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A limited LCA of bio-adipic acid: Manufacturing the nylon-6,6 precursor adipic acid using the benzoic acid degradation pathway from different feedstocks(2011) Biotechnology and Bioengineering, 108 (6), pp. 1298-1306.

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Limited LCA of Bio-Adipic Acid

J.B.J.H. van Duuren[1,2,3,5], B. Brehmer[4**], A.E. Mars[1,5], G. Eggink[1,2,5], V.A.P. Martins dos Santos[3,5***], J.P.M. Sanders[4]

[1]Wageningen UR Food & Biobased Research, P.O. Box 17, 6700AA Wageningen, The Netherlands

[2]Wageningen University, Department Bioprocess Engineering, Bomenweg 2, 6781 EV Wageningen, The Netherlands

[3]Helmholtz Centre for Infection Research, Systems and Synthetic Biology, Inhoffenstraβe 7, 38124 Braunschweig, Germany

[4]Wageningen University, Valorization of Plant Production Chains, Bornsesteeg 59, 6708PD Wageningen, The Netherlands

[5]Kluyver Centre for Genomics of Industrial Fermentation, P.O. Box 5057, 2600 GA Delft, The Netherlands

September 2010

[*]Corresponding author. mailing address: Helmholtz Centre for Infection Research, Inhoffenstrasse 7, 38124 Braunschweig, Germany. Phone +49-315775682698. Fax +49-53164049. E-mail: joost.vanduuren@helmholtz-hzi.de

[**]Present address: Evonik Degussa GmbH, Creavis Technologies & Innovation, Paul-Baumann-Strasse 1, 45772 Marl, Germany

[***]Present address: Wageningen University, Laboratory of Systems and Synthetic Biology, Dreijenplein 10, 6703 HB Wageningen, The Netherlands

Abstract

A limited life cycle assessment (LCA) was performed on a combined biological and chemical process for the production of adipic acid, which was compared to the traditional petrochemical process. The LCA comprises the biological conversion of the aromatic feedstocks benzoic acid, impure aromatics, toluene or phenol from lignin to *cis*, *cis*-muconic acid, which is subsequently converted to adipic acid through hydrogenation. Apart from the impact of usage of petrochemical and biomass-based feedstocks, the environmental impact of the final concentration of *cis*, *cis*-muconic acid in the fermentation broth was studied using 1.85% and 4.26% *cis*, *cis*-muconic acid. The LCA focused on the cumulative energy demand (CED), cumulative exergy demand (CExD) and the CO_2 equivalent (CO_2eq) emission, with CO_2 and N_2O measured separately. The highest calculated reduction potential of CED and CExD were achieved using phenol, which reduced the CED by 29% and 57% with 1.85% and 4.26% *cis*, *cis*-muconic acid, respectively. A decrease in the CO_2eq emission was especially achieved when the N_2O emission in the combined biological and chemical process was restricted. At 4.26% *cis*, *cis*-muconic acid, the different carbon backbone feedstocks contributed to an optimized reduction of CO_2eq emissions ranging from 14.0 to 17.4ton CO_2eq/ton adipic acid. The bulk of the bioprocessing energy intensity is attributed to the hydrogenation reactor, which has a high environmental impact and a direct relationship with the product concentration in the broth.

Keywords

Adipic acid, Biotechnology, *cis*, *cis*-Muconic acid, Energy, Exergy, LCA

Introduction

Ever since its discovery in the late 1930's, nylon-6,6 has been intrinsically linked to the production of adipic acid, being the main intermediate reactant. Adipic acid is a high volume bulk petrochemical commodity as is reflected in its low selling price, 1315 – 1385$/ton (2008), 1120 – 1190$/ton (2009) and high consumption volumes, 2.6 Mton worldwide (2006) (ICIS Pricing and ICIS News). Adipic acid has several commercial production route options, all of which originate from fossil fuels. The most common is the nitric acid oxidation of a cyclohexanol/cyclohexanone mixture derived from cyclohexane. Cyclohexane itself originates from naphtha benzene and nitric acid originates from ammonia, which in turn is most commonly produced from natural gas. N_2O is a byproduct in the process of nitric acid oxidation (Shimizu et al., 2000). Adipic acid is thus associated with a high fossil fuel energy demand and a high level of N_2O greenhouse gas emission. The cumulative energy demand (CED), which includes the total fossil fuel feedstock cost and all subsequent process energy inputs, has been reported to be 104.2GJ/ton (Patel, 2003). As a consequence of modern catalytic decomposition techniques and tail gas treatment, on average the current state-of-the-art will reduce the 301kg/ton N_2O by 80%, which will be selected for comparison in this study (EcoInvent Databank V2.1).

