics and bacterial growth, p. 495-512. *In* S. D. Faust and J. V. Hunter (ed.), Organic compounds in aquatic environments. Marcel Dekker, Inc., New York.

9. **Nozawa, T., and Y. Maruyama.** 1988. Anaerobic metabolism of phthalate and other aromatic compounds by a denitrifying bacterium. J. Bacteriol. **170:**5778-5784.

10. **Nozawa, T., and Y. Maruyama.** 1988. Denitrification by a soil bacterium with phthalate and aromatic compounds as substrates. J. Bacteriol. **170:**2501-2505.

11. **Schink, B.** 1992. Syntrophism among prokaryotes, p. 276-299. *In* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), The prokaryotes, vol. I. Springer-Verlag, New York, N.Y.

12. **Schink, B., A. Brune, and S. Schnell.** 1992. Anaerobic degradation of aromatic compounds, p. 219-242. *In* G. Winkelmann (ed.), Microbial Degradation of Natural Products. VCH, Weinheim, Germany.

13. **Seitz, H. J., B. Schink, and R. Conrad.** 1988. Thermodynamics of hydrogen metabolism in methanogenic cocultures degrading ethanol or lactate. FEMS Microbiol. Lett. **55:**119-124.

14. **Seitz, H. J., B. Schink, N. Pfennig, and R. Conrad.** 1990. Energetics of syntrophic ethanol oxidation in defined chemostat cocultures; energy requirements for H_2 production and H_2 oxidation (1). Arch. Microbiol. **155:**82-88.

15. **Seitz, H. J., B. Schink, N. Pfennig, and R. Conrad.** 1990. Energetics of syntrophic ethanol oxidation in defined chemostat cocultures; energy sharing in biomass production (2). Arch. Microbiol. **155:**89-93.

16. **Smith, D. P., and P. L. McCarty.** 1989. Energetic and rate effects on methanogenesis of ethanol and propionate in perturbed CSTRs. Biotechnol. Bioeng. **34:**39-54.

17. **Smith, D. P., and P. L. McCarty.** 1989. Reduced product formation following perturbation of ethanol-and propionate-fed methanogenic CSTRs. Biotechnol. Bioeng. **34:**885-895.

18. **Stams, A. J. M.** 1994. Metabolic interactions between anaerobic bacteria in methanogenic environments. Antonie Leeuwenhoek. **66:**271-294.

19. **Stouthamer, A. H.** 1979. The search for correlation between theoretical and experimental growth yields, p. 1-47. *In* J. R. Quayle (ed.), International review of biochemistry, vol. #21. University Park Press, Baltimore.

20. **Thauer, R. K., K. Jungermann, and K. Decker.** 1977. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Rev. **41:**100-179.

21. **Thauer, R. K., and J. G. Morris.** 1984. Metabolism of chemotrophic anaerobes: old views and new aspects, p. 123-168. *In* D. P. Kelly and N. G. Carr (ed.), The Microbe 1984, part II: Prokaryotes and Eukaryotes. Cambridge University Press, Cambridge.

22. **Thiele, J. H., and J. G. Zeikus.** 1988. Control of interspecies electron flow during anaerobic digestion: significance of formate transfer versus hydrogen transfer during syntrophic methanogenesis in flocs. Appl. Environ. Microbiol. **54:**20-29.

23. **Wallrabenstein, C., and B. Schink.** 1994. Evidence of reversed electron transport in syntrophic butyrate or benzoate oxidation by *Syntrophomonas wolfei* and *Syntrophus buswellii*. Arch. Microbiol. **162:**136-142.

24. **Warikoo, V., M. J. McInerney, J. A. Robinson, and J. M. Suflita.** 1996. Interspecies acetate transfer influences the extent of anaerobic benzoate degradation by syntrophic consortia. Appl. Environ. Microbiol. **62:**26-32.

25. **Westerhoff, H. V., J. S. Lolkema, R. Otto, and K. J. Hellingwerf.** 1982. Thermodynamics of growth; non-equilibrium thermodynamics of bacterial growth, the phenomenological and the mosaic approach. Biochim. Biophys. Acta. **683:**181-220.

26. **Wu, M. W., and R. F. Hickey.** 1996. N-propanol production during ethanol degradation using anaerobic granules. Wat. Res. **30:**1686-1694.

Wastewater Treatment I: Hybrid Reactors

Abstract

The degradation of terephthalate as sole carbon and energy source was studied in UASB and hybrid reactors seeded with suspended methanogenic biomass. High terephthalate removal capacities were only obtained in the hybrid reactors due to adequate biomass retention. The influence of the temperature and pH on the terephthalate grown biomass were furthermore assessed.

Contents

6.1 Summary

The anaerobic degradation of terephthalate as sole substrate was studied in three anaerobic upflow reactors. Initially, the reactors were operated as Upflow Anaerobic Sludge Bed (UASB) reactors and seeded with suspended methanogenic biomass obtained from a full-scale down-flow fixed film reactor, treating wastewater generated during production of Purified Terephthalic Acid (PTA). The reactors were operated at 30, 37 and 55 °C. The terephthalate removal capacities remained low in all three reactors (< 4 mmol·l^{-1}·day^{-1} or 1 g-COD·l^{-1}·day^{-1}) due to limitations in biomass retention. Batch-experiments with biomass from the UASB-reactors revealed that within the mesophilic temperature range, optimal terephthalate degradation is obtained at 37 °C. No thermophilic terephthalate degrading culture could be obtained in either continuous or batch cultures. In order to enhance biomass retention, the reactors were modified to anaerobic hybrid reactors by introduction of two types of reticulated polyurethane (PUR) foam particles. The hybrid reactors were operated at 37 °C and seeded with a mixture of biomass from the UASB-reactors operated at 30 and 37 °C. After a lag period of approximately 80 days, the terephthalate conversion capacity of the hybrid reactors increased exponentially at a specific rate of approximately 0.06 day^{-1}, and high removal rates were obtained (40-70 mmol·l^{-1}·day^{-1} or 10-17 g-COD·l^{-1}·day^{-1}) at hydraulic retention times between 10 and 15 hours. These high removal capacities could be attributed to enhanced biomass retention by the development of biofilms on the PUR-carrier material as well as the formation of granular biomass. Biomass balances over the hybrid reactors suggested that either bacterial decay or selective washout of the terephthalate fermenting biomass played an important role in the capacity limitations of the systems. The presented results suggest that terephthalate can be degraded at high volumetric rates if sufficiently long sludge ages can be maintained, and the reactor pH and temperature are close to their optima[E].

[E] A modified version of this chapter has been published in *Biotechnol. Prog.* (1999), **15**: 347-357.

6.2 Introduction

With regard to the anaerobic treatability of PTA-wastewater controversy exists (Chapter 1, [15]). The applicable loading rates reported in literature vary from 1 to 22 g-COD·l^{-1}·d^{-1} [2, 4, 19, 22, 24], but degradation of terephthalate and *para*-toluate have only been demonstrated in bioreactors operated at low loading rates (< 4 g-COD·l^{-1}·day^{-1} [20, 21, 23, 24]). During preliminary studies we found that terephthalate (and *para*-toluate) degradation were rate limiting in upflow anaerobic sludge bed (UASB) reactors operated at 30°C and treating artificial PTA-wastewater [15]. Batch- and continuous experiments furthermore demonstrated that terephthalate degradation is strongly inhibited by the readily degradable wastewater constituents benzoate and acetate (Chapter 4, [5, 15]). In the UASB-reactors, substantial degradation of terephthalic acid was only observed in absence of these other substrates, but volumetric conversion rates remained low (3.9 g-COD·l^{-1}·d^{-1} ≈ 16 mmol·l^{-1}·d^{-1}) despite a hydraulic retention time of 24 hours [15]. The low terephthalate removal capacities of the reactors were found to be related to the low growth rate (≅ 0.04 d^{-1} at 30°C) of the terephthalate degrading biomass, suggesting the need of very high solid retention times. At a temperature of 37°C, enrichment cultures were found to have a higher specific growth rate of 0.09 day^{-1} (Chapter 3). With regard to the toxicity of terephthalate it was demonstrated that in pH-neutral environments, the dominant anionic form is not toxic to the different species involved in terephthalate degradation at the concentrations normally found in PTA-wastewater (<25 mM) [5, 15]. Based on these results, we suggested a two-stage anaerobic bioreactor concept for the anaerobic treatment of PTA-wastewater [15]. Through pre-removal of the readily degradable substrates acetate and benzoate in the first stage of such a system, the removal of terephthalate (and *para*-toluate) in the latter stages can be optimised.

This chapter deals with the optimisation of terephthalate removal from PTA-wastewater in the second stage of a two-stage bioreactor concept. Continuous reactor studies were conducted with terephthalate as sole carbon and energy source, herewith simulating complete pre-removal of acetate and benzoate from the wastewater. The initial experiments were conducted with UASB-reactors operated at 30, 37 and 55 °C. The reactor operated at 55 °C was run to study if a thermophilic culture could be cultivated on terephthalate, because the temperature of PTA-wastewater can be as high as 50 °C. In a latter stage of the continuous experiments, the reactors were modified to upflow hybrid reactors through introduction of polyurethane (PUR) carrier material. Batch experiments were used to determine the maximum specific terephthalate degradation rate and maximum specific growth rate of the

biomass cultivated in the reactors. Batch experiments were furthermore used to asses the pH and temperature dependency of terephthalate degradation.

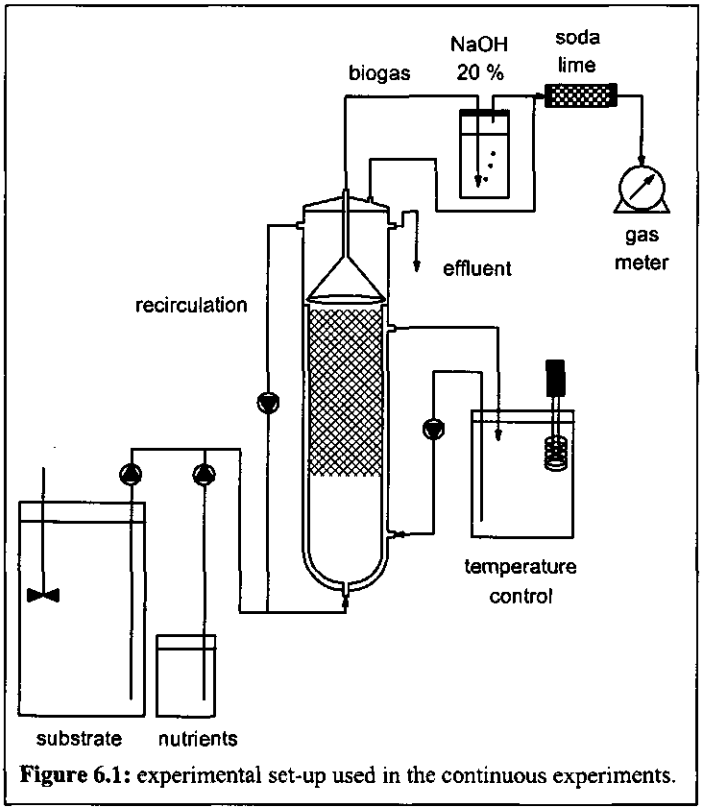


Figure 6.1: experimental set-up used in the continuous experiments.

6.3 Materials and methods

Continuous experiments. Three identical UASB-type reactors with a total working volume of 3.85 l. (inner diameter: main body: 80 mm, sedimentation zone: 120 mm) were used. The reactors were made of glass and equipped with an internal three phase separator as shown in Figure 6.1. Biogas produced was led through (i) a waterlock filled with a 20 % sodium hydroxide solution for carbon dioxide removal and for the control of the water level in the three phase separator, (ii) a column packed with soda lime pallets with indicator for water vapour removal and (iii) a wet gas meter (Meterfabriek Dordrecht, Dordrecht, The Netherlands) for quantification of the methane production. The temperature of the reactors was controlled by a thermostat-bath-circulator (Haake D8, Germany) connected to the double wall of the reactors.

All three reactors were fed with disodium terephthalate as sole carbon and energy source, from a continuously agitated 100 l. container. Substrate was prepared through dissolution of the desired amount of terephthalic acid in a sodium hydroxide solution of two times the molar terephthalic acid concentration. The final pH of the substrate was adjusted to 7 through addition of sodium hydroxide or hydrochloric acid from 1 M stock solutions. Nutrients were dosed from a separate container and contained (in mg·(l influent)$^{-1}$): NH_4Cl (1040), KH_2PO_4 (170), $(NH_4)_2SO4$ (170), $MgCl_2 \cdot 6H_2O$ (150), KCl (270), yeast extract (18) and trace elements (1 ml) as described by Huser et al [12]. Initially, $NaHCO_3$ (3000 mg·(l influent)$^{-1}$) was added to the influent to maintain a reactor pH of approximately 7.0. In a latter stage, sufficient bicarbonate alkalinity was generated through degradation of terephthalate, and $NaHCO_3$-dosage was stopped. Both substrate and nutrients were supplied to the reactors with multichannel peristaltic pumps (Gilson-Minipuls 2, Villiers-Le-Bel, France).

Table 6.1: Experimental set-up and initial operational parameters of the first and second run of the continuous experiments

Variable[1]	unit	**run 1**	**run 2**
name		R30/R37/R55	R1/R2/R3
reactor-type		UASB	hybrid
carrier		-	R1/R2: PUR-1[2] R3: PUR-2
temperature	(° C)	30/37/55	37
VLR	(mmol-TA·l^{-1}·d^{-1})	1.3	2.0
HRT	(hr)	36	15
XR	(gVS·l^{-1})	8.4	3.0
q_{TA}^{max}	(mmol-TA·gVS^{-1}·d^{-1})	<0.1 (30 °C)	0.5 (37 °C)
TAinfl	(mM)	1.8	1.3
recirculation	(l·hr^{-1})	-	R1: 6.0 R2/R3: -

[1] VLR: volumetric loading rate, HRT: hydraulic retention time, TA_{inf}: influent concentration terephthalate, X_R: concentration volatile solids in the reactor, q_{TA}^{max}: maximum specific terephthalate conversion rate.

[2] Characteristics of PUR-1 and PUR-2 are presented in Table 6.2.

Two experimental runs were performed and the initial operational characteristics during both runs are shown in Table 6.1. During the first run the reactors were operated as UASB (Upflow Anaerobic Sludge Blanket) reactors at 30, 37 and 55 °C. The reactors were seeded with 8.4 g-VS·(l reactor)$^{-1}$ suspended methanogenic sludge from a full scale down-flow fixed film reactor located at Geel, Belgium, treating wastewater generated during production of terephthalic acid and isophthalic acid [4]. Prior to inoculation of the reactors the sludge had been stored for 5 months at 4 °C. The initial specific activity of the seed sludge with terephthalate and acetate amounted respectively 0.1 and 5.3 mmol·g-VS^{-1}·day^{-1} at 30 °C, and had an ash-content of 27 %. In time, the loading rates were increased through increment of the influent concentration terephthalate.

Table 6.2: Characteristics of the two types of polyurethane (PUR) carrier material used in the anaerobic hybrid reactors.

		PUR-1	PUR-2
specific surface	$m^2 \cdot m^{-3}$	550	800
dimensions	mm	25*25*15	7*7*7
void volume	%	95	90

For the second run the reactors were inoculated with a mixture of the final biomass from R30 and R37. Two types of polyurethane carrier material (for characteristics see Table 6.2) were placed in the reactor below the three phase separator, herewith modifying the UASB reactors to hybrid reactors. The added amounts of carrier material resulted in a specific surface area per volume reactor of approximately 200 $m^2 \cdot m^{-3}$ in R1 and R2 and 375 $m^2 \cdot m^{-3}$ in R3. The carrier particles were randomly organized and occupied approximately 2/3 of the volume of the down-part of the reactor. The effluent of R1 was recycled with a peristaltic pump (Watson Marlow 502 S, Falmouth, UK) to obtain an upflow velocity of 3 m·hr^{-1}.

Batch experiments. Batch experiments were conducted in 120 or 320 ml serum bottles (liquid volume 25 or 70 ml respectively). The basal medium used in the batch experiments was similar as described in Chapter 2. The general procedure of the experiments was the following: nutrients and substrate from concentrated stock solutions were transferred to the bottles and demineralized water was dosed to obtain the desired liquid volume. Subsequently, the bottles were sealed with butyl rubber stoppers and aluminium crimp or screw caps, and the headspace was flushed with a N_2/CO_2-mixture (70/30 v/v). After flushing, 1 ml of a 30 g·l^{-1} $Na_2S \cdot 7\text{-}9H_2O$ stock solution was dosed, and the bottles were pre-incubated at the desired temperature in an orbital motion shaker. After the desired temperature had established, biomass was dosed to the serum bottles by syringe from a well

mixed sludge sample from the reactors. In case a significant part of the biomass was present in granular form, the granules were disintegrated with a blender prior to inoculation.

Determination of the maximum specific activities (q^{max}) of biomass from the bioreactors with terephthalate, benzoate or acetate (initial concentrations 5, 5 or 15 mM respectively) were performed at initial biomass concentrations of 1.5 to 2.5 g-VS·l^{-1}. For determination of the maximum specific activity on terephthalate of biomass washed out from the bioreactors, 25 ml of reactor effluent was sampled by syringe from the sedimentation zone and incubated with 1.0 ml from a terephthalate stock solution (0.12 M). In this case no additional nutrients or bicarbonate were supplied. Activity determinations were based on measured methane concentrations in the headspace during a one ore two day period.

Table 6.3: Initial concentrations of CO_2 and $NaHCO_3$ and the corresponding calculated initial and final (after complete degradation of 6 mM terephthalate) pH-values in the experiment for determination of the maximum specific growth rate on terephthalate at different pH-values.

exp.	CO_2	$NaHCO_3$	$pH_{initial}$	pH_{final}
	%	mM	-	-
1	100	4.8	5.6	6.1
2	75	12	6.1	6.3
3	95	48	6.6	6.6
4	30	48	7.1	7.0
5	10	48	7.6	7.4

Maximum specific growth rates (μ^{max}) on terephthalate were determined with biomass from R37 at different temperature and pH-values. The experimental procedure was comparable to the specific activity measurements, except that lower initial biomass concentrations (0.3 to 0.6 g-VS·l^{-1}) and (in some cases) higher terephthalate concentrations were used. Besides the methane concentration in the headspace, terephthalate concentrations in the medium were measured in time.

In order to asses the pH-dependency of anaerobic terephthalate degradation, biomass from R37 was incubated with terephthalate at initial pH-values ranging from 5.6 to 7.6. To obtain pH-values in this range, different CO_2/$NaHCO_3$ ratio's were used as shown in Table 6.3. The final pH in the bottles was calculated after complete degradation of 6 mM terephthalate according to the following stoichiometry:

$$C_8H_4O_4^{2-} + 6.5H_2O \longrightarrow 3.75CH_4 + 2HCO_3^- + 2.25CO_2 \qquad (6.1)$$

All batch experiments were performed at least in duplicate.

Analyses. The concentration of aromatic acids was determined by high pressure liquid chromatography (HPLC). The methane concentration in the headspace of the serum bottles, the hydrogen concentration in the biogas and the concentration and composition volatile

fatty acids in the liquid were determined by gas chromatography (GC). A detailed description of these methods can be found in Chapter 2.

The concentration of total and volatile solids (TS and VS) in sludge samples was determined without filtration, after drying and incineration of the samples according to Standard Methods for Examination of Water and Wastewater [1]. Measured solids concentrations were corrected for the measured concentration terephthalate in the samples. Determination of the concentration of total and volatile solids in the effluent of the bioreactors was performed after filtration, using glass fibre filters (GF 52, Schleicher and Schuell, Dassel, Germany).

Calculations. The maximum specific activity (q^{max}) was defined as the maximum substrate conversion rate related to the initial biomass concentration. The maximum substrate conversion rate was calculated from measured methane concentrations in the headspace corrected for the stoichiometry of the conversion, using linear regression. If non-methanogenic substrates are applied, this method is only valid if no intermediates accumulate throughout the experimental period, as was checked by measurement of the accumulating concentration volatile fatty acids.

Analyses of the temperature dependency of the maximum specific terephthalate conversion rate (q_{TA}^{max}) was based on the Arrhenius equation for the temperature dependency of chemical reactions:

$$q_{TA}^{max}(T) = A \cdot e^{\frac{-E_a}{R \cdot T}} \tag{6.2}$$

where $q_{TA}^{max}(T)$ (mmol-TA·g-VS^{-1}·d^{-1}) is the maximum specific terephthalate degradation rate at the absolute temperature T (K), A is the frequency factor (same unit as $q_{TA}^{max}(T)$), E_a (kJ·mol^{-1}) is the activation factor, and R (kJ·mol^{-1}·K^{-1}) is the gas constant. This equation is only valid at temperatures lower than the optimum temperature and contrary to enzyme kinetics, the constants A and E_a have only limited physiological meaning when microbial cultures are used. Values for A and E_a can be estimated through linearization of $\ln\left(q_{TA}^{max}(T)\right)$ versus T^{-1} within a limited range of temperatures lower than the optimum temperature.

After assessment of the optimum temperature (T_{opt}), the specific terephthalate degradation rate at temperatures lower than the optimum temperature can be calculated from $q_{TA}^{max}(T_{opt})$ and the estimated value of E_a according to:

$$q_{TA}^{max}(T) = q_{TA}^{max}(T_{opt}) \cdot \exp\left(-E_a \cdot \frac{T_{opt} - T}{R \cdot T \cdot T_{opt}}\right) \quad (6.3)$$

For incorporation of the temperature dependency in Monod-based equations for microbial growth ($\mu_{TA}^{max}(T)$), an equivalent equation was used. It was assumed that the microbial yield is independent of the temperature and maintenance and/or decay are neglected. Exponential substrate (terephthalate) depletion and product (methane) formation curves can consequently be described using the following equations:

$$C_{TA}(t) = C_{TA}(0) + \frac{R_{TA0}(T)}{\mu_{TA}^{max}(T)} \cdot \left(1 - \exp\left(\mu_{TA}^{max}(T) \cdot t\right)\right) \quad (6.4)$$

$$C_{CH_4}(t) = C_{CH_4}(0) - f_{TACH_4} \cdot (1 - \eta_{XTA}) \cdot \frac{R_{TA0}(T)}{\mu_{TA}^{max}(T)} \cdot \left(1 - \exp\left(\mu_{TA}^{max}(T) \cdot t\right)\right) \quad (6.5)$$

where $C_{TA}(t)$ and $C_{CH4}(t)$ (mM) are the terephthalate and methane concentration as a function of time (t, day), $R_{TA0}(T)$ (mM·day^{-1}) is the initial volumetric terephthalate consumption rate as a function of the temperature (T), f_{TACH4} (mol-CH_4·mol-TA^{-1}) is the number of moles methane produced per mole terephthalate, and η_{XTA} (e-mol-X·e-mol-TA) is the electron-yield of biomass (X) on terephthalate. Through substitution of functions equivalent to equation 6.3 in equation 6.4 and 6.5, a relation describing the temperature dependency of $R_{TA0}(T)$ and $\mu_{TA}^{max}(T)$ is obtained. Herewith, exponential terephthalate consumption and methane production curves, measured at different temperatures can be described using the $R_{TA0}(T_{opt})$ and $\mu_{TA}^{max}(T_{opt})$ as only variables.