An alternative production route for adipic acid could occur via biological conversion of benzoic acid or other aromatics to *cis*, *cis*-muconic acid, which can be easily converted to adipic acid by hydrogenation under slightly elevated pressure (3.5bar) and electric potential for 3 hours (Draths and Frost, 1993). Several bacteria have been described that convert benzoic acid to *cis*, *cis*-muconic acid (Bang and Choi, 1995; Mizuno et al., 1988; Schmidt and

Knackmuss, 1984). Recently a derivative of *P. putida* KT2440 (*P. putida* KT2440-JD1), which can no longer use benzoic acid as a carbon source, but still co-metabolizes benzoic acid to *cis*, *cis*-muconic acid when grown on glucose was isolated (van Duuren(1) et al., submitted). *P. putida* KT2440 is a soil bacterium with a versatile metabolism. It is able to convert several aromatic compounds, such as derivates of lignin proceeding from the recycling of plant derived material (Jiménez et al., 2002). Furthermore, the genome has been sequenced, the strain is genetically accessible, and it is the first Gram-negative soil bacterium that was certified as a safety strain by the Recombinant DNA Advisory Committee (Federal register. Certified host-vector systems; Nelson et al., 2002; Ramos et al., 1987). In this respect, it has significant potential for biotechnological applications, particularly those involving bioremediation and biotransformation (Wackett, 2003). One of the most influential parameters in the life cycle performance of chemical production systems is the feedstock (Hatti-Kaul et al., 2006). Various *P. putida* species have been described that can metabolize other aromatic compounds (Feist and Hegeman, 1969; Panke et al., 1998; Tao et al., 2004; Wackett, 2003). Since *P. putida* is genetically accessible, it is likely that *P. putida* KT2440-JD1 can be modified in such a way that it can convert the petrochemical feedstocks benzoic acid, toluene, and impure aromatics (e.g. collected from waste of the benzene, toluene, xylene (BTX) process), as well as phenol from the biomass-based feedstock lignin to *cis*, *cis*-muconic acid (Haigler et al., 1992).

P. putida KT2440-JD1 has the highest measured specific production rate of *cis*, *cis*-muconic acid (van Duuren(1) et al., submitted). The highest final concentration of *cis*, *cis*-muconic acid that has been achieved was 4.4% in a bioprocess with a fed-batch process using *Arthrobacter* sp. (Mizuno et al., 1988). A final concentration of *cis*, *cis*-muconic acid of 1.85% with *P. putida* KT2440-JD1 during a small-scale pH-stat fed-batch process was

reached. At this concentration, benzoic acid was only converted to the intermediate catechol; known to be toxic to cells (van Duuren(2) et al., submitted). It may be possible to optimize this conversion leading to a higher final concentration of *cis, cis*-muconic acid, because benzoic acid can be converted by *P. putida* KT2440-JD1 in the presence of up to 4.26% of *cis*, *cis*-muconic acid (vanDuuren(2) et al., submitted).

In this study, the overall goal was to assess the possibility to reduce the environmental impact of the current petroleum-based adipic acid production by comparing different carbon backbone feedstocks (petrochemical and biomass-based) for the combined biological and chemical production of adipic acid using a limited life cycle assessment (LCA). Moreover, the environmental impact of an increase in the final concentration of *cis*, *cis*-muconic acid was studied at two final fermentation broth concentrations of 1.85% (option 1) and 4.26% (option 2). At 104.2GJ/ton, 60.2kgN_2O/ton and 5.1tonCO_2/ton there is a large potential to reduce the environmental impact of adipic acid production by employing bio-processing techniques based on aqueous solutions.

Methodology

Biological production of cis, cis-Muconic Acid

cis, *cis*-Muconic acid has been produced by *P. putida* KT2440-JD1 in a small-scale pH-stat fed-batch process using benzoic acid as a feedstock (van Duuren(2) et al, submitted). The data rendered from this study were used in the limited LCA as a model process regarding the assessment of conditions of biological production systems.

Release of N_2O in the Petrochemical Industry

Catalytic techniques to decompose N_2O to elemental nitrogen and oxygen (an exothermic reaction) operate at elevated temperatures and can effectively reduce the overall N_2O emission and if the decomposition of the tail gas is performed under adiabatic conditions the heat of the reaction will continue the decomposition to a total emissions reduction of 99.9% (Shimizu et al., 2000). In these recently outfitted production plants the resulting emission of N_2O can be as low as 0.3kg per ton of adipic acid (EcoInvent Databank V2.1). On a worldwide scale, only an average of 80% N_2O emission reduction is actually realized with these tail-gas abatement technologies. In this respect, the production of adipic acid is still associated with for more than 44.8Mton CO_2 equivalent (CO_2eq) or about 60.2kg N_2O emission per ton of adipic acid. This average will be used to denote the current state-of-the-art reduction potential in this study.

LCA Goal and Scope

The standard methodology of LCA based on the ISO14040 series for environmental management systems was followed to a great extent with the exception that only the CED and cumulative exergy demand (CExD) were documented with the CO_2eq emission (CO_2 and N_2O measured separately) as the only environmental considerations (International Organization for Standardization, 2006). This specific setup of LCA was performed due to the interest of the study. The apparent environmental impact, namely the reduction of fossil fuel and greenhouse gas emissions of the compared production routes was to be assessed. Exergy, the quality of energy or maximum obtainable work, was mainly included to provide further depth in the energy efficiency of the processes and as an indication of future energy efficiency improvements. The cradle was set at the point of base fossil fuel delivery (naphtha, diesel, natural gas, etc.) including their respective extraction values. The grave was set at the factory gate for nylon-6,6 production, essentially the delivery point of a pure adipic acid. The

boundary was effectively confined to the process. This particular LCA technique is called an exergetic life cycle assessment (or E-LCA), but is in essence a limited exergetic cradle-to-factory gate assessment.

Data and System

GaBi and its associated datasets were used to accumulate life cycle inventory (LCI) data (PE International, GaBi 4). It is vital to set realistic and comparable factory gates in the LCI. The carbon contained in feedstocks of naphtha products from the petrochemical route is not yet released into the environment but is contained in the chemical end product. With this perspective, the analysis was partially taken beyond the factory gate and to the grave, when decades or centuries from now the nylon is presumably disintegrated or incinerated. Only at this stage will the petrochemical material feedstocks receive an extra emission charge, which was already incorporated according to common LCA practice. As the final products are identical, any other emissions associated with combustion would be the same and cancel each other out.

Bioprocessing Demands

Aspen^{+} was used to calculate the internal process energy requirements of the experimental set-up. Figure 1 highlights the system, unit operations and input streams involved. Two final broth concentrations of *cis*, *cis*-muconic acid of 1.85% (option 1) and 4.26% (option 2) were studied. Several process considerations were made in light of the experimental bioreactor, hydrogen reactor, and the succeeding separation and product isolation techniques:

- "FERMENT" – Bioreactor: 30°C, 1bar and 51 hours residence time
- "HYDROGEN" – Hydrogenation reactor: 30°C, 3.5bar, 3h and electrically charged

- "EVAPORAT" – Evaporator: 100°C, 3.5bar to remove water
- "CONDENSE" – Condenser: 20°C, 1bar to recover a large portion of the evaporator duty
- "CRYSTAL" – Crystallizer: 50°C, 1bar to isolate product

The heat streams were based on a simple fuel boiler system that has a heating efficiency of 85% energy and 45% exergy (Szargut et al., 1988). The hydrogenation reactor is pressurized and electrically charged having an overall electric production efficiency mix of 45% energy and 35% exergy.