The net growth rate in the anaerobic hybrid bioreactors during the exponential increase in the terephthalate removal capacity (RC, mol·l^{-1}·day^{-1}) was calculated from the cumulative methane production with equation 6.5. Using this equation for bioreactor data the net growth rate minus biomass wash-out is calculated. Calculations were performed during time periods that the effluent concentrations terephthalate exceeded 2 mM in order to fulfil the assumption of zero-order substrate consumption.

Calculation of the biomass yield for growth of the mixed population on terephthalate (Y_{XTA}, g-VS·mol^{-1}) in the anaerobic bioreactors was based on the mass-balance for terephthalate degrading biomass in a completely mixed reactor with biomass retention:

$$\frac{dX_R}{dt} = Y_{XTA} \cdot q_{TA}^{max} \cdot \frac{TA}{K_{TA} + TA} \cdot X_R - \frac{Q_{inf}}{V_R} \cdot X_{eff} \quad (6.6)$$

where X_R and X_{eff} (g-VS·l^{-1}) are the concentrations volatile solids in the reactor and the effluent respectively, V_R is the volume of the reactor (l), Q_{inf} is the influent flow rate (l·day^{-1}), and Y_{XTA} is the biomass yield for growth on terephthalate (g-VS·mol^{-1}). If effluent concentrations of terephthalate (TA) exceed significantly the apparent half saturation concentration for terephthalate degradation (TA >> K_{TA}), we can substitute $q_{TA}^{max} \cdot TA/(K_{TA} + TA) \cdot X_R$ by the average volumetric removal capacity of the reactor (RC_{TA}, mol·l^{-1}·d^{-1}). In case of steady state conditions in the reactor the biomass yield for growth on terephthalate can consequently be calculated according to:

$$Y_{XTA} = \frac{Q_{inf} \cdot X_{eff}}{RC_{TA} \cdot V_R} \tag{6.7}$$

The average biomass retention time (SRT, day) in the bioreactors is calculated according to:

$$SRT = \frac{\frac{RC_{TA}}{q_{TA}^{max}} \cdot V_R}{X_{eff} \cdot Q_{inf}} \tag{6.8}$$

Calculation of the half saturation constant for acetate (K_{C2}, mM) is based on the mass balance of acetate over the reactor:

$$\frac{dC2}{dt} = \frac{Q_{inf}}{V_R} \cdot (C2_{inf} - C2) + q_{TA}^{max} \cdot \frac{TA}{K_{TA} + TA} \cdot X_R \cdot f_{TAC2} \cdot (1 - \eta_{X_{TA}TA}) - q_{C2}^{max} \cdot \frac{C2}{K_{C2} + C2} \cdot X_R \tag{6.9}$$

where $\eta_{X_{TA}TA}$ (e-mol-X_{TA}·e-mol-TA^{-1}) is the electron yield of the terephthalate fermenting biomass as defined in Chapter 3 and f_{TAC2} (mol-C2·mol-TA^{-1}) is the number of moles acetate formed per mol terephthalate according to the stoichiometry of terephthalate fermentation:

$$C_8H_4O_4^{2-} + 8H_2O \longrightarrow 3C_2H_3O_2^- + 3H^+ + 2HCO_3^- + 3H_2 \tag{6.10}$$

If (i) no acetate is present in the influent, (ii) the effluent concentration acetate is small compared to the concentration of acetate generated due to terephthalate fermentation, (iii) the concentration of terephthalate is high compared to the half saturation constant for terephthalate (K_{TA}) and (iv) using the steady state assumption ($dC2/dt = 0$), the half saturation constant for acetate can be calculated from measured maximum specific substrate conversion rates for terephthalate and acetate, and the average effluent concentration acetate:

$$\frac{C2}{K_{C2}+C2}=\frac{q_{TA}^{max}\cdot f_{TAC2}\cdot(1-\eta_{X_{TA}TA})}{q_{C2}^{max}} \qquad (6.11)$$

6.4 Results

Continuous experiments in UASB-type reactors (run 1). Three UASB reactors, operated at 30, 37 and 55 °C (R30, R37 and R55) were run for a period of 130 days. The initial operational parameters are shown in Table 6.1. Despite the inoculation of the reactors with sludge obtained from a full-scale reactor that actively degraded terephthalate, the assessed initial maximum specific terephthalate degrading activity was very low (see Table 6.1). Probably the long storage time preceding the inoculation negatively affected the terephthalate degrading activity.

During most of the experimental period, a slow increase of the terephthalate removal capacity was found in R30 and R37. During approximately the last month of operation, no further increase of the removal capacity was observed. The average operational parameters and treatment performance during this month are presented in Table 6.4. This table furthermore shows the biomass concentration in the reactors at the termination of the experimental period, and the assessed maximum specific terephthalate degrading activity of biomass from the reactors at the operational temperatures. Even though the maximum specific terephthalate degradation rate (q_{TA}^{max}) of the biomass had increased significantly throughout the experimental period, the terephthalate removal capacity of the systems remained low due to the low biomass concentrations in the reactors. No granulation of the biomass was observed. Since good growth could be obtained in batch experiments, biomass retention was identified to be the main factor limiting the terephthalate removal capacity of the UASB-reactors operated at 30 and 37 °C.

Table 6.4: Average values for the treatment performance of the UASB reactors operated at 30 and 37 °C during the last month of operation

par.(1)	unit	R30	R37
VLR	$mmol \cdot l^{-1} \cdot d^{-1}$	4.0	6.0
HRT	hr	30	20
TAinf	mM	5.0	5.0
RC	$mmol \cdot l^{-1} \cdot d^{-1}$	2.0	3.8
XR	$gVS \cdot l^{-1}$	5.3	4.6
q_{TA}^{max}	$mmol \cdot gVS^{-1} \cdot d^{-1}$	0.25 (0.01)	0.59 (0.09)

(1) RC: removal capacity, for other abbreviations see Table 6.1

The terephthalate removal capacities calculated from the methane production rate corresponded well (within 15 %) to the removal capacities calculated from the effluent terephthalate concentrations. This indicates that little if any accumulation of intermediates occurred, as was confirmed by very low effluent acetate concentrations and hydrogen partial pressures in the biogas throughout the experimental period (< 0.3 mM and < 4 Pa respectively).

The terephthalate removal capacity of the reactor operated at 55 °C remained below 0.5 $mmol \cdot l^{-1} \cdot d^{-1}$. Combined with the observation that only limited conversion (< 10 %) of terephthalate was found in batch experiments incubated at 55 °C, it was concluded that either the seed material did not contain thermophilic terephthalate degrading organisms, or that the operational conditions were not adequate for cultivation of these organisms. No further efforts were made to treat terephthalate under thermophilic conditions.

Temperature and pH-dependency of the terephthalate grown biomass. The maximum specific terephthalate degradation activities of sludge samples from the three reactors at 20, 30, 33, 37, 40, 43 and 55 °C were measured in batch experiments. The results, presented in Figure 6.2, show that within the temperature range of 20 to 40 °C, a reasonable description of the temperature dependency of the maximum specific terephthalate degrading activity of biomass from R30 and R37 is obtained with an apparent activation energy (E_a) of 55 $kJ \cdot mol^{-1}$. The specific terephthalate degrading activity of biomass from R30 is 43 % lower compared to biomass from R37. At 43 °C the terephthalate degradation rates were low and decreased in time.

In experiments conducted with biomass from R55 and incubated at 30 to 40 °C, we found accumulation of acetate in time. Highest acetate concentrations were observed in experiments performed at 37 °C and the accumulated concentration amounted approximately 8 mM after 10 days of incubation. Exponential methane formation was observed after approximately 6 days of incubation. In all the experiments performed at 55 °C only limited terephthalate conversion was observed (<0.5 mM).

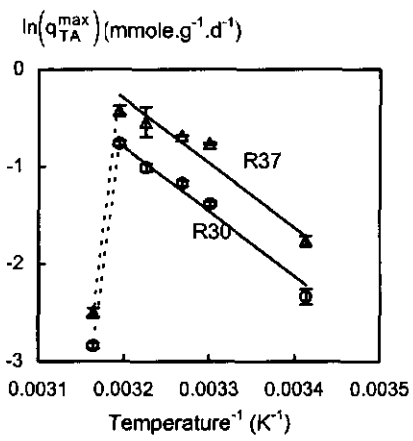


Figure 6.2: Temperature dependency of the maximum specific activity with terephthalate (q_{TA}^{max}) of biomass from the UASB-reactors R30 (O) and R37 (Δ). Solid drawn lines were calculated using an activation energy of 55 kJ·mole^{-1}.

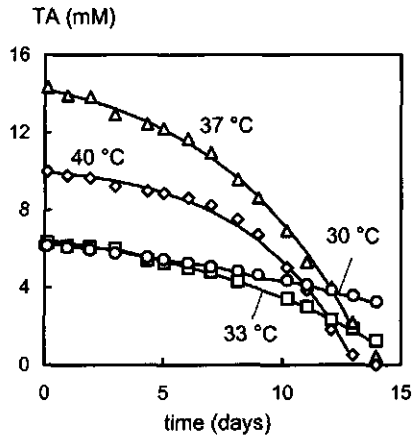


Figure 6.3: Terephthalate (TA) degradation by biomass from R37 at 30 (O), 33 (□), 37 (Δ) and 40 (◇) °C. Lines were calculated using an activation energy of 90 kJ·mole^{-1} and equal initial biomass concentrations, except for the experiment at 40 °C, where an initial biomass concentration 3.2 times smaller than in the other experiments had to be used in order to describe the measured data.

The temperature dependency of the specific growth rate of the terephthalate degrading biomass from R37 was investigated by incubation of a small amount of biomass (0.5 g-VS·l^{-1}) with terephthalate at 30, 33, 37, 40 and 43 °C (Figure 6.3). The exponential terephthalate depletion curves were analysed using Equation 6.4, and a satisfactory description of the curves measured at 30, 33 and 37 °C could be obtained using a value for E_a of 90.5 kJ·mol^{-1}. For description of the terephthalate depletion curve measured at 40 °C, an initial volumetric terephthalate degradation rate 3.2 times smaller as those used at 30-37 °C had to be applied. This suggests that a part of the terephthalate degrading activity in the inoculum was lost at 40 °C. The estimated value for μ_{TA}^{max} (T_{opt}) was 0.23 day^{-1} at 40 °C. At 43 °C less than 0.5 mM terephthalate was degraded.

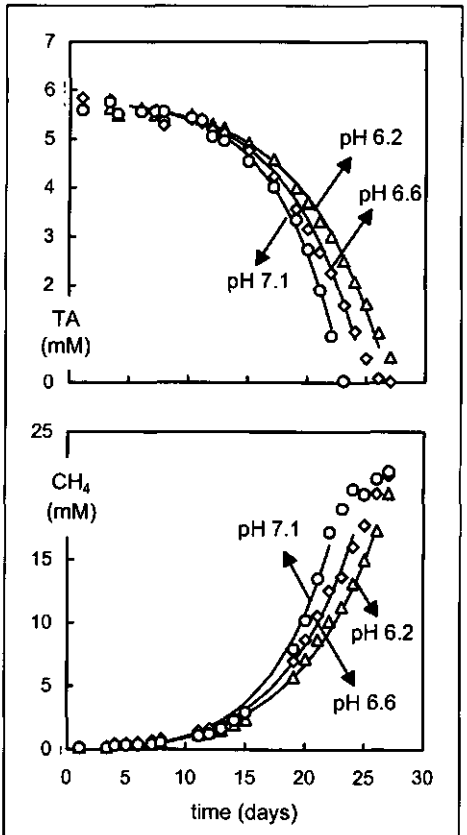


Figure 6.4: Terephthalate (TA, top graph) degradation and concomitant methane (CH_4, bottom graph) production by biomass from R37 at three different pH-values. Drawn lines were calculated using an equal initial volumetric conversion rate and the maximum specific growth rates shown in figure 5.

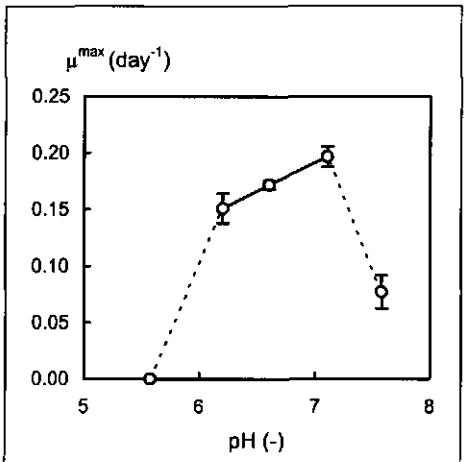


Figure 6.5: pH-dependency of the maximum specific growth (μ_{TA}^{max}) rate of biomass from R37 on terephthalate.

The pH-dependency of the growth rate of terephthalate degrading biomass from R37 was assessed in batch experiments at initial pH-values ranging from 5.6 to 7.6. pH-values within this range were obtained through application of different CO_2/$NaHCO_3$ ratios in the serum bottles (see Table 6.3). The calculated final pH-values after complete degradation of 6 mM terephthalate (Table 6.3) corresponded within 0.1 pH-unit to measured values.

The data presented in Figure 6.4 reveal that the differences observed in terephthalate degradation and methane production at initial pH-values between 6.1 and 7.1 are small. Both terephthalate degradation and methane production could be well described with Equation 6.4 and 6.5, using equal values for R_{TA0} and maximum specific growth rates ($\mu_{TA}^{max}(pH)$) as shown in Figure 6.5. The lag-phases prior to terephthalate degradation were approximately 5 days in all cases and were not included in the estimation of the maximum specific growth rate. The observation that both terephthalate degradation and methane production could be described with Equation 6.4 and

6.5 suggests that no intermediates accumulate throughout the experiment. This was confirmed by the measured low concentrations acetate (< 0.5 mM). In the experiment performed at an initial pH of 7.6, the lag-phase prior to terephthalate degradation was much longer (± 25 days) as compared to those observed at pH-values between 6.1 and 7.1. After this lag period, only a slow increase in the terephthalate consumption rate was observed, suggesting a low growth rate (Figure 6.5). At an initial pH of 5.6, accumulation of about 1.6 mM acetate was observed after 15 days of incubation. No further degradation of terephthalate or methane formation did occur. The resulting pH-dependency of the growth rate of the biomass from R37 is shown in Figure 6.5.

Table 6.5: Average values for the treatment performance of the hybrid reactors treating terephthalate as sole substrate during the last month of operation

par.[1]	unit	R1	R2	R3
VLR	$mmol \cdot l^{-1} \cdot d^{-1}$	51	61	84
HRT	hr	15	13	10
TAinf	mM	32	33	35
RCTA	$mmol \cdot l^{-1} \cdot d^{-1}$	44	51	71
RCCH4	$mmol \cdot l^{-1} \cdot d^{-1}$	42	52	68

[1] RC_{TA}: removal capacity based on measured effluent concentrations terephthalate; RC_{CH4}: terephthalate removal capacity based on measured methane production rates; for other abbreviations see Table 6.1.

Continuous experiments in hybrid reactors (run 2). In the second run of the continuous experiments, polyurethane (PUR) carrier material was introduced in the reactors in order to enhance biomass retention. The reactors were seeded with a mixture of biomass from R30 and R37 and they were operated at 37 °C. The initial operational parameters are shown in Table 6.1.

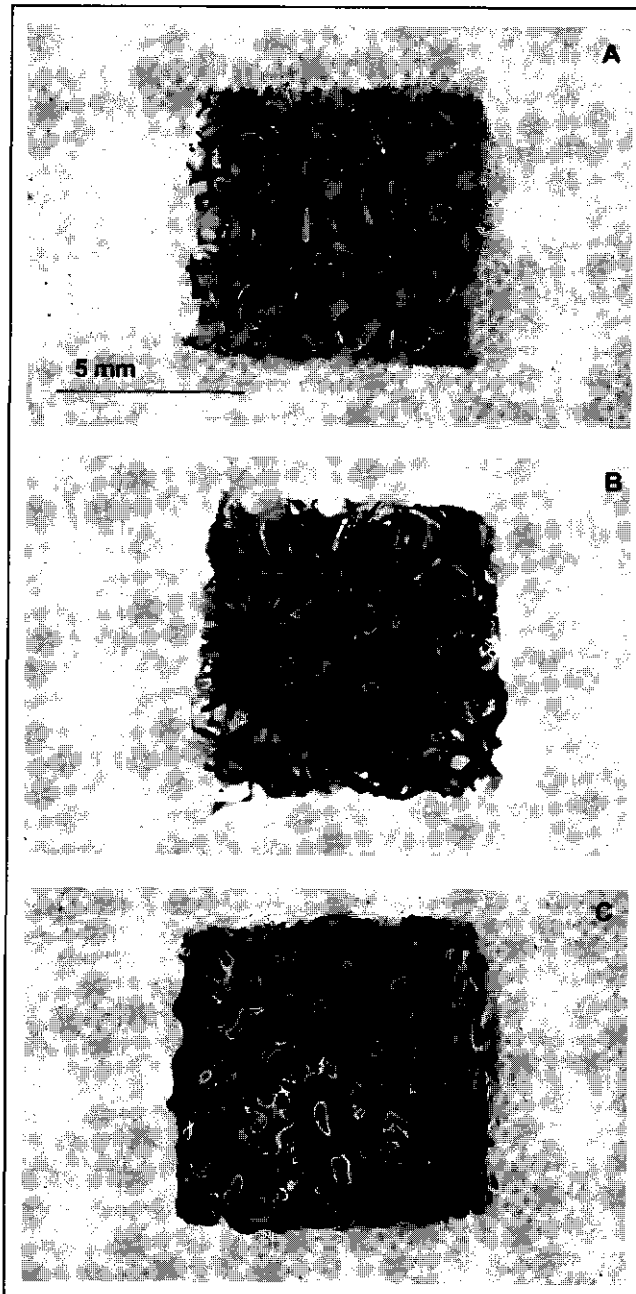


Figure 6.6: Biofilm formation on the carrier material from R3: carrier without biofilm (A), biofilm formation after 65 (B) and 110 (C) days of operation.

The averaged values for the imposed volumetric loading rate (VLR) and the assessed terephthalate removal capacity (RC) based on measured methane production rates throughout the experimental period of 211 (R1) or 234 (R2 and R3) days are shown in Figure 6.7. The treatment performance of the three reactors can be divided into three periods: (i) a lag period of approximately 50 days where only limited terephthalate removal is observed ($RC<1$ mmol·l^{-1}·day^{-1}), (ii) a period of exponential increase of the removal capacity of the reactors up to approximately day 115, and (iii) from this day until the end of the experiment the removal capacity of the reactors fluctuated (R1 and R2) or decreased slightly in time (R3). The final terephthalate removal rates of the reactors were much higher compared to the UASB-reactors of the first run, despite the shorter hydraulic retention times (Table 6.5). The terephthalate removal capacities as calculated from effluent concentrations

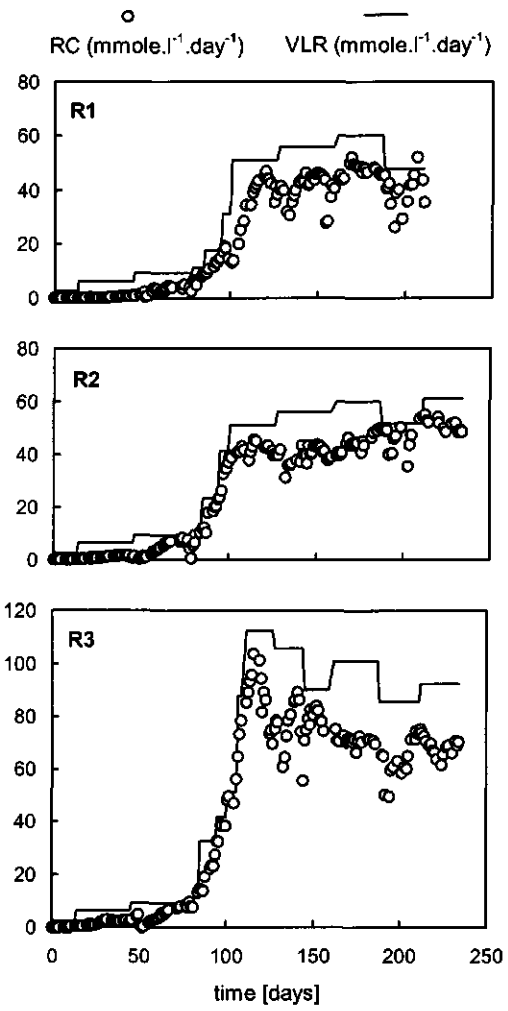


Figure 6.7: Volumetric removal capacity (RC) based on measured methane production rates and averaged volumetric loading rates (VLR) in R1 (top graph) to R3 (bottom graph).

terephthalate, corresponded well to the removal capacities calculated from the methane production rate. This suggests that terephthalate is completely converted into methane and inorganic carbon according to Equation 6.1, as was confirmed by the low effluent concentrations acetate and hydrogen partial pressures in the biogas throughout the experimental period (<0.5 mM and <5 Pa respectively).

During the period of exponential increase of the removal capacity of the reactors, biofilm formation on the carrier material clearly manifested (Figure 6.6). After approximately 110 days of continuous operation, biofilms in R3 occupied completely the void volume of the carrier particles. Due to the lower specific surface area and higher void volume of the carrier particles used in R1 and R2, these kept their open structure throughout the experimental period. During the exponential increase of the terephthalate removal rates, an increase in the biomass concentration in the bottom part of the reactors (beneath the carrier material) was observed. After approximately 110 days of operation the formation of oval shaped granules, ranging in size from 0.5 to 3 mm, was observed in the bottom part of the reactors. At the end of the experimental period, a dense layer of granular biomass was present in R2 and R3, occupying approximately 15-20 % of the volume beneath the three phase separator. In R1 less granular biomass was formed. In time the formation of channels

biomass concentrations indicate that biomass retention fluctuates around an average value which approximates steady state. A second assumption made during calculation of the reactor yield is that the microbial composition of biomass washing out from the reactor equals the biomass in the reactor. The correctness of this assumption was confirmed by the comparable maximum specific activities on terephthalate that were measured with biomass from the effluent as well as the sludge bed (Table 6.6).

Table 6.7: Estimated values of various parameters for description of the treatment performance of the anaerobic hybrid reactors. Parameter values obtained with a previously described terephthalate grown enrichment culture (enr.c.) are shown for comparison.

reactor	RC_{TA}	SRT	$\frac{SRT}{HRT}$	Y_{XTA}	K_{C2} [2]	$\frac{X_{Ferm}}{X_{AcM}}$ [3]	μ_{TA}^{net} [1]	
	$mmol \cdot l^{-1} \cdot d^{-1}$	day	-	$g \cdot mole^{-1}$	mM	$g \cdot g^{-1}$	day^{-1}	
R1	48	35	52	10.2	0.3	0.73	0.029	(0.062)
R2	53	42	63	10.0	0.5	0.54	0.024	(0.071)
R3	74	46	107	9.2	0.5	0.52	0.022	(0.062)
enr.c.	-	-	-	8.6	0.4	1.1	0.094	

[1] The net growth rate on terephthalate in R1 to R3 was calculated according to $\mu_{TA} = Y_{XTA} \cdot q_{TA}^{max}$. Values between brackets represent net growth rates as calculated during the exponential increase of the volumetric removal capacities in the reactors, neglecting biomass wash-out.

[2] The apparent half saturation constant (K_{C2}) for acetoclastic methanogenesis was calculated using Equation 6.11 and an average effluent concentration acetate of 0.3 mM.

[3] The concentration ratio of terephthalate fermenting organisms (X_{ferm}) versus acetoclastic methanogens (X_{AcM}) was calculated from measured maximum specific substrate conversion rates shown in Table 6.6, related to the values obtained with the enrichment culture: $q_{FermTA}^{max} = 23$ and $q_{AcM}^{max} = 98$ $mmol \cdot g^{-1} \cdot day^{-1}$.

Taken these considerations into account, the calculated SRT-values presented in Table 6.7 can be regarded as reasonable approximations of the average SRT. The SRT-values are comparable for the three reactors. The solid retention time related to the hydraulic retention (SRT/HRT) is considerably higher for R3, suggesting that the total surface of carrier material available for biofilm formation (R1/R2: 200 $m^2 \cdot m^{-3}$, R3: 375 m^2/m^{-3}) played a role in the efficiency of biomass retention. The terephthalate removal rates in R1 and R2 were highly comparable throughout the entire experimental period, suggesting that the differences in hydraulic regime imposed to these reactors hardly affected biomass retention.

Biomass growth in the anaerobic hybrid reactors. The measured maximum specific terephthalate degrading activity of biomass in the anaerobic hybrid reactors (average value is 2.5 mmol·g^{-1}·day^{-1}, Table 6.6) is much lower than the value of 10.9 mmol·g^{-1}·day^{-1} obtained with the previously described methanogenic enrichment culture (Chapter 3). Assuming that (i) the reactors were in steady state and (ii) the terephthalate concentration in the reactor exceeded considerably the apparent half velocity constant for terephthalate degradation (K_{TA}), the net growth rate in the reactors can be calculated from the biomass yield in the reactor and the measured maximum specific terephthalate conversion rate according to $\mu_{TA}^{net} = Y_{XTA} \cdot q_{TA}^{max}$. From the data presented in Table 6.7 it can be seen that the values for μ^{net} obtained using this method are lower than those calculated from the exponential increase of the terephthalate removal capacity during day 85-105, even though biomass wash-out was neglected using this latter method.

A possible reason for the lower net growth rates in the biofilm reactors during the latter period is the influence of mass transfer limitations, leading to substrate depletion in the biofilm. This may result in a negative growth rate (decay) in the centre of the biofilm. Combined with the positive growth rate in the biofilm near the bulk liquid, this may lead to lower values for the net growth rate calculated for the presumed steady state. However, the effluent terephthalate concentrations between day 150 and 200 (2 to 6 mM) were much higher than the K_{TA}-value of 0.1 mM which we estimated for R2 (Figure 6.8). This suggests that mass transfer limitations have been of minor importance in R2 and that no, or only limited substrate limitations occurred during the time period for calculation of the net growth rate based on the steady state assumption. Contrary to R1 and R2, biofilms in R3 occupied completely the void volume of the PUR-support particles, suggesting that mass transfer limitations may have played a more important role in this reactor. It furthermore is interesting to note that the decrease in the terephthalate removal capacity in R3 from day 120 (± 100 mmol·l^{-1}·day^{-1}) to 130 (± 70 mmol·l^{-1}·day^{-1}) occurred at the time that the PUR-particles became saturated with biomass (Figure 6.6). Presumably, the increase in the biofilm thickness may have contributed to the observed decrease in the capacity in this reactor.

Another aspect that may affect the net growth rate of the terephthalate degrading biomass in the bioreactors is the development of different concentration ratios of the individual species involved in the syntrophic degradation of terephthalate. As a result of different decay rates or different retention times in the reactor, the concentration ratio of the species in terephthalate degradation may vary. The estimated concentration ratio of terephthalate fermenting organisms versus acetoclastic methanogens, and the apparent half saturation constant for acetoclastic methanogenesis (K_{C2}) are shown in Table 6.7 for biomass from the anaerobic

hybrid reactors as well the enrichment culture. It is evident from the data in this table that even though the calculated values for K_{C2} for the hybrid reactors and the enrichment culture are comparable, the relative concentration of acetoclastic methanogens is higher compared to terephthalate fermenting organisms in the hybrid reactors. This suggests that either selective wash-out of the terephthalate fermenting culture occurs, or bacterial decay plays a more important role in the growth of the terephthalate fermenting culture compared to the acetoclastic methanogens.

In summary we suggest that either bacterial decay or selective washout of the terephthalate fermenting culture played an important role in the capacity limitation of the reactors. Mass transfer limitations due to the formation of thick biofilms appeared to play only a minor role.

Influence of the temperature and the pH on anaerobic terephthalate degradation. The temperature dependency of the specific terephthalate degrading activity of biomass from R30 and R37 in the temperature range of 20 to 40 °C could well be described with an apparent activation energy (E_a) of 55 kJ·mol^{-1}, corresponding to a Q_{10} of 2.0. For description of the temperature dependency of the specific growth rate of the terephthalate degrading biomass, a significantly higher value for E_a of 90 kJ·mol^{-1} (Q_{10}=3.2) was estimated. No clear reason could be identified for the difference in the estimated E_a-values.

Both values for E_a suggest that the anaerobic terephthalate degradation is kinetically controlled. If transport processes are rate limiting, a lower value for E_a ($\approx$ 20 kJ·mol^{-1}) is expected to be found. Based on an extensive literature research, Henze and Harremoës suggested a similar average value for Q_{10} of 2.7 for anaerobic conversions within the mesophilic temperature range [11].

Terephthalate degradation proceeded well within the pH-range of 6.1 - 7.1, with preference for a pH of 7.1. At an initial pH of 5.6 accumulation of acetate occurred, which may have contributed to the absence of growth at this pH. No acetate accumulation was observed at higher pH-values, suggesting that terephthalate degradation strongly depends on the rapid removal of acetate. The terephthalate degrading biomass was found to have a low tolerance for pH-values exceeding 7.5.

The estimated maximum specific growth rates of the sludge sampled from R37, as determined during the temperature- and the pH-experiment at 37 °C and pH 7.1 (respectively 0.16 and 0.19 day^{-1}), are significantly higher than the previously determined value with the terephthalate grown enrichment culture (0.094 day^{-1}, Chapter 3). Various reasons may have contributed to this observation: (i) besides "true growth", reactivation of the culture may have contributed to the observed increase in the terephthalate degradation rate, resulting in

an overestimation of the growth rate in the experiments with biomass from the reactors, (ii) the biomass cultivated in the reactors may contain a higher concentration of methanogens, relative to the concentration of terephthalate fermenting bacteria, and this may increase the overall growth rate of the mixed culture when grown on terephthalate [26], and (iii) both cultures may have a different microbiological composition with different kinetic properties.

UASB versus hybrid reactors. Both UASB and hybrid reactors have successfully been applied for the mesophilic treatment of a large variety of wastewaters, including wastewaters containing aromatic compounds [6, 10, 14, 16-18]. In both reactor types the formation and retention of methanogenic granular sludge has been observed, resulting in high biomass concentrations and high volumetric substrate removal rates. Considering that normally both reactor-types are dimensioned and operated in a similar way, the top part of the reactors - i.e. a combined internal three phase separator and settler for UASB-reactors, and a stationary packed bed in hybrid reactors - is the main difference between both reactor-types. Consequently, any differences in treatment performance between both reactor-types primarily can be attributed to differences in biomass retention and the formation and retention of granular biomass. The hybrid reactors used in our investigations differ from those described in literature, because they were equipped with an internal settler besides a layer of polyurethane (PUR) carrier material beneath the three phase separator.

The terephthalate removal rates found in the anaerobic hybrid reactors exceeded the rates in UASB-reactors by a factor of more than 10. The high removal rates obtained in the hybrid reactors can primarily be attributed to the high solid retention times (SRT) in these reactors (see Table 6.7). From the data shown in Table 6.4, a much lower SRT-value of 11 days was roughly estimated for the UASB-reactors operated at 30 and 37 °C, despite the longer hydraulic retention times in these reactors.

Formation of granular biomass was only observed in the hybrid reactors, i.e. following the formation of dense biofilms on the PUR support material. Presumably, the granulation in the hybrid reactors can be attributed to the accumulation of biofilm particles detached from the carrier material. The settled biofilm particles may serve as nuclei for granule formation in the lower part of the reactor. Probably due to the absence of such nuclei in the suspended inoculum of the UASB-reactors, no granulation was observed in these reactors. Enhanced granulation and biomass retention in hybrid versus UASB-reactors seeded with suspended biomass has previously been observed by Liangming et al. [19] during their work on the anaerobic treatability of artificial PTA-wastewater. Similar observations were made by Fiebig and Dellweg [7] in their study on acetate degradation in a UASB and a hybrid reactor. The high removal rates resulting from the effective colonisation of the PUR reticulated foam

particles furthermore confirms the suitability of this type of carrier as a microbial colonisation matrix [3, 9, 13, 25, 27].

Practical implications. The main practical achievement of the work described in this paper is that high rate degradation of terephthalate (> 40 $mmol \cdot l^{-1} \cdot day^{-1}$ or 10 $g\text{-}COD \cdot l^{-1} \cdot day^{-1}$) can be accomplished in anaerobic bioreactors if (i) a high sludge age can be obtained, (ii) the temperature is close to its optimum value of 37 °C and (iii) pH-values are between 6.1 and 7.1 with preference for a pH of 7 (and acetate concentrations are low). The maximum specific terephthalate degrading activity of biomass grown in the anaerobic hybrid reactors (± 2.5 $mmol \cdot g^{-1} \cdot day^{-1}$) is much higher than previously reported values of 0.1 to 0.3 $mmol \cdot g^{-1} \cdot day^{-1}$ obtained with biomass from terephthalate fed anaerobic bioreactors [2, 5, 15].

Previously we described that the easily degradable substrates in PTA-wastewater (acetate and benzoate) strongly inhibit the degradation of terephthalate (Chapter 4, [5, 15]). Based on these results we suggested to use a two-stage reactor system for anaerobic pre-treatment of PTA-wastewater. The results described in this chapter provide extra support for this hypothesis. In the first stage of a two-stage system, acetate and benzoate can be degraded at high rates [8, 18], while in the second stage high-rate degradation of terephthalate can be obtained if the previously defined boundary conditions are fulfilled. It is postulated that using this approach significantly higher volumetric capacities (> 15 $g\text{-}COD \cdot l^{-1} \cdot day^{-1}$) can be obtained as those described in literature (0.5 - 4 $g\text{-}COD \cdot l^{-1} \cdot day^{-1}$ [2, 4, 15, 22, 24, 28]). The application of a staged reactor concept for the anaerobic treatment of a mixture of acetate, benzoate and terephthalate will be described in Chapter 7.

References

1. **APHA.** 1985. Standard methods for the examination of water and wastewater, 16th ed. American Public Health Association, Washington D.C., USA.
2. **Cheng, S. S., C. Y. Ho, and J. H. Wu.** 1997. Pilot study of UASB process treating PTA manufacturing wastewater. Presented at the 8th Int. Conf. on Anaerobic Digestion, Sendai, Japan, May 25-29, 1997.
3. **Derycke, D., and W. Verstraete.** 1986. Anaerobic treatment of domestice wastewater in a lab and a pilot scale polyurethane carrier reactor. Presented at the Water treatment conference, Anaerobic treatment, a grown-up technology, Amsterdam, The Netherlands.
4. **Duffel, J. v.** 1993. Anaerobe behandeling van organische zuren. Presented at the National Conference on Anaerobic Treatment of Complex Wastewaters, Breda, The Netherlands.
5. **Fajardo, C., J. P. Guyot, H. Macarie, and O. Monroy.** 1997. Inhibition of anaerobic digestion by terephthalic acid and its aromatic by products. Water Sci. Technol. **36**:83-90.

6. **Fang, H. H. P., T. Chen, Y. Y. Li, and H. K. Chui.** 1996. Degradation of phenol in wastewater in an upflow sludge blanket reactor. Wat. Res. **30:**1353-1360.

7. **Fiebig, R., and H. Dellweg.** 1985. Comparison between the process performance of an UASB-reactor and an UASB-fixed film combination with an acetic acid enrichment culture. Biotechnol. Let. **7:**487-492.

8. **Frankin, R. J., J. van Gils, and R. J. F. Wermeling.** 1994. Full-scale anaerobic treatment of Shell wastewater containing benzoate with the Biothane UASB process. Presented at the 7th IAWQ Int. Symp. Anaerobic Digestion, Cape Town, South Africa.

9. **Fynn, G. H., and T. N. Whitmore.** 1982. Colonisation of polyurethane reticulated foam biomass support particles by methanogen species. Biotechnol. Let. **4:**577-582.

10. **Guiot, S. R., and L. van den Berg.** 1985. Performance of an upflow anaerobic reactor combining a sludge blanket and a filter treatinf sugar waste. Biotechnol. Bioeng. **27:**800-806.

11. **Henze, M., and P. Harremoes.** 1983. Anaerobic treatment of wastewaters in fixed film reactors - a literature review. Wat. Sci. Technol. **15:**1-101.

12. **Huser, B. A., K. Wuhrmann, and A. B. J. Zehnder.** 1980. *Methanothrix soehngenii* gen. nov. sp. nov., a new acetotrophic non hydrogen oxidizing methane bacterium. Arch. Microbiol. **132:**1-9.

13. **Huysman, P., P. v. Meenen, P. v. Assche, and W. Verstraete.** 1983. Factors affecting the colonization of non porous and porous packing materials in model upflow methane reactors. Biotechnol. Let. **5:**643-648.

14. **Kennedy, K. J., S. S. Gorur, C. A. Elliott, E. Andras, and S. R. Guiot.** 1989. Media effects on performance of anaerobic hybrid reactors. Wat. Res. **23:**1397-1405.

15. **Kleerebezem, R., J. Mortier, L. W. Hulshoff Pol, and G. Lettinga.** 1997. Anaerobic pre-treatment of petrochemical effluents: terephthalic acid wastewater. Wat. Sci. Tech. **36:**237-248.

16. **Lettinga, G.** 1995. Anaerobic digestion and wastewater treatment systems. Antonie Leeuwenhoek. **67:**3-28.

17. **Lettinga, G., L. W. Hulshoff Pol, I. W. Koster, W. M. Wiegant, W. de Zeeuw, A. Rinzema, P. C. Grin, R. E. Roersma, and S. W. Hobma.** 1984. High-rate anaerobic wastewater treatment using the UASB reactor under a wide range of temperature conditions. Biotechnol. Gen. Eng. Rev. **2:**253-283.

18. **Li, Y. Y., H. H. P. Fang, T. Chen, and H. K. Chui.** 1995. UASB treatment of wastewater containing concentrated benzoate. J. Environ. Eng. **121:**748-751.

19. **Liangming, X., C. Yuxiang, and Z. Xiangdong.** 1991. The anaerobic biological treatment of high strength petrochemical wastewater by a hybrid reactor. Presented at the International Conference on Petroleum Refining and Petrochemical Processing, Beijing, China, September 11-15.

20. **Macarie, H., and J. P. Guyot.** 1992. Inhibition of the methanogenic fermentation of p-toluic acid (4-methylbenzoic acid) by acetate. Appl. Microbiol. Biotechnol. **38:**398-402.

21. **Macarie, H., and J. P. Guyot.** 1995. Use of ferrous sulphate to reduce the redox potential and allow the start-up of UASB-reactors treating slowly biodegradable compounds: application to a wastewater containing 4-methylbenzoic acid. Environ. Tech. **16:**1185.

22. **Macarie, H., A. Noyola, and J. P. Guyot.** 1992. Anaerobic treatment of a petrochemical wastewater from a terephtalic acid plant. Water Sci. Technol. **25:**223-235.

23. **Neilson, A. H., A. S. Allard, P. A. Hynning, and M. Remberger.** 1988. Transformations of halogenated aromatic aldehydes by metabolically stable anaerobic enrichment cultures. Appl.Environ.Microbiol. **14:**2226-36.

24. **Pereboom, J. H. F., d. G. Man, and I. T. Su.** 1994. Start-up of full scale UASB-reactor for the treatment of terephtalic acid waste water. Presented at the 7th International Symposium on Anaerobic Digestion, Cape Town, South Africa.

25. **Poels, J., J. Van Assche, and W. Verstraete.** 1984. High rate anaerobic digestion of piggery manure with polyurethane sponges as support material. Biotechnol. Letters. **6:**747-752.

26. **Powell, G. E.** 1984. Equalisation of specific growth rates for syntrophic associations in batch cultures. J. Chem. Tech. Biotechnol. **34B:**97-100.

27. **Sharma, S., C. Ramakrishna, J. D. Desai, and N. M. Bhatt.** 1994. Anaerobic biodegradation of a petrochemical wastewater using biomass support particles. Appl. Microbiol. Biotechnol. **40:**768-771.

28. **Young, J. C.** 1997. Anaerobic treatability of phthalic acid production wastewaters. Presented at the 8th International Conf. on Anaerobic Digestion, Sendai, Japan, May 25-29, 1997.

Wastewater Treatment II: Two-stage Reactor

Abstract

The degradation of a terephthalate, benzoate and acetate mixture was studied in two-stage UASB-reactors. It was found that pre-removal of acetate and benzoate in a two-stage reactor concept enabled high-rate treatment of PTA-wastewater after a relatively short start-up period.

Contents

7.1 Summary

The feasibility of high-rate anaerobic pre-treatment of wastewater generated in the production of purified terephthalic acid (PTA) was studied in a lab-scale two-stage upflow anaerobic sludge blanket (UASB) reactor system. The artificial influent of the system contained the main organic substrates of PTA-wastewater: acetate, benzoate and terephthalate. Three reactors, operated in parallel, were used for the second stage. The reactors were seeded with a suspended terephthalate degrading culture, with and without additional methanogenic granular sludge (two different types). The first stage UASB-reactor was seeded with methanogenic granular sludge. All reactors were operated at 37 °C and pH 7.0.

During the first 300 days of operation a clear distinction between the biomass grown in both reactor stages was obtained. In the first stage, acetate and benzoate were degraded at a COD-removal efficiency of 95 % and a volumetric loading rate of 40 g-COD·l^{-1}·day^{-1} within 25 days of operation. No degradation of terephthalate was obtained in the first stage during the first 300 days of operation, despite operation of the reactor at a decreased volumetric loading rate with acetate and benzoate of 9 g-COD·l^{-1}·day^{-1} from day 150. Batch incubation of biomass from the reactor with terephthalate showed that the lag-phase prior to terephthalate degradation remained largely unchanged, indicating that no growth of terephthalate degrading biomass occurred. From day 300 onwards, however, terephthalate degradation was observed in the first stage, and the biomass in this reactor could successfully be enriched with terephthalate degrading biomass, resulting in terephthalate removal capacities of approximately 15 g-COD·l^{-1}·day^{-1}. Even though no clear reason could be identified why (suddenly) terephthalate degradation was obtained after such a long period of operation, it is suggested that the solid retention time as well the prevailing reactor concentrations acetate and benzoate may have played an important role. From the first day of operation, terephthalate was degraded in the second stage reactors. In presence of methanogenic granular biomass, high terephthalate removal capacities were obtained (15 g-COD·l^{-1}·day^{-1}) within approximately 125 days of operation.

From the results obtained it is concluded that terephthalate degradation is the bottleneck during anaerobic treatment of PTA-wastewater. Pre-removal of acetate and benzoate in staged bioreactor reduces the length of the lag-period prior to terephthalate degradation in latter stages, and enables high rate treatment of PTA-wastewater[§].

[§] A modified version of this chapter has been submitted for publication.

7.2 Introduction

Considering the composition of PTA-wastewater, a distinction can be made between compounds that are anaerobically readily degradable (acetate and benzoate) and compounds that are slowly degradable (terephthalate and *para*-toluate). In a preliminary study we demonstrated that during anaerobic treatment of a mixture of acetate, benzoate and terephthalate in lab-scale UASB-reactors, degradation of terephthalate was strongly rate limiting [8]. Only in absence of acetate and benzoate a substantial terephthalate degradation was achieved. The troublesome degradation of terephthalate could largely be attributed to (i) the combined effect of inhibition by acetate and benzoate and (ii) the slow growth rate of terephthalate degrading biomass at the applied operational temperature of 30 °C. These results were confirmed with a methanogenic enrichment culture grown on terephthalate (Chapter 3 and 4). The results obtained with this culture furthermore showed that the activity of the culture was almost completely lost by imposing feedless periods of only a few hours, as well as through co-incubation with high concentrations of acetate or benzoate. Still, by improving biomass retention through introduction of polyurethane carrier material and by working at the optimised temperature of 37 °C, high volumetric terephthalate removal rates (10-17 g-COD·l^{-1}·day^{-1}) could be obtained in anaerobic hybrid reactors seeded with suspended methanogenic biomass and fed with terephthalate as sole carbon and energy source (Chapter 6).