Feed-Level Demands for Bacterial Growth

For each mol *cis*, *cis*-muconic acid produced, 2 mol sodium hydroxide, and 2.28 mol (option 1) or 2.12 mol (option 2) hydrogen chloride were incorporated to maintain the fermentation broth at pH 7 during the fermentation and to acidify the fermentation broth to pH 2.5. Benzoic acid and *cis*, *cis*-muconic acid dissociate in water at pH 7 to the more soluble form of benzoate and *cis*, *cis*-muconate. The various streams flowing into the bioreactor need to be handled individually to assess their CED, CExD and emissions levels:

- Salts, nutrients and buffer: based on best available technology of the fertilizer industry (Brehmer et al., 2008; International Fertilizer Association)
    - Salts: $MgCl_2 \cdot H_2O$ – dolomite limestone mining (Brehmer(1), 2008; US Department of Energy)
        - CED: 0.1GJ/ton, CExD: 0.2GJ/ton
        - CO_2: 5.4kg/ton, N_2O: 0.0kg/ton

- Nutrient: $(NH_4)_2SO_4$ – ammonia and sulphuric acid production (Brehmer(1), 2008, Brehmer and Sanders, 2007)
  - CED: 3.7GJ/ton, CExD: 4.1GJ/ton
  - CO_2: 228.0kg/ton, N_2O: 208.0kg/ton
- K_2HPO_4 – phosphorous acid and potassium oxide production (Brehmer(1), 2008)
  - CED: 1.8GJ/ton, CExD: 2.6GJ/ton
  - CO_2: 117.0kg/ton, N_2O: 0.0kg/ton
- Buffer: $NaH_2PO_4 \cdot 2H_2O$ – mineral mining and phosphorous acid production (Brehmer(1), 2008; US Department of Energy)
  - CED: 0.5GJ/ton, CExD: 1.0GJ/ton
  - CO_2: 33kg/ton, N_2O: 0.0kg/ton

- Sodium hydroxide (EcoInvent Databank V2.1; PE International, GaBi 4)
  - CED: 24.8GJ/ton, CExD: 32.1GJ/ton
  - CO_2: 1063.6kg/ton, N_2O: 0.0kg/ton
- Hydrogen chloride (EcoInvent Databank V2.1; PE International, GaBi 4)
  - CED:4.1GJ/ton, CExD: 4.5GJ/ton
  - CO_2:235.3kg/ton, N_2O: 6,8kg/ton

Glucose, like any biomass feedstock, is commonly believed to be carbon neutral consuming as much atmospheric CO_2 as is emitted through its eventual decomposition (biological combustion). Nevertheless, because fossil fuels still are consumed, greenhouse gases are emitted throughout the agricultural cultivation and feedstock preparation process chain.

Figure 2 is a simple process diagram of the steps involved in glucose production with the following results:

- Glucose: based on sucrose content of sugar beets for glucose production in Holland (Brehmer(2), 2008; Ministry of Agriculture Nature and Food Quality)
  - CED: 8.3GJ/ton, CExD: 8.7GJ/ton
  - CO_2: 508.0kg/ton, N_2O: 23.3kg/ton

Bioreactor-Feedstock Demands

Four different potential feedstocks were used in the LCA: benzoic acid, impure aromatics, and toluene are petrochemical based and phenol from lignin is biomass based. Since these feedstocks have different energetic and exergetic values and various sources of origin, along with the additional process energy needed to acquire the fraction, their CED, CExD and CO_2eq emission values must be assessed separately.

To obtain benzoic acid and toluene, oil production and refinery are taken into account. Furthermore, as described the stoichiometric carbon content was included in the carbon dioxide emission calculations. An exergy ratio of 1.05 was adapted for the naphtha-based chemicals benzoic acid and toluene, which corresponds to the standard ratio of chemical energy/exergy of naphtha products.

Impure aromatics have the potential to be converted to *cis*, *cis*-muconic acid by *P. putida* KT2440-JD1. These compounds (e.g. benzoic acid) are primarily collected during the BTX purification process. Therefore impure aromatics are considered a major waste product, as they are an uneconomical residue from the distillation of crude oil. Being a waste product, no additional processing inputs are allocated to their production. Consequently, impure aromatics

have a CED and CExD demand coupled directly to their chemical formation energy/exergy and no extra CO_2eq emissions are considered.

Lignin is a special case being a highly complex molecule. Cleaving lignin via biological delignification (white rot fungi) will release countless different types of aromatic molecules. Due to the vast quantity and diversity of the molecules, economic delignification and isolation techniques are currently unavailable. In any case, phenol derived from lignin is present in higher concentrations and will receive its own theoretical energetic process burden based on its chemical formation energy/exergy and associated agricultural system.