Para-toluate is poorly degradable in methanogenic environments (Chapter 2, [7]) and consequently its removal in anaerobic bioreactors proceeds only at very low rates [10, 11]. Therefore *para*-toluate normally needs to be removed in an aerobic post-treatment step. However, since *para*-toluate generally contributes to only 5 to 15 % of the COD-load, high volumetric removal capacities and treatment efficiencies can be obtained during anaerobic pre-treatment, even when *para*-toluate is not degraded. The aromatic PTA-wastewater constituents benzoate, terephthalate and *para*-toluate were furthermore shown to be hardly toxic to acetoclastic and hydrogenotrophic methanogens [5, 8].

In order to avoid inhibition of terephthalate degradation by acetate and/or benzoate, we previously suggested a two-stage reactor concept, enabling the spatial separation of acetate and benzoate removal in a first stage, and degradation of terephthalate (and *para*-toluate) in a second stage (Chapter 4, [8]). Staged reactor systems can be obtained through application of anaerobic bioreactors in series, or through application of reactor-systems in which plug-flow is enhanced. Several concepts of integrated staged anaerobic bioreactors have been described in literature [2, 6, 9, 12, 13]. In this chapter the results obtained with a two-stage UASB-reactor concept treating a mixture of acetate, benzoate and terephthalate will be described,

together with the characteristics of the biomass cultivated in both stages. Based on the results obtained, the practical applicability of a high-rate two-stage reactor concept for pre-treatment of PTA-wastewater will be discussed.

7.3 Materials and Methods

Continuous experiments. The first stage of the two-stage reactor concept consisted of a UASB-type reactor with a total working volume of 3.85 l. (inner diameter main body: 80 mm, sedimentation zone: 120 mm). The reactor, made of glass, was equipped with an internal three phase separator as shown in the flow sheet presented in Figure 7.1. Biogas produced in the first stage was (i) scrubbed with a 20 % sodium hydroxide solution for carbon dioxide removal, (ii) led through a column packed with soda lime pallets with indicator for water vapour removal and (iii) a wet gas meter (Meterfabriek Dordrecht, Dordrecht, The Netherlands) for quantification of the methane production. The temperature of the reactor was controlled at 37 °C by a thermostat-bath-circulator (Haake D8, Germany) connected to the double wall of the reactor.

The substrate and a large fraction of the effluent of the first stage were mixed in a mixing vessel and pumped into the reactor at flow rate of 15 l·hour^{-1} to maintain a liquid upflow velocity of 3 m·hour^{-1}. The pH in the mixing vessel was maintained at 7.0 ± 0.1 through dosage of sodium hydroxide (50 mM). Biogas produced in the first stage was led over the mixing vessel in order to obtain a comparable carbon dioxide partial pressure in the reactor and the mixing vessel. The substrate- (terephthalate, benzoate and acetic acid) and nutrient-solutions were pumped to the mixing vessel from separate containers. Disodium terephthalate was prepared in a continuously agitated 100 l. container through dissolution of the desired amount of terephthalic acid in a sodium hydroxide solution. Nutrients, acetic acid and sodium benzoate were fed to the mixing vessel from 10 l. containers. The composition of the nutrient solution applied has been described in Chapter 2. Acetic acid was dosed as an acid to enable pH-control solely by dosing sodium hydroxide and to minimise the sodium concentration of the medium.

The effluent of the first stage was collected in an external settler to for sedimentation of washed-out biomass and to avoid introduction of biomass from the first stage into the second stage. Accumulated biomass washed out from the first stage was removed from the settler at regular intervals.

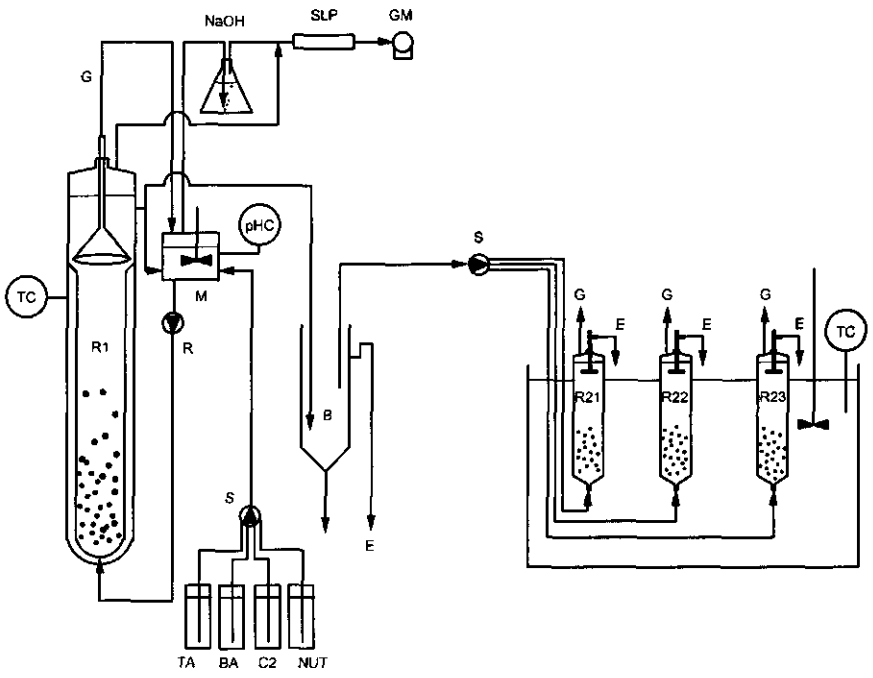


Figure 7.1: Flow sheet of the experimental set-up applied in the continuous experiments. R1: recirculated UASB-reactor (first stage); R21, R22 and R23: parallel operated UASB-reactors (second stage); M: mixing vessel; S: multichannel pump; R: recirculation pump; B: sedimentation reactor; G: biogas; E: effluent; NaOH: 20 % sodium hydroxide solution; SLP: column filled with soda lime pallets with colour indicator; TC: temperature control; pHC: pH-control; GM: Gas Meter; TA, BA, C2 and NUT: containers with stock-solutions disodium terephthalate, sodium benzoate, acetic acid and nutrients respectively.

The second stage of the two-stage reactor system consisted of three identical 0.95 l. UASB-reactors operated in parallel. The reactors were made of glass and had a inner diameter: 60 mm. The reactors were equipped with a simple three phase separator consisting of a reversed T-connection piece. Biogas produced in these reactors was scrubbed with a 20 % sodium hydroxide solution and quantified by liquid displacement using Mariot bottles. The reactors were placed in a temperature controlled (37 °C) water bath. Substrate was fed to the reactors from the settler using a multichannel peristaltic pump (Gilson-Minipuls 2, Villiers-Le-Bel, France).

Start-up and operational procedures. The first stage of the two-stage reactor (R1) was inoculated with methanogenic granular sludge from a full scale Internal Circulation (IC)

reactor [15] treating potato starch processing wastewater. The acetoclastic methanogenic activity of the seed sludge was 0.72 g-COD·g-VS^{-1}.d^{-1} at 37 °C and pH 7. The length of the lag-phases required to degrade 2 mM benzoate and terephthalate in batch experiments amounted approximately 9 and 61 days respectively (Chapter 2). Initially, the reactor was operated with acetic acid and benzoate as sole substrates in COD-equivalent amounts at a loading rate (VLR) of 4.0 g-COD·l^{-1}·day^{-1} and a hydraulic retention time (HRT) of 7 hours. In time the VLR of the reactor was increased by increasing the concentration acetic acid and benzoate in the influent. From day 40 day of operation, terephthalate was added to the influent, initially at a concentration of 0.60 g-COD·l^{-1}. In time the concentration of terephthalate in the influent was increased stepwise to 2.5 g-COD·l^{-1} to study whether the increased concentration terephthalate affected benzoate and acetate conversion in the reactor.

After 67 days of operation of R1, the three reactors of the second stage (R21, R22 and R23) were started up. All three reactors were inoculated with 1.5 g-VS·l^{-1} crushed biomass from a lab-scale anaerobic hybrid reactor treating terephthalate as sole carbon and energy source (Chapter 6). The initial specific terephthalate degrading activity of this biomass amounted to 0.48 g-COD·g-VS^{-1}·day^{-1} (2.0 mmol·gVS^{-1}·day^{-1}). In order to assess whether granular sludge could function as a carrier material for the terephthalate degrading suspended culture, granular biomass was added to R21 and R22. The biomass added to R21 was the original seed material used (25 gVS·l^{-1}) for inoculation of R1, and granular biomass removed from R1 (42 gVS·l^{-1}) was used as additional seed material for R22.

Batch experiments. Batch experiments were used to determine the specific activity of the biomass cultivated in the reactors with acetate, benzoate or terephthalate. The experiments were performed at 37 °C in 320 ml serum bottles using the general procedure described elsewhere (Chapter 6). The length of the lag-period prior to terephthalate degradation for biomass from R1 was determined in a similar manner as described in Chapter 2.

Analyses. The concentrations of benzoate and terephthalate were determined by high pressure liquid chromatography (HPLC). The methane concentration in the headspace of the serum bottles used in the batch experiments, the hydrogen concentration in the biogas and the concentration and composition of volatile fatty acids in the liquid were determined by gas chromatography (GC). A detailed description of these methods can be found in Chapter 2.

The concentration of total and volatile solids (TS and VS) in sludge samples was determined without filtration, after drying and incineration of the samples according to Standard Methods for Examination of Water and Wastewater [1]. Granular sludge samples were

sieved (mesh 0.5 mm) prior to determination of the concentration TS and VS. Measured concentrations were corrected for the measured substrate concentration in the samples.

Calculations. The maximum specific activity determined in the batch experiments was calculated using the method described in Chapter 6. The specific growth rate of biomass unadapted to terephthalate was determined from the exponential methane production curve as described in Chapter 3. The length of the lag phase required to obtain terephthalate degradation was defined as the time needed to obtain 1 mM terephthalate degradation, for easy comparison with previously determined values (Chapter 2).

7.4 Results

The anaerobic breakdown of PTA-wastewater constituents (acetate, benzoate and terephthalate) was studied in a two-stage bioreactor concept. Initially, only the first stage (R1) was started up with acetate and benzoate as organic substrates in COD-equivalent concentrations. Even though the biomass used as inoculum for R1 had not been grown on benzoate, the capacity of the reactor to degrade benzoate increased rapidly. Within 25 days of operation R1 was able to degrade the acetate-benzoate mixture at a volumetric loading rate (VLR) of 40 g-COD·l^{-1}·day^{-1} and a hydraulic retention time of 7 hours with a COD-removal efficiency exceeding 95 % (data not shown).

Following the initial start-up procedure, the operation of R1 can be divided into three time-periods (see Table 7.1):

Period I, Reactor R1 (day 25-150): The main objectives during this period were (i) to asses if terephthalate degrading capacity could be obtained in R1 at high VLR-values for benzoate and acetate, and (ii) to assess if increasing terephthalate concentrations in the influent would inhibit acetate and/or benzoate removal.

From day 40 onwards terephthalate was supplied to the reactor at an initial concentration of 0.6 g-COD·l^{-1}. Until day 60 the influent terephthalate concentration was increased stepwise to a concentration of 2.5 g-COD·l^{-1}. It was found that terephthalate did not affect the degradation of either benzoate or acetate and showed an inert behaviour in the reactor.

On day 70 of operation 42 g volatile solids were removed from R1 (reducing the volatile solids concentration in R1 from 40.0 to 28.9 g-VS·l^{-1}) for inoculation of one of the reactors of the second stage (R22). To avoid accumulation of acetate and/or benzoate in the effluent of the first stage, the VLR with benzoate and acetate was temporarily lowered to 30 g-COD·l^{-1}·day^{-1}. Within one week, however, the loading rate could be resumed to 46 g-COD·l^{-1}·day^{-1} without affecting the treatment efficiency for acetate and benzoate.

Table 7.1: Averaged values for the operational parameters of the first stage (R1) of the two-stage anaerobic system.

		time period		
parameter[1]	unit	I	II	III
day number	-	25-150	150-330	330-398
HRT	hour	4.7-5.3	2.9-5.2	2.2-2.9
VLR_{BA}	g-COD·l^{-1}·d^{-1}	23	4.5	0-52
VLR_{C2}	g-COD·l^{-1}·d^{-1}	23	4.5	0-60
VLR_{TA}	g-COD·l^{-1}·d^{-1}	2.5-12	12-22	25-40
η_{BA}	%	88-100	98-100	55-100
η_{C2}	%	95-100	86-100	98-100
RC_{TA}	g-COD·l^{-1}·d^{-1}	0	0-15	5-22
day: biomass	g-VS·l^{-1}	0: 23.0 137: 36.7	230: 26.2	398: 54.5

[1] HRT: hydraulic retention time, VLR: volumetric loading rate, RC: removal capacity, η: COD-removal efficiency, BA: benzoate, C2: acetate, TA: terephthalate.

During the last 20 days of period I, the treatment efficiency of R1 for benzoate declined slightly, resulting in an increase in the effluent concentration benzoate from 100-200 mg-COD·l^{-1} to 500-800 mg-COD·l^{-1}. Besides benzoate, small amounts (<100 mg-COD·l^{-1}) of carboxycyclohexane accumulated in the effluent, suggesting that the methanogenic consumption of intermediately formed reducing equivalents was inhibited. Since no identifiable modification in the operation was imposed to the reactor, a distinct reason for the decrease in the treatment efficiency for benzoate could not be identified.

Throughout period I, the effluent concentrations terephthalate were not different from the influent concentrations, suggesting that no adsorption or biodegradation of terephthalate occurred. The absence of net-growth of terephthalate degrading biomass in the sludge from R1 was confirmed by results from batch experiments incubated with terephthalate as sole carbon and energy source (Table 7.2). Even though the length of the lag phase required to degrade the 1 mM terephthalate decreased slightly compared the inoculum of the reactor (30 to 40 days versus 61 days in the inoculum (Chapter 2)), it is evident that no significant amounts of terephthalate degrading organisms had accumulated in R1 throughout period I.

Table 7.2: Characteristics of biomass from R1 sampled after 70, 137, 194 and 234 days of operation

		day nr.			
parameter[1]	unit	70	137	194	234
q_{C2}^{max}	g-COD·gVS^{-1}·d^{-1}	1.33	0.95	1.00	0.83
q_{BA}^{max}	g-COD·gVS^{-1}·d^{-1}	0.79	0.60	0.78	0.50
lag_{TA}	day	42	30	40	51
μ_{TA}	day^{-1}	0.11	0.12	0.10	0.13

[1] q_{C2}^{max} and q_{BA}^{max}: maximum specific acetate and benzoate degradation rate respectively; lag-TA: lag period required to obtain 50 % terephthalate degradation; μ_{TA}: estimated growth rate on terephthalate.

Period II, reactor R1 (day 150-330). During the second period the VLR with acetate and benzoate was lowered from 46 to 9 g-COD·l^{-1}·day^{-1}, to assess if terephthalate degradation could be achieved at this lower loading rate. Due to the decrease in the loading rate, effluent concentrations acetate and benzoate were lower compared to period I (0-60 versus 100-300 mg-acetate-COD·l^{-1} and 0-30 versus 100-200 mg-benzoate-COD·l^{-1}).

Despite the lowered VLR with benzoate and acetate, no terephthalate degradation was observed from day 150 to 300 (see Figure 7.2). The absence of terephthalate degradation capacity of the biomass in R1 was confirmed by a batch-experiment with sludge sampled from the reactor on day 234 (see Table 7.2). The length of the lag-period required to obtain 2 mM terephthalate degradation was comparable to values measured during period I.

At day 234 of operation, sludge was removed from R1 and mixed with granular sludge used for the initial inoculation of R1. Part of this sludge mixture was used for re-inoculation of one of the second stage reactors (R23), while the rest of the biomass was returned into R1. Herewith the biomass concentration in R1 remained unchanged (≈26.2 g-VS·l^{-1}). The removal of acetate and benzoate in R1 was hardly affected by this procedure and remained as high as 95-100 %.

From approximately day 300 onwards, significant terephthalate removal was obtained in R1. The terephthalate removal capacity increased within 30 days to approximately 15 g-COD·l^{-1}·day^{-1}. The degradation of terephthalate in R1 was confirmed by the increased methane production rates. Throughout the period of increasing terephthalate removal, the effluent concentrations acetate and benzoate dropped to values lower than the detection limit of the corresponding analytical methods (approximately 5 and 20 mg-COD·l^{-1} for acetate and benzoate respectively). Prior to this period of increasing terephthalate removal in R1, effluent concentrations acetate were low (10-50 mg-COD·l^{-1}), but always higher than the detection limit.

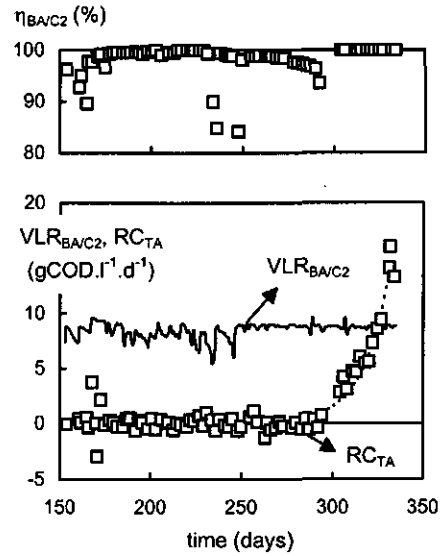


Figure 7.2: Operational performance of R1 during period II. The bottom graph shows the volumetric loading rate with benzoate and acetate ($VLR_{BA/C2}$, solid line) and the terephthalate removal capacity (RC_{TA},). The top graph shows the removal efficiency for acetate and benzoate.

Period III, reactor R1 (day 330-398). Throughout period III the operational parameters of R1 were varied for short periods of time. Variations imposed to the system were accomplished by varying the influent concentrations acetate and benzoate. Herewith the HRT remained unchanged at approximately 2.5 hours. The VLR for terephthalate was kept significantly higher compared to the terephthalate removal capacity and therefore terephthalate was present in excess in the reactor at all times. The effect of the varying loading rates with acetate and benzoate on the terephthalate removal capacity is shown in Figure 7.3. The following characteristic periods can be distinguished:

- Up to day 348 the VLR with benzoate and acetate was kept constant at values around 10 and 8 g-COD·l^{-1}·day^{-1} respectively. During this period the terephthalate removal capacity reached a stable value of approximately 16 g-COD·l^{-1}·day^{-1},
- at day 348 the VLR with benzoate and acetate was increased to 17 and 10 g-COD·l^{-1}·day^{-1} respectively. This resulted in an immediate decrease in the terephthalate removal capacity from 16 to 13 g-COD·l^{-1}·day^{-1}.

- when the dosage of benzoate was stopped on day 353, the terephthalate removal capacity increased to 16 g-COD·l^{-1}·day^{-1}, while termination of acetate acid dosage on day 355 led to a further increase of the terephthalate removal capacity to 20 g-COD·l^{-1}·day^{-1},
- returning the benzoate and acetate load on day 357 to 17 and 10 g-COD·l^{-1}·day^{-1} respectively, led to return of the terephthalate removal capacity to its previous value of 16 g-COD·l^{-1}·day^{-1},
- from day 360 to 367, the acetate VLR was gradually increased to 60 g-COD·l^{-1}·day^{-1}. Despite this large increase, the acetate concentration in the effluent increased to a maximum value of only 75 mg-COD·l^{-1}, and the terephthalate removal capacity decreased concomitant to the increase in acetate loading to 5 g-COD·l^{-1}·day^{-1},
- at day 367 the acetate loading rate was decreased to 19 g-COD·l^{-1}·day^{-1}, resulting in an increase in the terephthalate degrading capacity to 15 g-COD·l^{-1}·day^{-1} within a period of 6 days .

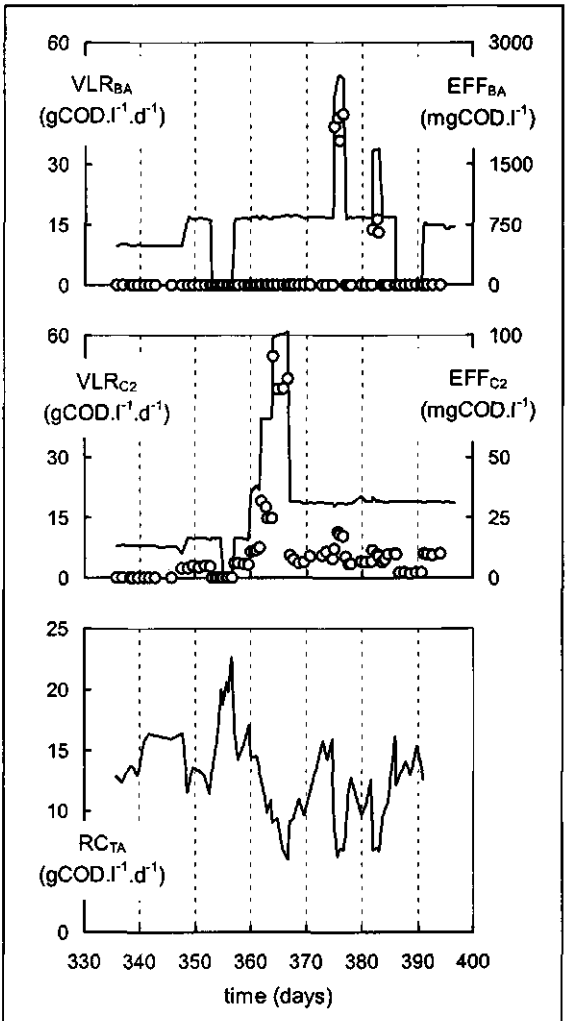


Figure 7.3: Operational performance of R1 during period III. The two top graphs show the volumetric loading rate (VLR, solid lines, left y-axis) with benzoate (BA) and acetate (C2) and the corresponding effluent concentrations (O, right y-axis). The bottom graph shows the terephthalate removal capacity (RC_{TA})

- on day 375 and day 382 the benzoate loading rates were during a two day period increased to 52 and 33 g-COD·l^{-1}·day^{-1} respectively, resulting in effluent benzoate concentrations of 2.0 and 0.7 g-COD·l^{-1}·day^{-1}. These high benzoate concentrations in the

effluent resulted in a decrease of the terephthalate removal capacity to 6 g-COD·l^{-1}·day^{-1} in both cases.

Second stage reactors; R21, R22 and R23. As a result of inoculation of R21, R22 and R23 with a small amount of suspended terephthalate degrading biomass, terephthalate degradation was observed from the first day of operation of the second stage (day 67). Initially the reactors were operated at a VLR of approximately 1 g-COD·l^{-1}·day^{-1} and a HRT of 50 hours. Due to the high treatment efficiency of R1 for benzoate and acetate, terephthalate was the main carbon and energy source in the influent of R21 to R23.