The potential feedstocks are:

- Benzoic acid: derived from oxidized toluene (Patel, 2003; Szargut et al., 1988)
  - CED: 62.4GJ/ton, CExD: 65.5GJ/ton
  - CO_2: 3735.0kg/ton, N_2O: trace ≈ 0.0kg/ton
- Impure aromatics: derived from an oil cracker (Internal calculations)
  - CED: 25.4GJ/ton, CExD: 27.4GJ/ton
  - CO_2: 0.0kg/ton, N_2O: 0.0kg/ton
- Toluene: derived from benzene (Panke et al., 1998; Patel, 2003; Szargut et al., 1988)
  - CED: 56.4GJ/ton, CExD: 59.2GJ/ton
  - CO_2: 4190.0kg/ton, N_2O: trace ≈ 0.0kg/ton
- Phenol derived from lignin: derived from wheat stover grown under Dutch conditions (Brehmer(1), 2008; Brehmer(2), 2008)
  - CED: 11.2GJ/ton, CExD: 13.8GJ/ton
  - CO_2: 682.0kg/ton, N_2O: 3.9kg/ton

Limitations

For the conversion of benzoic acid to *cis*, *cis*-muconic acid no net consumption of co-factors like NADH are required, while for each mol toluene or phenol one mol NADH is produced or consumed, respectively. Hence the biomass yield (g dry cell weight (dcw)/g glucose) might increase with toluene and decrease with phenol. This hypothesis and its impact on the overall system dynamics were not taken into account. Furthermore, other aromatic feedstocks are also foreseeable, but were not assessed.

Apolar compounds such as phenol and toluene are generally not well soluble in water and often toxic to whole cells as they accumulate in the cytoplasmic membrane. As a consequence the membrane loses its integrity and increases its permeability (Ramos et al, 2002). Technical solutions to this problem are two-liquid-phase media, gas-phase biocatalysis and feeding under controlled conditions such as the pH-stat fed-batch process (Schmid et al., 2001). For the LCA, the pH-stat fed-batch process is the only biological production system considered.

Residual minerals can be separated from the product by (re-)crystallization from methanol (Mizuno et al., 1988). This option and the impact on the overall system dynamics was however not taken into account.

Calculations

Bioreactor System

In the small-scale pH-stat fed-batch process a molar production yield (mol *cis*, *cis*-muconic acid acid/mol benzoic acid) of 96% was achieved with *P. putida* KT2440-JD1 (van Duuren(2)

et al., submitted). This is further assumed for all four different feedstocks, as they are all similar aromatic compounds that can be converted to *cis*, *cis*-muconic acid.

Energy and Emission

Aspen$^+$ lists the resulting energy duties of the internal bioprocessing in terms of flows, i.e. GJ/h. Consequently, the bioprocessing demands in CED, CExD and CO_2eq emission values per ton pure adipic acid can be calculated for both final broth concentrations of *cis*, *cis*-muconic acid and are presented in Table I. The feed-level demands for growth, maintenance, and pH regulation are listed in Table II for the bioreactor at both final broth concentrations of *cis*, *cis*-muconic acid per ton pure adipic acid, which provide the basis for calculating the associated CED, CExD and CO_2eq emission values. Hereby it is assumed that for option 2 the duration and medium consumption is similar, independent of the different specific productivity and final concentration of *cis*, *cis*-muconic acid. Furthermore, for each of the four feedstocks based on the stoichiometric conversion rate and respective feed level per final product, the associated CED, CExD and CO_2eq emission per ton pure adipic acid were calculated (Table III). Figure 3 illustrates the resulting potential CED, CExD and CO_2eq emissions induced for option 1 and 2 compared to the traditional petrochemical production route of adipic acid. As described, the greenhouse gas emissions are combined and expressed as the total CO_2eq emission, but special consideration should be paid to the major reduction potential N_2O.

Results and Discussion

Reduction of Fossil Fuel and Related Greenhouse Gas Emission

The production of *cis*, *cis*-muconic acid from benzoic acid by *P. putida* KT2440-JD1 in a pH-stat fed-batch process (van Duuren(2) et al., submitted) was used as a model system for the limited LCA of a combined biological and chemical process for the production of adipic acid. The data in Table II and III reveal that glucose, sodium hydroxide and the feedstock for the carbon backbone of *cis, cis*-muconic acid play important roles in the total fossil fuel energy demand and greenhouse gas emission of the system. For the low final product concentration of option 1, use of the chemical pure laboratory benzoic acid feedstock did not contribute to an environmental impact decrease (Figure 3). Compared to benzoic acid, impure aromatics led to an increase in the overall fossil fuel reduction potential: for option 1 from -17.8GJ/ton and -35.3GJ/ton to 15.4GJ/ton and -1.2GJ/ton CED and CExD, respectively and for option 2 from 10.7GJ/ton and 2.0GJ/ton to 43.9GJ/ton and 36.2GJ/ton CED and CExD, respectively. This reveals the huge potential of employing impure aromatics over benzoic acid as a feedstock. The robustness and productivity of *P. putida* KT2440-JD1 with impure aromatics however remains to be determined. The other pure petrochemical feedstock toluene has a neutral fossil fuel reduction potential for option 1 at 0.0GJ/ton CED and -16.7GJ/ton CExD. The most sustainable option would be using the biobased material lignin for phenols: for option 1 and 2 the highest calculated reduction potential of CED (30.4 and 58.9 GJ/ton respectively) and CExD (13.8 and 51.1 GJ/ton respectively) are achievable. When phenol from lignin is used as a carbon source the CED would decrease 29% (30.4 GJ/ton) for option 1 and 57% (58.4 GJ/ton) for option 2 compared to the traditional petroleum-based production route. As the biological delignification and isolation techniques improve, a switch to a lignin-based feedstock is foreseeable (Chakar and Ragauskas, 2004).

For both product concentrations the CO_2eq emissions are reduced for all of the different carbon backbone feedstocks (petrochemical and biomass-based). For option 2 the reduction is

higher, which relates to a lower consumption of glucose and ammonium sulphate per ton product. A reduction in CO_2eq emission can especially be achieved when the N_2O emission is also restricted in the combined biological and chemical process for the production of adipic acid (Figure 3). As a first option, it might be advantageous to use the protein rich co-product slurry of the glucose production to offset ammonium sulphate of the feed-level demands. This would reduce the N_2O emissions of the feed-level demands for ammonium sulphate to practically zero, achieving a decrease of an average of 47% or 38% of the CO_2eq emissions for option 1 and 2, respectively. Therefore, in Figure 3 the optimized CO_2eq extinction is added representing these calculations. For option 1 and 2, the optimized CO_2eq emissions concern a reduction in the magnitude ranging from 9.8 to 13.1 and 14.0 to 17.4 ton CO_2eq/ton adipic acid, respectively.

Increasing the final product concentration of *cis*, *cis*-muconic acid in the bioreactor will result in a reduction of glucose and NPK per ton product reducing the environmental impact, assuming that the requirements of *P. putida* KT2440-JD1 for growth and production are constant. By increasing the final concentration less water is present, which directly results in less bioprocessing energy per ton adipic acid. The required energy is directly proportional to the concentration of *cis*, *cis*-muconic acid in the broth. It is imperative that any future biotechnological work should aim for the highest solid loading possible. The bulk of the bioprocessing energy is attributed to the hydrogenation reactor, therefore this effect is greater for this process step than any other unit. As shown in option 2 with 4.26% *cis*, *cis*-muconic acid, the energy required for the hydrogenation was 15.1GJ/ton whereas in option 1 with only 1.85% 35.1GJ/ton was required (Table I). In option 2 together with the lower feed-level demands this reduced the bioprocessing energy by more than 50%, which even facilitated an energy mitigation potential for benzoic acid. A further doubling of the final product

concentration to beyond 8% would result in a highly competitive system, which might be feasible since *P. putida* KT2440-JD1 can grow in mineral medium up to 8.7% *cis*, *cis*-muconic acid (van Duuren(2) et al., submitted). Alternatively, the high energy intensity of the hydrogenation-evaporation process of the *cis*, *cis*-muconic acid-containing fermentation broth could be reduced by extracting *cis*, *cis*-muconic acid from the acidified broth with e.g. cold diethyl ether (Schmidt and Knackmus, 1984). This will result in a much higher concentration of *cis*, *cis*-muconic acid in cold diethyl ether, and the evaporation will require much less energy since the boiling temperature of cold diethyl ether (34.6°C) is much lower than water. Furthermore, the solvent used for extraction purifies the product from salts and should have a high extraction efficiency, since this has a large influence on the environmental impact.