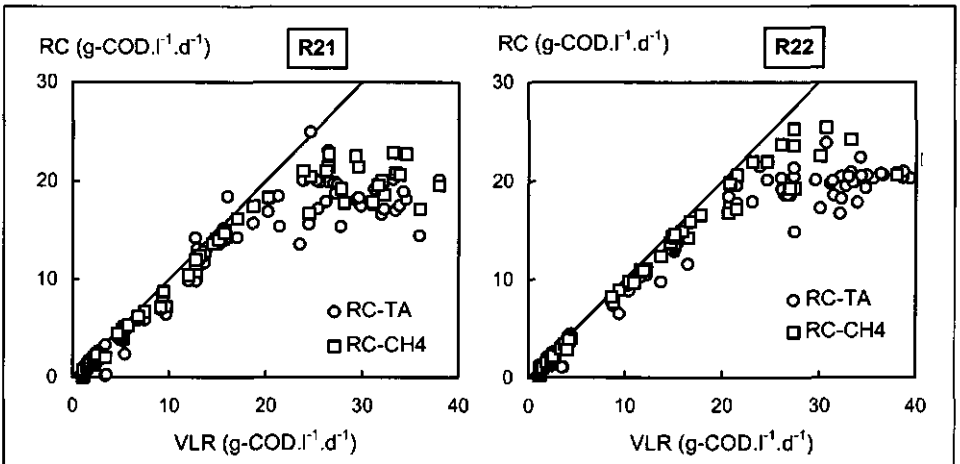


Figure 7.4: The COD-removal capacity, calculated from methane production rates (RC-CH4) and in- and effluent concentration terephthalate (RC-TA), as a function of the volumetric loading rate (VLR) in R21 (left graph) and R22 (right graph). Drawn lines represent a COD-removal efficiency of 100 %.

In time, the VLR of the reactors was gradually increased by increasing the influent flow rate. R21 and R22, that were inoculated with granular biomass besides the terephthalate degrading biomass, the VLR could gradually be increased to 15 g-COD·l^{-1}·day^{-1} after 125 days of operation (day 192). Treatment efficiencies exceeded 80 % throughout this period, as can be seen from Figure 7.4. At this time the HRT was as low as 4 hours. Even though the removal capacity of R22 increased slightly faster compared to R21, the differences observed in the treatment performance between both reactors were insignificant. During period III of operation of R1 the influent concentrations terephthalate of the second stage fluctuated strongly resulting in VLR-values ranging from 20 to 40 g-COD·l^{-1}·day^{-1} at a HRT of 2 hours in R21 and R22. Despite these strong fluctuations, the removal capacity of terephthalate

remained around 20 g-COD·l^{-1}·day^{-1} in both reactors. The final concentration of volatile solids in R21 and R22 amounted 32.9 and 52.2 g-VS·l^{-1}.

Compared to reactor R21 and R22, the terephthalate removal capacity of R23 remained considerably lower. The maximum capacity was found to be 2 g-COD·l^{-1}·day^{-1} at a HRT of 12 hours. Even though some floc formation occurred in this reactor, its low removal capacity primarily could be attributed to the low biomass concentrations (final biomass concentration on 230 day: 5.2 g-VS·l^{-1}).

At day 234, R23 was reinoculated with biomass from R1, supplemented with the original seed sludge. In this way an equal biomass concentration (≈ 26 g-VS·l^{-1}) and composition was obtained in the reactors R1 and R23,. Consequently, the removal of terephthalate in both reactors could readily be compared. Until day 274 no terephthalate degradation was observed in R23, but from this day onwards the terephthalate removal capacity increased exponentially at a comparable rate as calculated for R1 (≈ 0.08 day^{-1}, data not shown). Herewith the lag-phase prior to terephthalate degradation amounted 40 days, which is highly comparable with the length of the lag period prior to terephthalate degradation observed in the batch experiment performed on day 234 (see Table 7.2). The terephthalate removal capacity of R23 increased from day 274 onwards to values comparable to those obtained with R21 and R22 (15-20 g-COD·l^{-1}·day^{-1}) within 75 days of operation.

Observed irregularities in the treatment performance of the second stage reactors. At day 234, when the modifications were made to R1 and R23 as described above, the terephthalate removal capacity in R21 and R22 was almost completely lost, as shown for R22 in Figure 7.5. For a period of approximately 20 days almost no terephthalate conversion and biogas production, was observed in both reactors. Following this period, the terephthalate removal capacity recovered exponentially to values comparable to those observed prior to the upset of the reactors. The apparent growth rate, calculated from the exponential

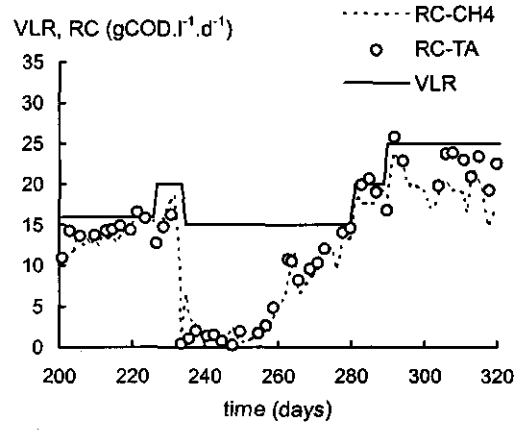


Figure 7.5: Volumetric loading rate (VLR) and COD-removal capacity calculated from methane production rates (RC-CH4) and in- and effluent concentrations terephthalate (RC-TA) in R22 from day 200 to 320.

increase of the terephthalate removal capacity from day 250 to 263, amounted 0.19 day^{-1}. This value is considerably higher as those estimated for exponential growth in R1 and R23, suggesting that besides bacterial growth, reactivation of the terephthalate degrading biomass played a role in the recovery of the terephthalate degrading activity of R21 and R22.

Table 7.3: Specific activities (expressed as g-substrate-COD·g-VS^{-1}·day^{-1}) of sludge from R22 and R23 incubated with acetate, benzoate and terephthalate on day 241 and 317 of operation.

	R22		R23	
substrate	day 241	day 317	day 241	day 317
acetate	0.37	0.94	0.38	1.17
benzoate	0.21	0.48	0.16	0.57
terephthalate	0.06	0.43	0.02	0.29

In order to assess whether specifically the terephthalate degrading biomass was inhibited in R21 and R22, the specific activity was measured of biomass sampled on day 241 from the reactors with acetate, benzoate and terephthalate. Measured values were compared to those obtained with biomass sampled from R21 and R22 on day 317. At this time both reactors were actively degrading terephthalate at a rate of approximately 20 g-COD·l^{-1}·day^{-1}. From the data presented in Table 7.3 it can be seen that all specific activities were significantly lower at day 241, compared to day 317. However, while the activities with benzoate and acetate at day 241 are 2 to 3 times lower, the specific activities with terephthalate are 7 and 17 times lower in R21 and R22 respectively. These data suggest that mainly the terephthalate degrading biomass had been inhibited.

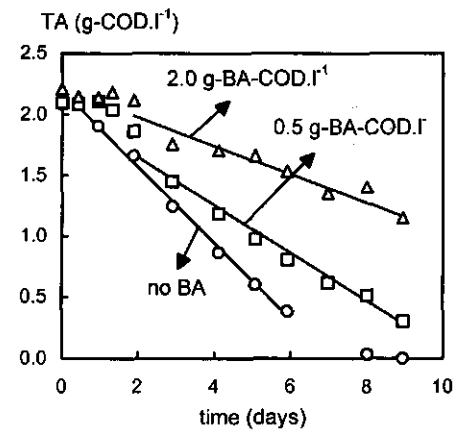


Figure 7.6: Terephthalate (TA) degradation by sludge from R22, incubated without benzoate (BA, o), and with 0.5 ( ) and 2.0 g-BA-COD·l^{-1} (Δ). BA was completely degraded within 0.8 and 2.5 days in the experiments incubated with 0.5 and 2.0 g-BA-COD·l^{-1} respectively. Drawn lines correspond to TA-degradation rates of 0.31, 0.19 and 0.12 g-TA-COD·l^{-1}·day^{-1} for incubation without, and with 0.5 and 2.0 g-BA-COD·l^{-1} respectively.

Previous research with a terephthalate

degrading enrichment culture revealed that short periods without substrate may result in a complete loss of the terephthalate degrading activity. The terephthalate degrading biomass could be resumed through dosage of low concentrations of benzoate to the culture (Chapter 4). In order to assess if dosage of benzoate to R21 and R22 could enhance the recovery of the terephthalate degrading capacity, batch experiments were performed with biomass sampled from R21 and R22 at day 239, and incubated with mixtures of terephthalate and benzoate (see Figure 7.6). The results reveal that benzoate did not improve the terephthalate degrading activity. The terephthalate degradation rate after complete removal of benzoate even decreased with increasing benzoate dosage. It was furthermore observed that terephthalate degradation was completely inhibited by benzoate at initial concentrations as low as 2.1 mM. These results are comparable to those obtained with the terephthalate degrading enrichment culture, when no period without substrate was imposed to the culture (Chapter 4).

7.5 Discussion

General performance. The results presented above demonstrate that high-rate anaerobic pre-treatment of PTA-wastewater in a two-stage UASB-reactor is possible. Benzoate and acetate can be degraded in the first stage of such a staged reactor concept at very high rates (> 40 g-COD·l^{-1}·day^{-1}), while terephthalate can be degraded in a latter stage at moderate rates (15-20 g-COD·l^{-1}·day^{-1}). Taken into account that normally in PTA-wastewater (i) acetate, benzoate and terephthalate correspond to 80-90 % of the COD-load, (ii) the total COD-concentration of PTA-wastewater amounts to 6 g-COD·l^{-1}, and (iii) acetate, benzoate and terephthalate are present in COD-equivalent concentrations, the results suggest that a COD-removal efficiency of approximately 80 % can be reached at an overall volumetric loading rate of 25 g-COD·l^{-1}·day^{-1} and a hydraulic retention time of 5.6 hours (first stage: 2.4 hours; second stage 3.2 hours). Herewith it is assumed that the rest-fraction of the COD-load of PTA-wastewater consists primarily of *para*-toluic acid, which is anaerobically degraded at only very low rates (Chapter 2, [10, 11]) and should therefore be removed in an aerobic post-treatment step.

Compared to previously reported data on anaerobic pre-treatment of PTA-wastewater, summarised in Table 1.4, the volumetric COD-removal capacities described in this chapter are considerably higher. So far, terephthalate degradation has only been reported in anaerobic bioreactors operated at low VLR-values of 2.5 to 4.5 g-COD·l^{-1}·day^{-1}. Herewith this is the first study that evidently shows that high-rate treatment of a mixture of acetate, benzoate and terephthalate at short hydraulic retention times is possible.

Terephthalate degradation: the bottleneck in anaerobic PTA-wastewater treatment. The work described here confirms our previous observation that terephthalate degradation represents the bottleneck in anaerobic PTA-wastewater treatment [8]. Since terephthalate corresponds normally to 30 to 50 % of the COD-load in PTA-wastewater, degradation of this compound is essential for successful implementation of anaerobic treatment. Several properties of the terephthalate degrading biomass play a role in the problematic terephthalate degradation in anaerobic treatment systems: (i) the long lag-period prior to terephthalate degradation with anaerobic granular sludge or digested sewage sludge (44 to 61 days, [Chapter 2]), (ii) the slow growth rate as determined with a terephthalate grown methanogenic enrichment cultures (μ^{max} = 0.094 day^{-1} at 37°C) and estimated from reactor data (neglecting biomass wash-out: μ^{max} = 0.04 day^{-1} at 30°C to 0.06-0.08 day^{-1} at 37°C, this chapter and Chapter 3 and 6, [8]), (iii) the severe inhibition of terephthalate degradation at low concentrations of acetate, benzoate or molecular hydrogen (Chapter 4, [5, 8]), and (iv) the loss of terephthalate degrading activity due to short periods without substrate, or through incubation with high concentrations benzoate or acetate besides terephthalate (Chapter 4, [5], Figure 7.6). We furthermore observed strong inhibition of terephthalate degradation in R21 and R22 from day 130 to 160 of operation. So far we were unable to identify a clear reason for this loss in terephthalate degrading capacity, but the activity measurements (Table 7.3) revealed that the inhibition of acetate and benzoate degrading activities were less severe. Apparently, anaerobic terephthalate degradation is far more sensitive to minor environmental changes compared to anaerobic benzoate and acetate degradation, and may be troublesome to obtain and to maintain in anaerobic bioreactors

Despite these intrinsic limitations, successful enrichment of biomass on terephthalate can be achieved in anaerobic bioreactors, and consequently high removal rates can be obtained. High terephthalate removal rates in bioreactors of the second stage were only obtained when they were inoculated with granular biomass. In the reactor seeded with suspended terephthalate degrading biomass (R23), only low removal rates (2 g-COD·l^{-1}·day^{-1}) were obtained due to limitations in biomass retention. Co-inoculation with granular biomass enabled high terephthalate removal rates (15-20 g-COD·l^{-1}·day^{-1}) at short hydraulic retention times (2 hours). Since the granular biomass itself only had a very low potential for terephthalate degradation, with lag-phases prior to terephthalate degradation of 30 to 60 days, it appears that initially the granular biomass acted as an efficient carrier for enhanced retention of the terephthalate degrading culture. Based on these results we suggest that the presence of nuclei for biofilm formation in the UASB-reactors is essential for effective biomass retention required for high-rate treatment of terephthalate. Previously we used a

similar line of reasoning to explain the superior treatment performance of anaerobic hybrid reactors versus UASB-reactors seeded with suspended methanogenic biomass (Chapter 6).

Single reactor versus a two-stage reactor concept. Experiences with staged anaerobic bioreactor concepts reported in literature are primarily focused on easily degradable substrates such as carbohydrates. In these reactors, acidification primarily proceeds in the first stages of the process, whereas in the latter stages the volatile fatty acids produced are converted to methane and carbon dioxide [2, 6, 9, 12, 13]. The two-stage anaerobic bioreactor concept used during this study was obtained by placing two UASB-reactors in series. To avoid introduction of biomass from the first stage into the second, biomass washed out from R1 was removed by sedimentation. Contrary to the multi-stage bioreactors described in literature, we obtained a clear spatial separation of biomass in both treatment steps by using this approach.

During the first 300 days of the experiment, terephthalate degradation occurred only in the second stage of the system. The length of the lag-phase prior to terephthalate degradation found for biomass from R1 remained between 30 and 50 days (Table 7.2), suggesting that hardly any net-accumulation of terephthalate degrading biomass had been obtained in R1. Based on biomass yields on acetate and benzoate (Chapter 3) and measured biomass concentrations, we roughly estimated the solid retention time (SRT) in R1 at 20 to 25 days throughout period I, and at 66 to 92 days during period II. Even though the higher SRT-values calculated for period II may have contributed to the gain of terephthalate degrading capacity in R1 starting from day 300, obviously the SRT cannot fully account for the absence of terephthalate degradation during the first 300 days of operation because the estimated SRT-values are considerably higher than the doubling time of the terephthalate degrading culture ($\approx$ 7 days). Therefore we presume that besides the SRT other aspects, such as the prevailing reactor concentrations acetate and benzoate, play a role in the complete absence of terephthalate degradation throughout the first 300 days of operation of R1. This explanation is confirmed by comparison of the operational performance of R1 and R23 from day 234 onwards. At this day, both reactors were re-inoculated with the same sludge at equal concentrations. Operational differences between both reactors were that (i) R1 was fed with acetate and benzoate besides terephthalate at a low rate (9 g-COD·l^{-1}·day^{-1}), and (ii) the HRT was significantly lower in R1 (2.5 hours in R1 versus 12 hours in R23). Due to these operational differences the lag phase required to obtain terephthalate degradation in R23 amounted to approximately 40 days in R23 and 66 days in R1. Insufficient data are available to elucidate to what extent the SRT and/or the inhibition of terephthalate degradation by

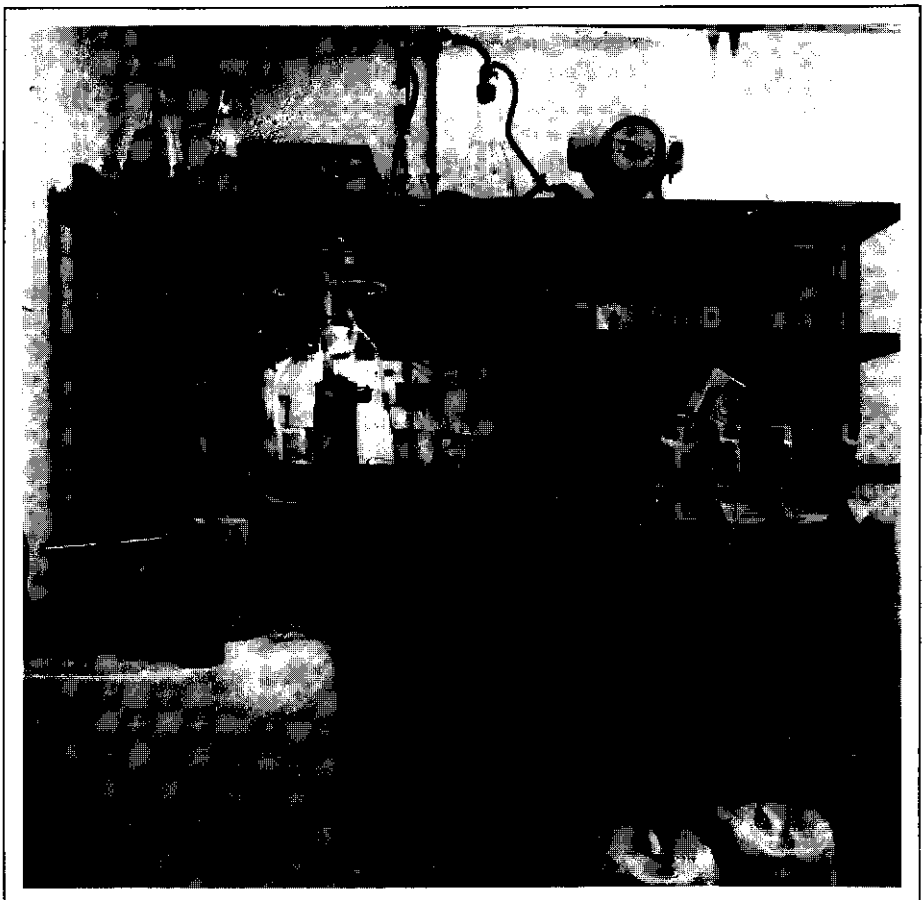
acetate, benzoate and/or hydrogen are the dominant factors in the extremely long lag-phase prior to terephthalate degradation observed in R1.

In this respect it is relevant to note that after terephthalate degradation was obtained in R1, this reactor showed a high tolerance to increased loading rates with acetate and benzoate. Contrary to the batch experiment with biomass from R21 and R22 (Figure 7.6), no complete inhibition of terephthalate degradation was observed at benzoate concentrations as high as 2.0 g-COD·l^{-1}. Furthermore, at volumetric loading rates with acetate and benzoate as high as 60 and 16 g-COD·l^{-1}·day^{-1} respectively, no complete inhibition of terephthalate was obtained. During operation of R1 at moderate loading rates with acetate and benzoate (≈ 15-25 g-COD·l^{-1}·day^{-1}), comparable volumetric terephthalate removal capacities could be obtained as in R21 and R22 (15-20 g-COD·l^{-1}·day^{-1}). It should be emphasised, however, that varying the loading rates with benzoate and acetate did strongly affect the terephthalate degradation in R1, resulting in terephthalate removal capacities between 5 and 20 g-COD·l^{-1}·day^{-1}. It can furthermore be speculated that long-term operation at elevated acetate and benzoate loading rates will lead to a reduced terephthalate removal capacity due to the unfavourable growth kinetics on terephthalate, compared to those on acetate or benzoate.

In summary it can be concluded that the main advantage of the two-staged anaerobic reactor system is reduction of the lag-phase prior to terephthalate degradation. As a result of pre-removal of acetate and benzoate in the first stage, a high terephthalate degrading capacity in the second stage can be obtained at prolonged SRT-values and minimised concentrations acetate and benzoate. Terephthalate degradation only can be obtained in the first stage when the reactor concentrations acetate and benzoate are sufficiently low and the SRT is high. Still, the observation that strongly fluctuating acetate and benzoate loading rates primarily affect the terephthalate removal capacity, suggests that anaerobic degradation of terephthalate in a second stage remains to be preferred.

Practical implications. A combined low rate anaerobic pre-treatment and aerobic post-treatment of PTA-wastewater was shown to be an economically feasible alternative for the complete aerobic treatment by Amoco Petrochemicals Inc. [3, 4, 14]. This company has built four full-scale down-flow fixed film reactors for anaerobic pre-treatment of PTA-wastewater. The applied VLR of the reactor located in Geel, Belgium is low (4.0 g-COD·l^{-1}·day^{-1}) and therefore the reactor is very big (15,200 m^3) [3]. The high investment costs for such a large reactor are compensated by the significantly lower operational costs compared to complete aerobic treatment due to lower nutrient requirements, lower excess sludge production and lower energy consumption .

High rate two-stage anaerobic pre-treatment of PTA-wastewater may represent an attractive alternative for the low rate system developed by Amoco Petrochemical Inc. Assuming that an overall VLR of 25 g-COD·l^{-1}·day^{-1} can be applied in a staged UASB-reactor concept, the reactor volume can be reduced by a factor 6. As emphasised above, the applicability of such VLR-values depends strongly on the biomass concentrations that can be maintained in the reactor. In this respect the availability of granular methanogenic biomass for start-up of the system, may have a large positive impact on the time needed for start-up of the system. If sufficient granular biomass is available, high rate degradation of acetate and benzoate in the first stage can be obtained within one month. However, start-up of the second stage for degradation of terephthalate will normally require at least 3 to 5 months, and even longer when using flocculent methanogenic biomass.



Experimental set-up used for two-stage anaerobic treatment of artificial PTA-wastewater

References

1. **APHA.** 1985. Standard methods for examination of water and wastewater, 16th ed. American Public Health Association, Washington D.C., USA.
2. **Bachmann, A., V. L. Beard, and P. L. McCarty.** 1985. Performance characteristics of the anaerobic baffled reactor. Wat. Res. **19:**99-106.
3. **Duffel, J. v.** 1993. Anaerobe behandeling van organische zuren. Presented at the National Conference on Anaerobic Treatment of Complex Wastewaters, Breda, The Netherlands.
4. **Ely, S. R., and G. P. Olsen.** 1989. AMOCO-Process for treatment of PTA-wastewater. USA patent 4,826,600.
5. **Fajardo, C., J. P. Guyot, H. Macarie, and O. Monroy.** 1997. Inhibition of anaerobic digestion by terephthalic acid and its aromatic by products. Wat. Sci. Technol. **36:**83-90.
6. **Grobicki, A., and D. C. Stuckey.** 1991. Performance of the anaerobic baffled reactor under steady state and shock-loading conditions. Biotechnol. Bioeng. **37:**344-355.
7. **Horowitz, A., D. R. Shelton, C. P. Cornell, and J. M. Tiedje.** 1982. Anaerobic degradation of aromatic compounds in sediments and digested sludge. Dev. Ind. Microbiol. **23:**435-444.
8. **Kleerebezem, R., J. Mortier, L. W. Hulshoff Pol, and G. Lettinga.** 1997. Anaerobic pre-treatment of petrochemical effluents: terephthalic acid wastewater. Wat. Sci. Tech. **36:**237-248.
9. **Lier van, J. B., F. Boersma, M. M. W. H. Debets, and G. Lettinga.** 1994. High rate thermophilic anaerobic wastewater treatment in compartmentalized upflow reactors. Wat. Sci. Technol. **30:**251-261.
10. **Macarie, H., and J. P. Guyot.** 1992. Inhibition of the methanogenic fermentation of p-toluic acid (4-methylbenzoic acid) by acetate. Appl. Microbiol. Biotechnol. **38:**398-402.
11. **Macarie, H., and J. P. Guyot.** 1995. Use of ferrous sulphate to reduce the redox potential and allow the start-up of UASB-reactors treating slowly biodegradable compounds: application to a wastewater containing 4-methylbenzoic acid. Environ. Tech. **16:**1185.
12. **Mulligan, C. N., B. F. Safi, J. Meunier, and J. Chebib.** 1993. Anaerobic treatment of cheese dairy wastewater using the SNC reactor. Water Poll. Res. J. Canada. **28:**597-619.
13. **Nachaiyasit, S., and D. C. Stuckey.** 1995. Microbial response to environmental changes in an anaerobic baffled reactor (ABR). Antonie van Leeuwenhoek. **67:**111-123.
14. **Shelley, S.** 1991. And the winner is... Amoco chemical. Chemical Engineering. **98:**90-93.
15. **Yspeert, P., T. L. F. M. Vereijken, S. Vellenga, and d. A. Vegt.** 1993. The IC-reactor for the anaerobic treatment of industrial wastewater. Presented at the Food Industry Environmental Conference, Atlanta, USA.

General Discussion

Abstract

A discussion on some practical aspects of the anaerobic treatment of phthalate isomers containing wastewaters is presented. Based on the results described in the previous chapters and considerations concerning reactor design and pH-control, we will work towards an optimised concept for design and operation of anaerobic PTA-wastewater treatment plants

Contents

8.1 Introduction

The aim of the work described in this thesis was to elucidate whether or not anaerobic treatment represents an attractive contribution to conventional aerobic treatment for treatment of phthalic acids containing wastewaters. Wastewater generated during production of phthalic acid isomers can be characterised as a concentrated mixture of non-complex and complex substrates. Normally, both categories represent approximately 50 % of the total organic contamination level of the wastewater. Acetate and benzoate can be ranked among the first category of compounds. Both compounds can be degraded at high-rates in anaerobic bioreactors, as has been demonstrated on lab- and full-scale [13, 15]. The phthalate isomers and the more reduced aromatic compounds represent the category of complex substrates. As outlined in chapter 1, the bottleneck in anaerobic treatment of these wastewaters is the degradation of phthalic acids. Therefore we focused our research on both microbiological and technological aspects of the anaerobic degradation of these compounds.

In this chapter some practical implications of the experimental results presented in Chapter 2 to 7, on anaerobic bioreactor design and operation will be discussed. Since various microbiological aspects are extensively discussed in Chapter 3 to 5, this chapter will focus on engineering aspects of full-scale treatment of phthalate isomers containing wastewaters. As the anaerobic degradation of terephthalate has been studied most extensively in our investigations, the discussion will consider the wastewater generated during terephthalic acid production (PTA-wastewater). First, recent developments regarding full-scale anaerobic treatment of PTA-wastewater are presented. Based on these experiences, we will work towards an optimised concept for anaerobic treatment of PTA-wastewater taking into account considerations concerning reactor design (Paragraph 8.3), and caustic soda requirements for pH-control (Paragraph 8.4).

8.2 Full-scale PTA-wastewater treatment

In recent years a relatively large number of anaerobic bioreactors has been constructed for pre-treatment of PTA-wastewater (Table 8.1). It appears that since Amoco demonstrated in the late 1980's that low-rate anaerobic pre-treatment of PTA-wastewater is an economically attractive alternative for conventionally applied aerobic treatment methods [9, 10, 21], various other PTA-producers became interested in anaerobic pre-treatment of their waste as well.

In the past decade more than 10 full-scale anaerobic bioreactors have been constructed for PTA-wastewater treatment (Table 8.1). The data presented in Table 8.1 show that most reactors have been designed to operate at low to moderate volumetric loading rates (2-10 kg-COD·m^{-3}·day^{-1}). Combined with the fact that PTA-production normally occurs at quite a large scale, this resulted in the installation of large reactor volumes. As a consequence, some of the anaerobic reactors listed in Table 8.1 are among the biggest in the world. Due to the large volumes, the anaerobic bioreactors are normally constructed in 2 to 4 compartments. Besides the down-flow fixed-film reactors constructed by Amoco, both hybrid and UASB-reactors have been installed for PTA-wastewater treatment. The final post-treatment of the anaerobic effluent of most of the treatment plants listed in Table 8.1, is achieved in conventional activated sludge systems. Some PTA-producers, such as Eastman Petrochemical Inc, USA, rely completely on aerobic treatment technology. This company recently constructed PTA-production plants with aerobic wastewater treatment systems in The Netherlands and Spain.

Besides the recent full-scale applications mentioned in Table 8.1, some PTA-producers are currently working on a new generation of PTA-production plants that include anaerobic treatment of the generated waste. Dupont, that took over the PTA-production capacity from ICI, is currently developing anaerobic treatment technology for implementation in future PTA-projects. Downstream of PTA-production, i.e. in the production of polyester from PTA and ethylene glycol, the anaerobic treatment technology is becoming more important as well, as can be deduced from the recently constructed Biobed® reactors by Biothane in Greece and Turkey.

As Amoco only published few of their experimental, as well as full-scale experiences, design criteria for anaerobic bioreactors treating PTA-wastewater remain unclear. Moreover, it appears to be very hard to acquire reliable information with regard to the treatment performance of the anaerobic bioreactors listed in Table 8.1. Petrochemical companies generally are quite reluctant in providing information about the amount and composition of the generated waste because this may provide insight in the efficiency of their production processes. The closeness of these companies seriously hampers the development of environmental treatment technologies for wastestreams generated in petrochemical production processes, such as PTA-wastewater. The literature information on lab-scale studies dealing with anaerobic PTA-wastewater treatment are furthermore scarce and controversial as outlined in Chapter 1. Consequently, anaerobic treatment of PTA-wastewater can hardly be regarded as an accepted technology.

Table 8.1: Full-scale anaerobic bioreactors that have been constructed in recent years for anaerobic (pre-)treatment of PTA-wastewater

year of construction	PTA-producer	Reactor-design	Location	reactor type	Volume (m^3)	COD-conc. ($g \cdot l^{-1}$)[c]	VLR ($g\text{-}COD \cdot l^{-1} \cdot d^{-1}$)	Remarks	Ref.
1998	Temex	IBTech	Mitatitlan, Mexico	UASB-derivative	3 * 6,000	8-12 (16)	2 -3	start-up 1999	[14]
1998	SASA	Biothane	Turkey	Biobed	2 * 1,000	6.5	13	start-up 1999	[5]
1998	Dupont	ADI	Taiwan	hybrid	14,000	6	6	start-up 1999	[8]
1996	ATV-Petro.	Paques	India	UASB	1,330	12	10 - 12	-	[19]
1994	Sam Nam Petrochemical	ADI	South-Korea	hybrid	2 * 1,100	20	10	No TA and p-T in wastewater	[2]
1994	Reliance Industries	ADI	Hazira, India	hybrid	2 * 3,700	5.7	6	-	[2]
1994	Tuntex	Hepe	Thailand	UASB	3*3.300	10	6	-	[12]
1993	Amoco[a]	Amoco	Geel, Belgium	DFF	15,000	3.7	4	see Chapter 1	[9]
1991	Tuntex	Grontmij	Tainan, Taiwan	UASB	4 * 1,700	6 (15)	4 (10)	see Chapter 1	[20]
1990	N.A.	BMRI[b]	Nanjing, China	UASB	4 * 3,000	9	6.3	-	[25]
1989	Capco	Amoco	Taiwan	DFF	2 * 5,000	10	3 - 4	-	

[a] Besides in Geel, Belgium, Amoco constructed anaerobic DFF-reactors for PTA-wastewater treatment in Joliet, USA, and in South-Korea.

[b] BMRI: Beijing Municipal Recearch Institute

[c] Values represent average concentrations, maximum concentrations are shown between brackets

8.3 Reactor technology

The commercial success of high-rate anaerobic bioreactor technology is based on the high concentrations of biomass that can be maintained in these reactors due to uncoupling of the solid and liquid retention time. High biomass concentrations combined with high specific substrate conversion rates, and adequate mixing to overcome mass transfer limitations, result in high volumetric substrate conversion rates and consequently small reactor volumes.

With regard to anaerobic terephthalate degradation, we demonstrated in Chapter 6 and 7 that both anaerobic hybrid reactors and UASB-reactors were capable of terephthalate degradation at moderate- to high-rates (10-20 g-COD·l^{-1}·day^{-1}). The good treatment performance of both reactor systems can be explained by the high biomass concentrations that could be maintained, combined with the high values for the maximum specific terephthalate conversion rates (q_{TA}^{max}) (see Table 8.2). The values for q_{TA}^{max} we measured with biomass from both reactor types (see Table 8.2) were much higher than those reported in literature (0.02 to 0.07 g-TA-COD·g-VS^{-1}·d^{-1}, [7, 11, 17]). The main objective of the continuous reactor studies was to elucidate if high terephthalate removal capacities could be obtained, and not to determine the minimum effluent concentrations that can be reached. Consequently, we worked at relatively high reactor concentrations terephthalate (>0.5 g-COD·l^{-1}) and therefore mass transfer limitations played only a minor role.

Table 8.2: Operational characteristics of anaerobic bioreactors described in Chapter 6 and 7 and treating terephthalate as main carbon and energy source.

reactor type:		UASB	Hybrid
name:		R22 and R23, Chapter 7	R1, R2 and R3 Chapter 6
C_X	(g-VS·l^{-1})	30 - 50	15 - 30
RC_{TA}	(g-TA-COD·l^{-1}·d^{-1})	15 - 20	10 - 17
q_{TA}^{max}	(g-TA-COD·g-VS^{-1}·d^{-1})	0.3 - 0.5	0.5 - 0.7

The successful enrichment of the terephthalate degrading biomass could be achieved in both reactor types despite major microbiological bottlenecks in the anaerobic degradation of terephthalate:

1. Even at the optimum temperature (37°C) and pH (~7.0), the growth rate of a terephthalate degrading culture was found to be very slow (μ_{TA}^{max} = 0.09 day^{-1}, Chapter 3),
2. the lag-period prior to terephthalate degradation with methanogenic granular sludge or digested sewage sludge was long (44 to 61 days, Chapter 2),
3. terephthalate degradation is strongly inhibited by benzoate and acetate (and molecular hydrogen) (Chapter 4, [11, 17]),
4. a terephthalate grown enrichment culture readily lost its capacity when incubated with high concentrations benzoate or acetate, or after a short period without substrate (Chapter 4).

With regard to the kinetic parameters for terephthalate degradation some controversial results were obtained. The maximum specific growth rate (μ_{TA}^{max}) for terephthalate degradation determined with a terephthalate grown enrichment culture (Chapter 3) and those calculated from the exponential growth phase in the hybrid and UASB-type reactors (Chapter 6 and 7) were of the same order of magnitude (0.09 and 0.06 to 0.08 day^{-1} respectively). However, the values calculated from batch experiments with biomass sampled from the hybrid reactors (Chapter 6) and those from the second stage reactors during the recovery period (Chapter 7) were significantly higher (0.17 to 0.21 day^{-1}). All these experiments were conducted at highly comparable environmental conditions; pH 7.0 and 37°C. Although other reasons cannot be excluded, likely the higher μ_{TA}^{max}-values measured with bioreactor biomass result from an overestimation of the true growth rate due to reactivation of the biomass (as described in chapter 6 and 7). Due to the indirect measurement of μ_{TA}^{max} - i.e. from the exponential increase of the substrate conversion rate - no distinction can be made between "true" growth and exponential reactivation. Undoubtedly, a direct determination of growth through measurement of biomass concentrations in time represents a superior method. However, a reliable measurement of an increase in biomass concentration with reactor sludge is not possible in batch-experiments due to the low biomass yield of many anaerobic bacteria and the high background concentration solids. The best method for determination of kinetic parameters is by using chemostat cultures. However, at low growth rates these kind of experiments are extremely laborious. Therefore we remain dependent on the methods described in this thesis, but interpretation of the results obtained should be handled with care.

The maximum specific terephthalate degradation rate (q_{TA}^{max}) calculated for the terephthalate grown enrichment culture (Chapter 3) was significantly higher than the values found for the biomass from the hybrid and UASB-type reactors fed with terephthalate as main substrate

(2.6 versus 0.3 to 0.7 g-TA-COD·g-VS^{-1}·d^{-1}). This indicates that in the anaerobic bioreactors inactive solids have accumulated in the sludge, likely due to bacterial decay (Chapter 6). However, the kinetic description of biomass dynamics in anaerobic bioreactors is highly complex and depends on various processes.

Biomass dynamics in high-rate anaerobic bioreactors. Important factors that may influence biomass dynamics in high-rate anaerobic bioreactors are (i) variations in solid retention times as function of the location in the biofilm, and (ii) kinetic parameters such as substrate consumption for growth-independent maintenance, bacterial decay, and subsequent lysis and hydrolysis of the cultivated biomass.

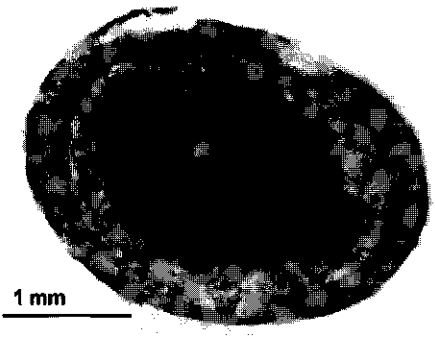


Figure 8.1: Cross section of granular biomass sampled from reactor R1 (Chapter 7) after 320 days of operation. The initial granular seed sludge can readily be distinguished as the black core of the granule

Variations in solid retention time can readily be predicted from microscopic inspection of granular biomass that has been subjected to a change in waste water composition. For example, the original granular biomass used for inoculation of the UASB-reactors described in Chapter 7 could still be distinguished in the core of the granules that had developed in the reactors after more than one year of operation. This suggests that the granular inoculum primarily functioned as carrier for the cultivated specific biomass. As the surface of the granule is subjected to shear forces in the reactor, biomass located on the outside of the granule may have considerably shorter solid retention times than biomass located in the core of the granule. This suggests that the solid retention time is a function of the location of the solids in the biofilm.

Combined with the limited insight available with regard to maintenance, decay and hydrolysis related processes in high-rate anaerobic bioreactors, it is concluded that the knowledge available for adequate kinetic description of biomass dynamics in high-rate anaerobic bioreactors is far from sufficient. Herewith the identification of reasons for the observed capacity limitations in these systems remains troublesome (Chapter 6 and 7). Furthermore, the design of high-rate anaerobic bioreactors remains strongly based on empirically defined grounds.

Hybrid versus UASB-reactors. As described above, both anaerobic hybrid and UASB-reactors were capable to degrade terephthalate at high rates (Chapter 6 and 7). However, a major difference between anaerobic hybrid reactors and UASB-reactors may manifest when scaling-up these systems from lab- to full-scale. At full-scale, mixing in both reactor-types is primarily obtained through biogas production. The biogas production per unit surface-area of reactor (Q_A, $m^3 \cdot m^{-2} \cdot hr^{-1}$) determines to a great extent the mixing in the reactor compartment. As this parameter is linearly related to the height of the reactor, Q_A-values are normally 15 to 30 times higher in full-scale reactors. Moreover, the liquid upflow velocity (v_{up}, $m \cdot hr^{-1}$) will be higher to the same extent. The higher Q_A-value in full-scale hybrid-reactors will give rise to significantly higher shear-forces in the fixed bed zone of the reactor, whereas this will have little impact on the sedimentation zone of a full-scale UASB-reactor. Consequently, the efficiency of biomass hold-up may be significantly lower in full-scale compared to lab-scale anaerobic hybrid reactors.

The fixed-bed section in hybrid reactors may improve the process of biomass granulation from suspended seed-material, as described in Chapter 6. The following subsequent steps could be identified in the granulation process in the anaerobic hybrid reactors: (i) formation of biofilms on the polyurethane carrier particles, (ii) after full colonisation of the carrier, biofilm detachment was observed, and (iii) the detached biofilm particles settled and served as nuclei for granule formation in the down-part of the reactor. Similar mechanisms for granule formation have been described for nitrifying, denitrifying, sulfate reducing and methanogenic bacteria [3, 4, 16]. In none of the UASB-reactors we observed biomass granulation from suspended seed-material.

Based on these arguments we suggest that for full-scale application, the UASB-reactor concept may remain superior over hybrid reactors, provided that granular biomass is available for inoculation. If no granular biomass is available, hybrid reactors likely need a shorter start-up time due to more effective formation of granular biomass. The time required for start-up of UASB-reactors with suspended biomass presumably can be shortened by introducing the proper nuclei for biofilm formation in the reactor compartment.

Single versus two-stage reactors. In Chapter 4 and 6 we suggested that physical separation of biomass degrading acetate and benzoate, and biomass degrading terephthalate may increase the overall capacity of a system treating PTA-wastewater. In such a two-stage reactor concept, the inhibition of terephthalate degradation by acetate and benzoate in the second stage can be avoided. Furthermore, the solid retention times (SRT-values) required for gaining sufficient capacity for the different substrates can be optimised for both reactor modules individually.

The experimental results described in Chapter 7 showed that the positive effect of staging on anaerobic terephthalate degradation will mainly manifest during start-up. Only after a period exceeding 300 days of operation a substantial degradation of terephthalate was observed in the first stage reactor. We attempted to explain these results based on the basis of a simple mathematical model (Appendix). The model considers the treatment of a mixture of acetate, benzoate and terephthalate at COD-equivalent concentrations in a single-stage reactor. The active biomass concentrations in the anaerobic bioreactor was assumed constant (20 gVS-COD·l^{-1}). Kinetic parameters as described in Chapter 3 were used. The initial concentration of terephthalate degrading biomass in non-adapted seed sludge was estimated from the data presented in Chapter 2 and amounted to 3 mg-VS-COD·l^{-1}.

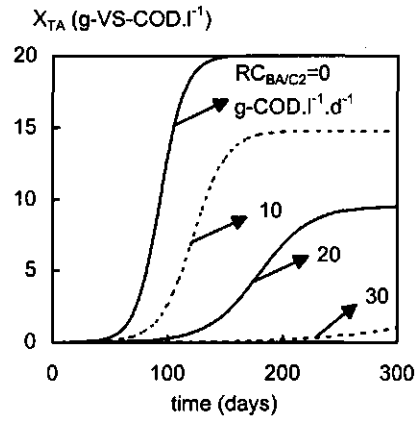


Figure 8.2: Development of the concentration terephthalate degrading biomass (X_{TA}) as function of time at four removal capacities for acetate and benzoate ($RC_{BA/C2}$).

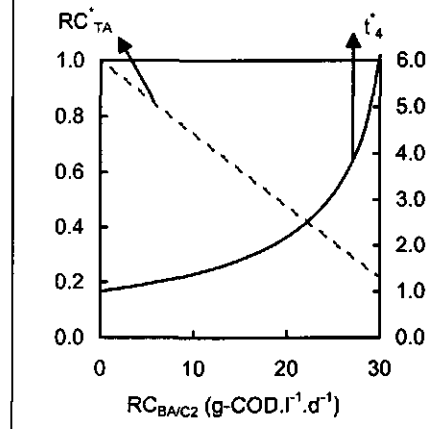


Figure 8.3: Relative steady state terephthalate removal capacity (RC^*_{TA}), and the relative time required to obtain a concentration terephthalate degrading biomass (t^*_4) of 4 g-VS-COD·l^{-1}, as a function of the combined removal capacity for benzoate and acetate ($RC_{BA/C2}$). The situation where terephthalate is the sole carbon and energy source is used as reference.

The change in the concentration of terephthalate degrading biomass (X_{TA}) as a function of time was calculated at four different removal capacities for acetate and benzoate. Results are shown in Figure 8.2. The relative time required to obtain a concentration terephthalate degrading biomass of 4 g-VS-COD·l^{-1} (i), and the relative steady state terephthalate removal capacity (ii) as a function of the removal capacity for acetate and benzoate are shown in Figure 8.3. As expected, the model predicts that in absence of acetate and benzoate the seed material in the reactor is slowly replaced by terephthalate degrading

biomass, until the active biomass in the reactor consists fully of terephthalate degrading biomass. Ideally, this situation would prevail in a second stage reactor. In presence of acetate and benzoate, two effects are observed: (i) the maximum concentration of terephthalate degrading biomass in the reactor is reduced, and (ii) the time needed to obtain this maximum concentration is significantly prolonged. As in the model calculations the inhibition of terephthalate degradation by benzoate and acetate were neglected, the trends observed in practical conditions may be even more pronounced. In general terms, the biomass yield on the readily degradable substrates (acetate and benzoate) and the total concentration of active biomass that can be achieved in the reactor(s) determine the extent of the positive effect of the application of two-stage reactor concept for treatment of a mixture of readily and slowly degradable substrates

The factors implemented in the model may have contributed to the extremely long lag-phase prior to terephthalate degradation observed in the first stage of the two-stage system (Chapter 7). The observation that following this lag-period (of approximately 300 days), the reactor achieved a relatively high capacity for terephthalate degradation (~ 15 g-COD·l^{-1}·d^{-1}) indicates that the biomass concentration in the reactor had to be increased significantly. This was confirmed by the measured increase in the total sludge concentration from approximately 30 to more than 50 g-VS·l^{-1} at this stage of the experiment. However, no conclusive explanation is available for the observed increase in the biomass concentration.