Biological Process

Compared to existing small-scale biological production systems (Bang and Choi, 1995; Mizuno et al., 1988; Schmidt and Knackmuss, 1984), the small-scale pH-stat fed-batch process with *P. putida* KT2440-JD1 has allowed for a further optimization of the biological production of *cis, cis*-muconic acid (van Duuren(2) et al., submitted). The volumetric productivity (g/L) of the process could be further improved by applying a cell-recycle system, since this will increase the cell density. Choi et al. (1997) were the first who addressed the cell-recycling issue for *cis*, *cis*-muconic acid production, and reached a volumetric productivity of 5.5 g/(L·h). Unfortunately, they were unable to maintain a continuous recycle system for more than 2 days due to membrane fouling. By combining the pH-stat fed-batch bioprocess with a cell retention system, the volumetric productivity of a cell-recycle bioreactor is expected to increase significantly, assuming that the pH-control still is accurate at high cell densities. While this only has a minor impact on the energy consumption (Table I

(bioreactor)), it does have a major impact on the economic parameters of an industrial scale bio-reactor. Another positive effect is that recycling of biomass would reduce the consumption of glucose and NPK, which lowers the environmental impact of the bioprocess. To retain the cells, new process technology in the form of membrane separation must first be resolved with cell fouling stipulating the main hurdle.

Conclusions of the Limited LCA

The environmental impact concerning the CED, CExD and CO_2eq emission of the manufacturing of the nylon-6,6 precursor adipic acid can be reduced by replacing the current petroleum-based chemical process with a combined biological and chemical process that is based on a small-scale pH-stat fed-batch bioprocess to produce *cis, cis*-muconic acid using *P. putida* KT2440-JD1 (van Duuren(2) et al., submitted). The usage of different carbon backbone feedstocks (both petrochemical and biomass-based), the final product concentration in the fermentation broth, and the level of N_2O emission have a large influence on the environmental impact. At a final concentration of 1.85% *cis, cis*-muconic acid in the fermentation broth, the CED is only improved when using impure aromatics or phenol derived from lignin. Should the bioprocess be further optimized to increase the final concentration of *cis, cis*-muconic acid to 4.26%, the CED and CExD are reduced for all the assessed feedstocks (benzoic acid, impure aromatics, toluene, and phenol). Additionally, the CO_2eq emissions for option 2 are reduced more as for option 1. In order to further reduce the CO_2eq emission compared to the petrochemical process, it is assumed that the N_2O emission can be actively decreased in the combined biological and chemical process by using the

protein rich co-product slurry of the glucose production to offset ammonium sulphate of the feed-level demands. The highest reductions were achieved with impure aromatics and phenol. Several possibilities exist to improve the environmental and economical impact of the bioprocess, like i) improvements of *P. putida* KT2440-JD1 by enhancing the catechol 1,2-dioxygenase activity, reducing the sensitivity of the enzyme to putative product inhibition by *cis, cis*-muconic acid in the cell, increasing the possibly limiting transport of *cis*, *cis*-muconic acid through the cell membrane, and extension of the range of aromatic feedstocks that can be co-metabolized to *cis, cis*-muconic acid; ii) enhancement of the volumetric productivity of the bioprocess and reduction of the demand for nutrients by recycling the biomass; iii) reduction of the energy demand of the hydrogenation-evaporation process by extraction of *cis, cis*-muconic acid from the fermentation broth with e.g. diethyl ether.

A huge potential for employing impure aromatics has been revealed as well as for phenol derived from the biological delignification and isolation techniques. Yet, the robustness and productivity of *P. putida* KT2440-JD1 with impure aromatics remains to be determined, while the biological delignification and isolation techniques have to be improved considerably before a switch to a lignin-based feedstock is foreseeable.

Acknowledgements

This research was carried out within the research programme of the Kluyver Centre for Genomics of Industrial Fermentation, which is part of the Netherlands Genomics Initiative.

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