8.4 pH-control

During the production of purified terephthalic acid (and isophthalic acid), the separation of the product is based on their poor solubility in water. Through washing the aromatic acid crystals with water, water-soluble impurities are removed. As an increased alkalinity of the water would decrease the efficiency of product separation, it is evident that the generated wastewater generally contains only trace amounts of alkalinity. Only during cleaning of production units with concentrated sodium hydroxide, significant amounts of alkalinity are introduced into the wastewater. This suggests that to enable anaerobic treatment of PTA-wastewater, substantial amounts of neutralising agents have to be dosed to maintain an optimal reactor pH, as is required for effective anaerobic treatment. Herewith dosage of neutralising agents may represent a large fraction of the operational costs [9].

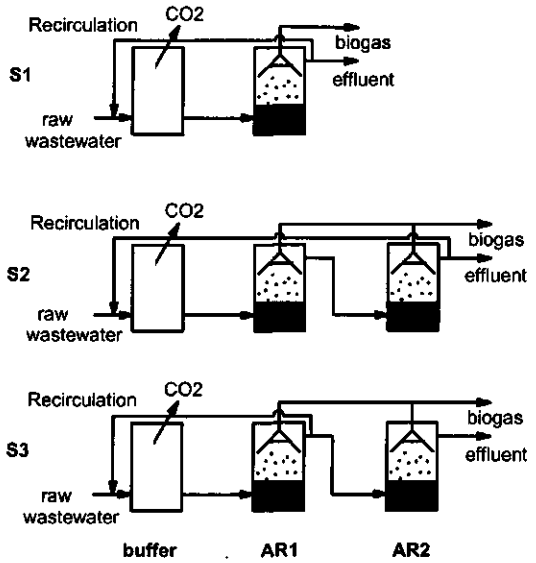


Figure 8.4: Flow charts (S1, S2 and S3) and general assumptions used for calculation of the alkalinity requirements during anaerobic pre-treatment of PTA-wastewater.

To gain insight in the alkalinity requirements for anaerobic treatment of PTA-wastewater, a mathematical model was developed that enables the calculation of chemical speciation (including precipitation). The model is based on chemical equilibrium constants and the wastewater composition. A detailed description of this model can be found elsewhere [24]. This chemical equilibrium model was coupled with a reactor model for calculation of the impact of recirculation flows and of biological degradation reactions on the required amount of neutralising agents.

Prior to conducting the reactor calculations, the solubility product of terephthalic acid was estimated from the water solubility at 25°C (see Table 1.1). The calculated value amounts $K_s(TA) = [H^+]\cdot[HTA^-] = 10^{-8.1}$ $mol^2\cdot l^{-2}$. All the other chemical equilibrium constants were obtained from standard text books [6, 22, 23]. Combined with the pKa-values for terephthalate (Table 1.1), this results in the chemical speciation of terephthalate in pure water as a function of the pH as shown in Figure 8.5.

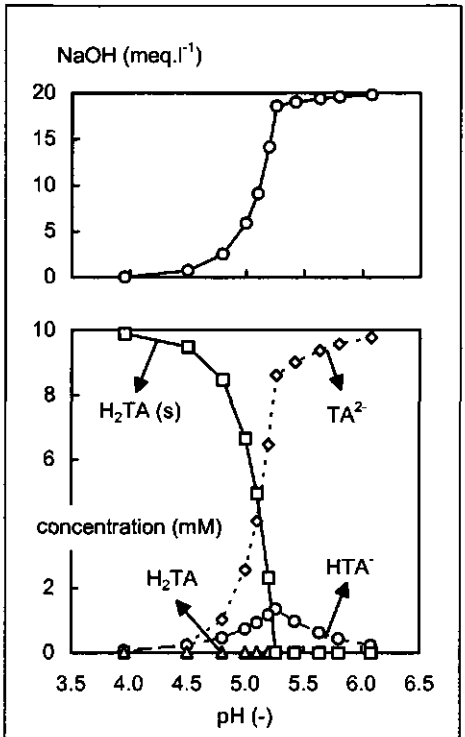


Figure 8.5: Speciation of terephthalate as a function of the pH (bottom graph) and the concentration of sodium hydroxide dosed. Without NaOH-dosage the pH amounts to 3.96.

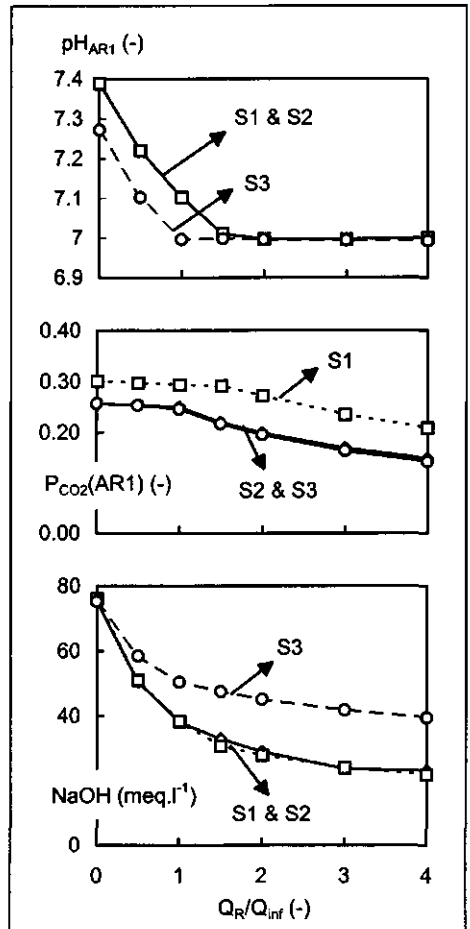


Figure 8.6: Influence of recirculation on the sodium hydroxide requirements for neutralisation (bottom graph), the CO_2-partial pressure in AR1 (middle graph) and the pH in AR1 (top graph). Boundary conditions and definitions are explained in Figure 8.4.

Chemical equilibrium calculations were conducted for wastewater treatment plants consisting of subsequently a buffer tank and either a single or two-stage anaerobic bioreactor configuration as shown in Figure 8.4. The caustic soda requirements were calculated in relation to the recirculation flow rate over the buffer tank. The pH-setpoint in the buffer tank was set at 5.5 to avoid terephthalic acid precipitation (see Figure 8.5). Carbon dioxide was stripped from the buffer tank at variable air-flow-rates and mass transfer limitations were neglected. Other assumptions made to enable the calculations are mentioned in Figure 8.4. The calculated required amounts of caustic soda that need to be dosed to the buffer tank (and the anaerobic

bioreactors) to obtain the pH-setpoints are shown in Figure 8.6. The results clearly demonstrate that a major reduction (upto 70 %) in caustic soda requirements can be obtained through applying recirculation of the effluent of the anaerobic reactor(s) to the buffer tank. Applying a recirculation factor exceeding four only has a limited impact on the caustic soda requirements. The results furthermore demonstrate that to avoid unfavourable high pH-values in the anaerobic reactors (> 7, see Chapter 6), the recirculation-factor should amount at least 1.

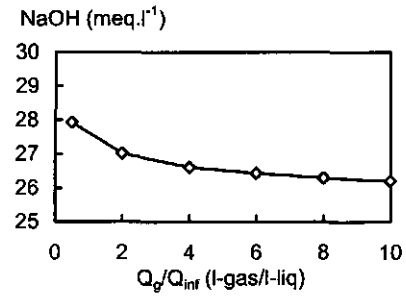


Figure 8.7: Influence of the gas-flow rate in the buffer (CO_2-stripping) reactor related to the influent flow rate (Q_g/Q_{inf}) on the caustic soda requirements for maintaining a pH of 5.5 in the buffer tank (at $Q_R/Q_{inf} = 2$).

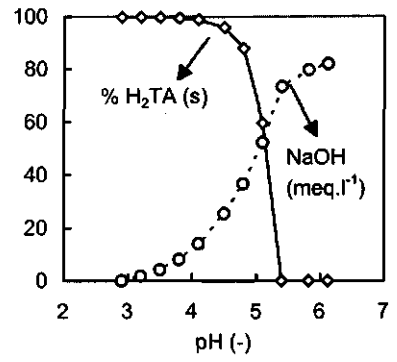


Figure 8.8: Percentage of terephthalate in the solid form (H_2TA [s]) as a function of the pH and the concentration sodium hydroxide required to obtain the pH-values, based on the wastewater composition presented in Figure 8.4.

At first sight the two-stage reactor (S2, Figure 8.4) was expected to require higher amounts of caustic soda for neutralisation, because non-degraded terephthalic acid in the first stage will requires additional caustic soda dosage. However, the calculations show that the caustic soda requirements in S1 and S2 are highly comparable. This can be attributed to the lower CO_2-partial pressure in AR1 that is obtained when degrading only a benzoate/acetate-mixture as in S2 and S3. Consequently, lower bicarbonate concentrations are required to maintain a pH of 7 in AR1. When recirculation is conducted from AR1 (S3, Figure 8.4) caustic soda requirements are significantly higher due to the high (undiluted) terephthalate concentration in AR1. It should be noted that when a two-stage system is operated in series, the hydraulic load of the system is twice the value of parallel operation. In a hydraulically limited system therefore parallel operation is preferred with regard to the caustic soda requirements.

Active stripping of carbon dioxide from the buffer tank will normally be necessary to overcome mass transfer limitations [23]. However, stripping with air may lead to the

introduction of significant amounts of oxygen and subsequent growth of aerobic biomass in the buffer tank. A reduction in the amount of air used for stripping can be obtained through gas-recirculation. Recirculation of the gas used for stripping will lead to an increase of the CO_2 partial pressure in the off-gas of the stripping tank. However, at a pH-setpoint of 5.5 in the stripping (buffer) tank, the driving force for carbon dioxide stripping is high. Therefore recirculation of air is possible and the caustic soda requirements are relatively independent from the CO_2-partial pressure as can be seen from Figure 8.7.

Pre-precipitation of terephthalic acid. Wastewater generated in both steps of the PTA-production process differ considerably in composition and strength. As outlined in Chapter 1 wastewater generated during production of CTA consists primarily of acetic acid, whereas during the purification of CTA, terephthalic acid containing wastewater is generated. Mixing of both wastestreams will enhance pre-precipitation of terephthalic acid, even in presence of significant amounts of caustic soda as can be seen from Figure 8.8. Pre-precipitation of terephthalic acid, may represent a feasible complementary step to biological treatment [26]. The generated solid waste can either be incinerated, or be used in polyester production processes that do not require highly purified terephthalic acid [25].

Closing the water cycle. Closing the water cycle in industrial production processes is becoming a hot topic these days [1]. However, it is evident that the effluent of a treatment plant that includes anaerobic bioreactors is not suitable for application in the terephthalic acid production process due to the high concentration of bicarbonate alkalinity. The utilisation of bicarbonate-rich water in the production process will increase the solubility of terephthalic acid, and therewith decrease the product yield. To remove the alkalinity of the effluent, hyperfiltration (reversed osmosis) can be used. The benefits of such a step are that water suitable for application in the production process is generated, while the concentrated bicarbonate stream can be used for neutralisation of the raw wastewater. Herewith the costs for caustic soda dosage can be substantially reduced. However, hyperfiltration is an expensive treatment method and whether this may represent an economically feasible solution is doubtful.

8.5 Concluding remarks

In Paragraph 8.3 we proposed that a two-stage system is preferred over a single stage reactor for the start-up of anaerobic bioreactors for treatment of PTA-wastewater. In a two-stage system the length of the lag-phase prior to terephthalate degradation can be significantly reduced. However, with regard to the caustic soda requirements in a hydraulically limited

system a single reactor or a two-stage system operated in parallel is preferred. Taken these considerations into account, and assuming a treatment plant consisting of two identical high-rate anaerobic UASB-reactors, the following steps are suggested to optimise the start-up procedure:

period 1: parallel operation.

During this first period both reactors should obtain sufficient capacity for acetate and benzoate removal. Optimal growth of the acetate and benzoate degrading biomass can be obtained if all biomass is subjected to sufficiently high substrate concentrations, as can easily be realised in a system where both reactors are operated in parallel. It should be noted that by removal of benzoate and acetate from the wastewater, normally treatment efficiencies of 30 to 60 % are obtained. Consequently the loading rate of an aerobic post-treatment step will be significantly reduced. If a sufficient amount of methanogenic granular sludge is available for start-up of the reactors, high treatment efficiencies can normally be obtained within one month of operation. However, when only a flocculent seed-sludge is available, such as digested pig-manure or (digested) sewage sludge, the first period may last several months.

Period 2: operation in series.

Once a high treatment efficiencies for benzoate and acetate has been achieved, the operation should gradually be changed to a sequential mode. Herewith optimal conditions can be established in the second stage reactor for the generation of terephthalate degrading capacity. Since the biomass in the second stage reactor has been fed with acetate and benzoate throughout period 1, it will be able to efficiently degrade the products of terephthalate fermentation (acetate and molecular hydrogen) and consequently an optimal environment is created in the second stage reactor for growth of terephthalate fermenting biomass. If the first stage reactor is capable to degrade benzoate and acetate at high efficiencies, part of the biomass in the first stage can be transferred to the second stage in order to optimise the conditions for terephthalate degradation further.

Period 3: optimisation.

If the system is capable to degrade efficiently acetate and benzoate in the first reactor and terephthalate in the second reactor, the operation can be optimised economically by minimising the caustic soda requirements. In hydraulically limited systems, this may include a gradual change to parallel operation of the two reactors, preferably combined with the transfer of biomass from the second stage to the first stage and vice versa.

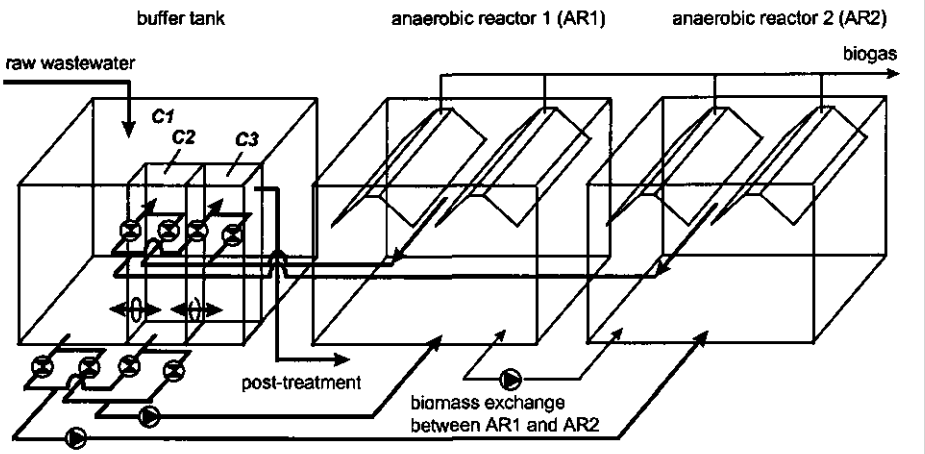


Figure 8.9: Flow-chart consisting of a buffer tank and two anaerobic bioreactors (AR1 and AR2) that can be operated in parallel and plug-flow. C1, C2 and C3 stand for the three compartments of the buffer tank.

The construction of a treatment plant that enables both operation in parallel or in series is not necessarily more complicated than construction of a system that can only be operated in one of these two modes. In this respect it is relevant to note that most of the full-scale anaerobic treatment plants listed in Table 8.1 consist of 2 or more reactor-compartments because of their large volumes. Herewith the construction of a system that can both be operated parallel or in series becomes a matter of installation of adequate piping, valves and pumps. In Figure 8.9 an example is shown of how a system consisting of a buffer tank and two anaerobic bioreactors (AR1 and AR2) may look like. To obtain parallel operation of both reactors, wastewater should be pumped from C1 (or C2) to AR1 and AR2, and the effluent should be returned to C3. For operation in series (with AR2 as the second stage), wastewater to AR1 should be pumped from C1, and to AR2 from C2. The effluent from AR1 should be returned to C2, and from AR2 to C3. For operation in series, both the flow rate to AR1 and AR2 should exceed the flow rate of the raw wastewater. For a gradual transfer from parallel to sequential operation, the influent valves should be equipped with flow controllers that enable mixed flow from C1 and C2.

The presented set-up not only looks attractive for wastestreams that consist of a mixture of slowly and rapidly degradable substrates, but also for wastestreams composed of substrates that are degraded at comparable rates. Operation in series will lead to increased substrate concentrations in the first stage, resulting in relatively high growth rates of the biomass. Due to the lower substrate concentrations, only limited or negative growth will occur in the second stage. Continuous operation in series with either AR1 or AR2 as first stage may

therefore lead to limitations in the biomass concentration in the second stage. This can be overcome either through transferring biomass from AR1 to AR2 and vice versa, or through switching operation using alternating AR1 and AR2 as first stage reactor. This second operational strategy is applied by ADI Systems Inc. for their anaerobic hybrid reactors [18]. In summary it is suggested that optimised treatment of non-toxic wastewater containing substrates that are degraded at comparable rates, is obtained in systems which can be regarded as completely mixed with regard to the biomass, and plug-flow for the wastewater.

With regard to the applicability of high-rate anaerobic PTA-wastewater treatment, we have to wait for the start-up experiences of Biothane Inc. with the newly constructed treatment plant in Turkey (Table 8.1). This two-stage Biobed® system is constructed to operate at high loading rates and in a sequential mode. Successful start-up and operation of this plant may provide the first full-scale reference for two-stage high-rate anaerobic treatment of PTA-wastewater.

Appendix

To get a quantitative idea of the impact of rapidly degradable substrates on the degradation of slowly degradable substrates in a single stage anaerobic bioreactor, a simple kinetic model was developed. As an example, a mixture of acetate, benzoate and terephthalate was used. Kinetic parameters for degradation of the different substrates as described in Chapter 3 were used.

The following assumptions were made to enable the calculations:

1. The total concentration active biomass in the reactor is constant,

This assumption is based on the observation that the active biomass concentrations in well-functioning anaerobic UASB-reactors varies within a limited range. A more mechanistic description of the biomass concentration is highly complex as described in Paragraph 8.3.

2. maintenance and decay related processes are neglected,
3. during start-up, terephthalate is present in excess ($C_{TA} >> K_{TA}$),
4. there is no mutual influence of the different substrates

The mass balances of the total active biomass concentration (X_{tot}, g-VS-COD·l^{-1}) and the concentration of terephthalate degrading biomass (X_{TA}, g-VS-COD·l^{-1}) over the reactor are:

$$\frac{dX_{tot}}{dt} = \mu_{TA}^{max} \cdot X_{TA} + Y_{X_{AcM}C2} \cdot RC_{C2} + Y_{X_{BA}BA} \cdot RC_{BA} - \frac{X_{tot}}{SRT} \qquad (8.1)$$

$$\frac{dX_{TA}}{dt} = \mu_{TA}^{max} \cdot X_{TA} - \frac{X_{TA}}{SRT} \tag{8.2}$$

where RC stands for volumetric removal capacity (g-COD·l^{-1}·d^{-1}), $Y_{X_{AcM}C2}$ and $Y_{X_{BA}BA}$ are the total (lumped) biomass yields for growth on acetate (C2) and benzoate (BA), and μ_{TA}^{max} represents the maximum specific growth rate on terephthalate (TA). As X_{tot} is assumed to be constant, Equation 8.1 can be solved for SRT and substituted into Equation 8.2, resulting in the following equation:

$$\frac{dX_{TA}}{dt} = a \cdot X_{TA} + b \cdot X_{TA}^{2} \tag{8.3}$$

where $a = \mu_{TA}^{max} - \frac{Y_{X_{AcM}C2} \cdot RC_{C2}}{X_{tot}} - \frac{Y_{X_{BA}BA} \cdot RC_{BA}}{Xtot}$ and

$$b = \frac{\mu_{TA}^{max}}{X_{tot}}$$

Integration allows for calculation of X_{TA} as a function of time:

$$X_{TA}(t) = \frac{a \cdot X_{TA}(0) \cdot e^{a \cdot t}}{a - b \cdot X_{TA}(0) + b \cdot X_{TA}(0) \cdot e^{a \cdot t}} \tag{8.4}$$

The reduction of the steady state concentration terephthalate degrading biomass as a function of the acetate and benzoate removal capacity can be calculated from:

$$\lim_{t \to \infty} X_{TA}(t) = \frac{a}{b} = X_{tot} - Y_{X_{AcM}C2} \cdot RC_{C2} - Y_{X_{BA}BA} \cdot RC_{BA} \tag{8.5}$$

and the time needed to obtain a given concentration terephthalate degrading biomass can be calculated from:

$$t = \frac{\ln\left|\frac{X_{TA}(t)}{X_{TA}(0)}\right| + \ln\left|\frac{b \cdot X_{TA}(0) - a}{b \cdot X_{TA}(t) - a}\right|}{a} \tag{8.6}$$

References

1. **Aalderink, H., E. v. Ierland, B. Klapwijk, G. Lettinga, M. Lexmond, and Terpstra (ed.).** 1999. Options for closed water systems: sustainable water managements, vol. 39. IAWQ, London, UK.
2. **ADI-Systems-Inc.** Salem, New Hampshire, USA. 1998. Reference list.
3. **Beeftink, H. H., and P. Staugaard.** 1986. Structure and dynamics of anaerobic bacterial aggregates in a gas-lift reactor. Appl. Environ. Microbiol. **52:**1139-1146.
4. **Benthum, W. v.** 1998. Integrated nitrification and denitrification in biofilm airlift suspension reactors: biofilm development, process design and hydrodynamics. Ph.D. Thesis. Delft University of Technology, Delft, The Netherlands.
5. **Biothane-Systems-International.** Delft, The Netherlands. 1999. Reference list.
6. **Buffle, J.** 1988. Complexation reactions in aquatic systems: an analytical approach. Ellis Horwood Limited, Chichester, UK.
7. **Cheng, S. S., C. Y. Ho, and J. H. Wu.** 1997. Pilot study of UASB process treating PTA manufacturing wastewater. Presented at the 8th Int. Conf. on Anaerobic Digestion, Sendai, Japan, May 25-29, 1997.
8. **Cocci, A. A.** ADI systems Inc, Salem, New Hampshire, USA. 1998. Personal commumnication.
9. **Duffel, J. v.** 1993. Anaerobe behandeling van organische zuren. Presented at the National Conference on Anaerobic Treatment of Complex Wastewaters, Breda, The Netherlands.
10. **Ely, S. R., and G. P. Olsen.** 1989. AMOCO-Process for treatment of PTA-wastewater. USA patent 4,826,600.
11. **Fajardo, C., J. P. Guyot, H. Macarie, and O. Monroy.** 1997. Inhibition of anaerobic digestion by terephthalic acid and its aromatic by products. Water Sci. Technol. **36:**83-90.
12. **Fan, J.** Hepe, Tainan, Taiwan. 1998. Personal communication.
13. **Fang, H. H. P., and G. M. Zhou.** 1997. Anaerobic degradation of benzoate and cresol isomers in sulfate-rich wastewater. Presented at the 8th Int. Conf. on Anaerobic Digestion, Sendai, Japan, May 25-29, 1997.
14. **Fernando, V. F., H. Macarie, M. V. Roberto, R. A. Guillermo, G. A. J. Fransisco, and P. V. Adolfo.** 1998. Acidity, biodegradability and solids limitations in an anaerobic reactor feed with aromatic acids as COD source. Presented at the Fifth Latin-American conference on anaerobic wastewater treatment, Viña del Mar, Chili.
15. **Frankin, R. J., J. van Gils, and R. J. F. Wermeling.** 1994. Full-scale anaerobic treatment of Shell wastewater containing benzoate with the Biothane UASB process. Presented at the 7th IAWQ Int. Symp. Anaerobic Digestion, Cape Town, South Africa.
16. **Houten, R. T. v.** 1996. Biological sulphate reduction with synthesis gas. PhD-thesis. Wageningen Agricultural University, Wageningen, The Netherlands.

17. **Kleerebezem, R., J. Mortier, L. W. Hulshoff Pol, and G. Lettinga.** 1997. Anaerobic pre-treatment of petrochemical effluents: terephthalic acid wastewater. Wat. Sci. Tech. **36**:237-248.

18. **Page, I. C., D. R. Wilson, A. A. Cocci, and R. C. Landine.** 1998. Anaerobic hybrid treatment of terephthalic acid wastewaters. Presented at the 71st Annual Water Environment Federation (WEF) Conference and Exposition, Orlando, Florida, USA, October 1998.

19. **Paques, B. V.** 1997. Reference List, Balk, The Netherlands.

20. **Pereboom, J. H. F., d. G. Man, and I. T. Su.** 1994. Start-up of full scale UASB-reactor for the treatment of terephtalic acid waste water. Presented at the 7th International Symposium on Anaerobic Digestion, Cape Town, South Africa.

21. **Shelley, S.** 1991. And the winner is... Amoco chemical. Chemical Engineering. **98**:90-93.

22. **Solomons, T. W.** 1983. Organic Chemistry, 4th ed. John Wiley &Sons, Inc., New York, USA.

23. **Stumm, W., and J. J. Morgan.** 1996. Aquatic chemistry, chemical equilibria and rates in natural waters. John Wiley and Sons, Inc., New York.

24. **Veeken, A.** 1998. Removal of heavy metals from biowaste, modelling of heavy metal behaviour and development of removal technologies. PhD-thesis. Wageningen Agricultural University, Wageningen, The Netherlands.

25. **Wang, K., and Y. Yang.** . 1993. Personal Communication.

26. **Xu, K., L. Xu, H. Dong, J. Sun, and Q. Zhang.** 1988. Treatment of alkaline terephtalic wastewater by flocculation sedimentation. Water Treatment. **3**:481-490.

Summary

Contents

9.1 Summary

Phthalic acids can be ranked among the group of compounds that play an important role in our human environment. Terephthalic acid can be found in polyester fibres and polyethylene terephthalate -PET- bottles, phthalic acid esters manufactured from *ortho*-phthalic acid is used as plasticizer in PVC, e.g. for toys, and isophthalic acid can be found in numerous polyester resins. With an annual world-wide production of approximately 4 kilogram per capita, phthalic acids comprise an important group of aromatic bulk-chemicals.

All three phthalic acid-isomers (*ortho*, *meta*- and *para*-benzene dicarboxylic acid) are primarily produced from their xylene analogues, derived from crude oil. During production of phthalic acids, wastestreams are generated with a high concentration of organic contamination. The work described in this thesis was aimed at answering the question if anaerobic biological treatment might represent an attractive alternative for conventional aerobic treatment technologies. Some principal advantages of anaerobic treatment over aerobic treatment are (i) the lower nutrient requirements (ii) the lower excess sludge production, and (iii) energy generation through production methane-rich biogas, instead of energy consumption for aeration. However, with regard to the anaerobic biodegradability of various aromatic wastewater constituents almost no information is available. This is particularly true for the phthalic acids, that normally represent 30 to 60 % of the Chemical Oxygen Demand (COD) load of the generated wastewater. We therefore focused our work on the anaerobic degradation of these compounds. Both microbiological (Chapter 2 to 5) and technological aspects of anaerobic phthalic acid degradation were studied (Chapter 6 and 7).

Microbiological aspects

The anaerobic biodegradability of phthalate isomers and some related compounds was studied using batch assays (*Chapter 2*). Two types of granular sludge and digested sewage sludge were used as inoculum. All phthalate isomers and their corresponding methyl esters were found to be degraded by all inocula studied, after lag periods ranging from 17 to 156 days. The observed order in the length of the lag-period prior to degradation was in all cases *ortho*-phthalate < terephthalate < isophthalate.

Using the biomass from the biodegradability experiments and bioreactor biomass, cultures were enriched on the three phthalate isomers (*Chapter 3*). Three enrichment cultures were obtained, degrading either *ortho*-phthalate isophthalate or terephthalate. All three cultures were capable of benzoate degradation without a lag-period. Kinetic parameters were determined for growth of the cultures on the phthalate isomers, benzoate and acetate. A three species model was developed for description of the intermediate acetate and molecular

hydrogen formation, and final production of methane from benzoate and terephthalate. Using this approach, a satisfactory description of the degradation of both substrate by the syntrophic cultures was obtained.

In *Chapter 4* some specific properties of the terephthalate degrading enrichment cultures are described. It was found that incubation of the culture with a mixture of terephthalate and either benzoate or acetate, resulted in an almost complete loss of the terephthalate degrading capacity. Furthermore, a period of only a few hours without substrate had a similar effect. The latter effect could be overcome by dosage of a low concentration of benzoate. Based on these results we suggest that terephthalate degradation is strongly dependent on the degradation of benzoate (presumably the first intermediate formed) in a kind of chain reaction.

In *Chapter 5* experiments with the phthalate isomers grown enrichment cultures, incubated with the phthalate isomers or a benzoate-phthalate mixture and bromoethanosulfonate are described. Bromoethanosulfonate is a specific inhibitor of methanogens. Using this approach, product formation from the phthalate isomers and benzoate fermenting cultures can be studied. It was found that reduction equivalents generated during oxidation of both the phthalates and benzoate were incorporated in benzoate under formation of cyclohexane carboxylate. The free energy change for both benzoate oxidation and reduction were found to be highly comparable, suggesting that within narrow energetic limits, benzoate oxidation and reduction may proceed simultaneously.

Technological aspects

Preliminary studies indicated that the anaerobic degradation of terephthalate is the rate limiting step in the anaerobic treatment of a acetate-benzoate-terephthalate mixture (*Wat. Sci. Technol.* (1997) **36**: 237-248). Acetate, benzoate and terephthalate are the main organic pollutants in wastewater generated during Purified Terephthalic Acid production (PTA-wastewater). Substantial terephthalate degradation in lab-scale Upflow Anaerobic Sludge Bed (UASB) reactors was only obtained after removal of benzoate and acetate from the influent, but terephthalate removal rates remained low. Based on these results we suggested to treat PTA-wastewater in a two-stage reactor concept, enabling pre-removal of acetate and benzoate.

Chapter 6 deals with the optimisation of terephthalate removal from PTA-wastewater in the second stage of a two-stage reactor concept. Reactor studies were conducted with terephthalate as sole carbon and energy source, herewith simulating complete pre-removal of acetate and benzoate. Three UASB-reactors were inoculated with suspended methanogenic

biomass and operated at 30, 37 and 55°C. Only low treatment capacities could be obtained in these reactors as a result of limitations in biomass retention. Batch assays with biomass sampled from the reactors indicated that optimised environmental conditions for terephthalate degradation comprised a temperature of 37 °C and a pH of 7.0. Introduction of polyurethane (PUR) static carrier and operation at 37°C significantly improved the treatment performance of the reactors. After a lag-period of approximately 80 days, the capacity of the reactors increased exponentially. Final treatment capacities in these reactors ranged from 10-17 g-COD·l^{-1}·day^{-1}. These high treatment capacities could largely be attributed to the effective colonisation of the PUR particles and subsequent formation of granular biomass.

In *Chapter 7* the results obtained with a two-stage UASB-reactor for treatment of an acetate-benzoate-terephthalate mixture are described. The first stage UASB-reactor was inoculated with unadapted methanogenic granular sludge and found to be capable of high-rate acetate and benzoate degradation (~ 40 g-COD·l^{-1}·day^{-1}) within one month of operation. Despite lowering of the acetate-benzoate loading rate to 9 g-COD·l^{-1}·day^{-1} from day 150 of operation, no terephthalate degradation was obtained in the first stage until day 300 of operation. From this day onwards, the terephthalate degrading capacity increased rapidly and the maximum terephthalate degradation rates obtained amounted to 15-20 g-COD·l^{-1}·day^{-1}. The reactor furthermore showed a large tolerance to overloading with benzoate and acetate. No clear reason could be identified with regard to the question why it took 300 days to obtain terephthalate degrading capacity in this reactor.

Three parallel operated UASB-reactors were used as second stage. The reactors were inoculated with a small amount of suspended terephthalate degrading biomass and granular methanogenic sludge was added to two reactors. Terephthalate degradation was observed from day 1 of operation and the terephthalate removal rates after 100 days of operation amounted to 15-20 g-COD·l^{-1}·day^{-1} in the reactors inoculated with granular sludge. From this work we conclude that the two-stage reactor concept primarily reduces the time required for start-up of anaerobic bioreactors treating PTA-wastewater. The two-stage reactor concept furthermore enables high-rate anaerobic treatment of PTA-wastewater at a short hydraulic retention time (25 g-COD·l^{-1}·day^{-1} and 6 hours respectively).

In *Chapter 8* engineering aspects of the anaerobic treatment of PTA-wastewater are discussed. Based on considerations concerning the optimal conditions for cultivation of the specific types of biomass, and considerations concerning sodium-hydroxide requirements for neutralisation, a gradual transition from initial operation in parallel to operation in series is suggested for start-up of a two-stage anaerobic bioreactor.

9.2 Samenvatting

Ftaalzuren behoren tot de groep van petrochemische producten die een belangrijke rol spelen in het menselijk milieu. Tereftaalzuur wordt gebruikt voor de produktie van kledingvezels en polyethyleen tereftalaat (PET) flessen voor koolzuurhoudende frisdrank. Esters van *ortho*-ftaalzuur worden toegepast als weekmaker in polyvinyl chloride in b.v. speelgoed, en isoftaalzuur wordt gebruikt bij de produktie van harsen.

Ftaalzuren worden middels chemische oxydatie geproduceerd uit xylenen, gewonnen uit aardolie. Als gevolg van de produktie en het gebruik van water gedurende de produktie van ftaalzuren worden afvalstromen gegenereerd met een hoge concentratie organische verontreiniging. Het doel van het in dit proefschrift beschreven onderzoek was om te bepalen of anaërobe biologische zuivering een goed alternatief, of aanvulling kan zijn voor aërobe zuiveringsmethoden voor deze afvalstromen. Principiële voordelen van anaërobe zuivering ten opzichte van aërobe zuivering zijn: (i) de lagere nutriënten behoefte, (ii) lagere biomassa produktie, en (iii) de produktie van energierijk biogas. Ten aanzien van de anaërobe afbreekbaarheid van de aromatische verbindingen in het afvalwater, is echter weinig informatie beschikbaar. Dit is met name het geval voor de ftaalzuren, die normaal gesproken 30-60 % van de totale organische verontreiniging omvatten van het gegenereerde afvalwater. Daarom is het onderzoek beschreven in dit proefschrift toegespitst op deze groep verbindingen. Zowel microbiologische (Hoofdstuk 2-5) als technologische (Hoofdstuk 6-8) aspecten van de afbraak van ftalaten zijn bestudeerd.

Microbiologische aspecten

De anaërobe afbreekbaarheid van ftalaten en verwante componenten zijn onderzocht met behulp van batch-experimenten (*Hoofdstuk 2*). Als entmateriaal werden twee typen methanogeen korrelslib en slijkgistingsslib gebruikt. De drie ftalaat isomeren en de bijbehorende methyl esters werden allen afgebroken door de drie entmaterialen, na een lag-fase die varieerde in lengte van 17 tot 156 dagen. De volgorde van de lengte van de lag-fase voorafgaande de afbraak van de drie ftalaat isomeren was in alle gevallen *ortho*-ftalaat<tereftalaat<isoftalaat.

De in de afbreekbaarheidsexperimenten gebruikte biomassa, en biomassa uit anaërobe bioreaktoren zijn vervolgens gebruikt voor ophoping van methanogene culturen op de ftalaat isomeren (*Hoofdstuk 3*). Op deze wijze werden drie ftalaat afbrekende syntrofe culturen verkregen die in staat waren om één van de ftalaat isomeren af te breken. De drie culturen konden benzoaat afbreken zonder lag-fase. De microbiële groei beschrijvende kinetische parameters van de drie culturen zijn bepaald voor de ftalaten, benzoaat en acetaat. Uitgaande

van de betrokkenheid van drie typen micro-organismen in de volledige afbraak van de ftalaten en benzoaat is een kinetisch model opgesteld. Met behulp van het model en gemeten en berekende parameters kon de accumulatie van de als intermediair gevormde acetaat en waterstof en de uiteindelijke produktie van methaan worden beschreven.

In *Hoofdstuk 4* worden een aantal specifieke eigenschappen van de tereftalaat afbrekende ophopingscultuur beschreven. Incubatie van de cultuur met een mengsel van tereftalaat en benzoaat of acetaat resulteerde in een nagenoeg volledig verlies van tereftalaat afbrekende capaciteit van de cultuur. Een vergelijkbaar effect werd waargenomen na een periode van enkele uren zonder substraat. Door dosering van een kleine hoeveelheid benzoaat na een periode zonder substraat, kon de tereftalaat afbrekende capaciteit gedeeltelijk worden geregenereerd. Op basis van deze experimentele resultaten stellen wij voor dat de afbraak van tereftalaat sterk afhankelijk is van de gelijktijdige afbraak van benzoaat (waarschijnlijk het eerste intermediair tijdens tereftalaat afbraak) in een soort kettingreaktie.

Hoofdstuk 5 beschrijft de experimenten waarbij de drie ophopingsculturen werden geïncubeerd met de ftalaat isomeren of een benzoaat-ftalaat mengsel in aanwezigheid van bromoethanosulfonaat. Bromoethanosulfonaat is een specifieke remmer van de methanogenese. Op deze manier kan produktvorming gedurende fermentatie van de aromatische substraten worden onderzocht. De resultaten gaven aan dat reduktie-equivalenten die worden gegenereerd gedurende benzoaat en ftalaat oxydatie worden geïncorporeerd in benzoaat onder vorming van carboxy-cyclohexaan. De vrije energie verandering voor benzoaat oxydatie en reductie waren in hoge mate vergelijkbaar, suggererende dat, binnen bepaalde energetische grenzen, benzoaat oxydatie en reductie gelijktijdig kunnen verlopen.

Technologische aspecten

Uit inleidende experimenten hebben we geconcludeerd dat de anaërobe afbraak van tereftalaat de shelheidsbepalende stap is in de afbraak van een acetaat-benzoaat-tereftalaat mengsel (*Wat. Sci. Technol.* (1997) **36**: 237-248). Acetaat, benzoaat en tereftalaat zijn de belangrijkste organische substraten in afvalwater dat wordt gegenereerd tijdens de produktie van PTA (Purified Terephthalic Acid). Substantiële tereftalaat afbraak in labschaal UASB (Upflow Anaerobic Sludge Bed) reaktoren werd alleen waargenomen na verwijdering van acetaat en benzoaat uit het influent, maar de tereftalaat verwijderingscapacitieit bleef laag. Op basis van deze resultaten hebben wij voorgesteld om voor anaërobe zuivering van PTA-afvalwater gebruik te maken van een tweetraps reaktor, teneinde de afbraak van tereftalaat in de tweede trap te kunnen optimaliseren door voorverwijdering van acetaat en benzoaat in de eerste trap.

De experimenten beschreven in *Hoofdstuk 6* waren gericht op de optimalisatie van de tereftalaat afbraak in de tweede trap van een tweetraps reactor concept. Teneinde de volledige voorverwijdering van acetaat en benzoaat te simuleren werden reactor-experimenten uitgevoerd met tereftalaat als enig substraat. Drie UASB-reactoren werden geënt met gesuspendeerd methanogeen slib en bedreven bij 30, 37 en 55°C. Als gevolg van beperkingen in biomassa retentie, konden slechts lage tereftalaat verwijderingscapiciteiten worden verkregen in deze reactoren. Uit batch-experimenten met slib uit de reactoren bleek dat optimale tereftalaat afbraak werd verkregen bij een temperatuur van 37°C en een pH van 7,0. Teneinde de biomassa retentie in de reaktoren te verbeteren werd vervolgens polyurethaan (PUR) dragermateriaal aan de reactoren toegevoegd en de operationele temperatuur werd op 37°C ingesteld. Na een lag-fase van ca. 80 dagen leidden deze maatregelen tot een exponentiële toename van de zuiveringscapaciteit van de reactoren. De uiteindelijke zuiveringscapaciteit die in de drie reactoren kon worden bewerkstelligd varieerde tussen 10-17 g-CZV(Chemisch Zuurstof Verbruik)·l^{-1}·dag^{-1}. Deze hoge zuiveringscapaciteiten waren het gevolg van de vorming van biofilmen op het PUR-dragermateriaal, gevolgd door de vorming van korrelslib.

In *Hoofdstuk 7* worden de resultaten besproken van experimenten met een tweetraps UASB-reactor voor de behandeling van een acetaat-benzoaat-tereftalaat mengsel. De eerste trap reactor werd geënt met ongeadapteerd methanogeen korrelslib en bleek binnen een maand in staat om acetaat en benzoaat af te breken met een hoge snelheid (ca. 40 g-CZV·l^{-1}·dag^{-1}). Ondanks het verlagen van de belasting van de reactor met benzoaat en acetaat tot 9 g-CZV·l^{-1}·dag^{-1} vanaf dag 150, werd gedurende de eerste 300 dagen geen afbraak van tereftalaat waargenomen in de eerste trap. Vanaf dag 300 nam de zuiveringscapaciteit ten aanzien van tereftalaat snel toe, en de uiteindelijke tereftalaat afbrekende capaciteit bedroeg 15-20 g-CZV·l^{-1}·dag^{-1}. Bovendien bleek de reactor in staat om sterke overbelastingen met acetaat en benzoaat te verwerken met slechts een beperkt verlies van tereftalaat afbrekende capaciteit. Geen eenduidig antwoord werd gevonden op de vraag waarom het 300 dagen duurde voordat de reactor in staat bleek om tereftalaat af te breken.

Drie parallel bedreven UASB-reactoren werden gebruikt als tweede trap. De drie reactoren werden geënt met een kleine hoeveelheid tereftalaat afbrekend slib, waaraan in twee reactoren methanogeen korrelslib werd toegevoegd. De met korrelslib geënte reactoren bleken na ca. 100 dagen in staat om tereftalaat af te breken met een snelheid van 15-20 g-CZV·l^{-1}·dag^{-1}. De belangrijkste conclusie uit deze experimenten is dat door toepassing van een tweetraps systeem voor de anaërobe behandeling van PTA-afvalwater, de benodigde tijd voor opstart van de zuivering aanzienlijk kan worden verkort.

In *Hoofdstuk 8* worden een aantal technologische aspecten van de anaërobe zuivering van PTA-afvalwater besproken. Aan de hand van overwegingen betreffende reactor ontwerp en de natronloog behoeften voor pH-sturing, wordt voor opstart van een tweetraps reactor voor behandeling van PTA-afvalwater een geleidelijke overgang in de bedrijfsvoering van parallel naar propstroom voorgesteld.

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[1] Krachtterm naar keuze invullen

Curriculum vitae

De auteur van dit proefschrift werd gedurende een donderende onweersbui geboren op 20 juli 1966 te Zevenaar. In 1984 behaalde hij het Havo-diploma aan de Scholengemeenschap Zuid te Enschede. Hetzelfde jaar werd een aanvang genomen met de studie Chemische Technologie, met als differentiatie Milieutechnologie, aan de HTS te Hengelo. In 1989 werd deze opleiding met succes afgerond. Zich nog niet uitgestudeerd wanend, werd vervolgens aan de Landbouwuniversiteit Wageningen de studie Milieuhygiëne gestart. De afstudeervakken werden uitgevoerd bij de vakgroep Milieutechnologie (anaërobe zuivering van verdund afvalwater) en de sectie Waterkwaliteitsbeheer van de vakgroep Natuurbeheer (modellering van de zuurstofhuishouding in de Ankeveense plassen). Na een afsluitende stage in Taiwan (opstart van een anaërobe zuivering voor de behandeling van PTA-afvalwater) werd de studie in 1992 afgerond. Aansluitend heeft hij gedurende ruim een half jaar gewerkt bij de vakgroep Milieutechnologie van de Landbouwuniversiteit Wageningen aan de afvalwater problematiek bij de suikerfabriek van de Suikerunie te Puttershoek. In 1993 werd een aanvang genomen met het in dit proefschrift beschreven onderzoek. Daarna heeft hij nog enkele korte projecten uitgevoerd bij dezelfde vakgroep, waaronder de modellering van chemische evenwichten in de waterkringloop van een papierfabriek. Voorafgaand aan de verdediging van zijn proefschrift heeft hij 3 maanden gewerkt bij de Staatsuniversiteit Moskou, te Rusland, aan de modellering van een zuivering voor varkensmest. Vanaf Januari 2000 zal de auteur van dit proefschrift als post-doc onderzoeker werkzaam zijn op het gebied van de stikstofkringloop aan de universiteit van Santiago de Compostela, te Spanje.