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The effect of fuel alcohol on monoaromatic hydrocarbon biodegradation and natural attenuation

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ABSTRACT. The proposed replacement of the gasoline oxygenate MTBE with ethanol represents potential economic and environmental quality benefits. However, these benefits may be offset to some extent by potential detrimental effects on groundwater quality and natural attenuation of released petroleum products. The objectives of this literature review are to bound the extent to which these impacts may occur, summarize the available information on the biodegradation of ethanol in the environment, assess the potential effect that biodegradation processes may have on the fate and transport of BTEX compounds, and provide recommendations for research to enhance related risk assessment and management decisions.

Ethanol that reaches groundwater aquifers is likely to be degraded at much faster rates than other gasoline constituents. If the carbon source is not limiting, a preferential degradation of ethanol over BTEX may be observed under both aerobic and anaerobic conditions. Depending on the extent of the release, ethanol may exert a high biochemical oxygen demand that would contribute to the rapid depletion of dissolved oxygen in the groundwater. Thus, ethanol will likely be degraded predominantly under anaerobic conditions. None of the potential ethanol metabolites that could accumulate in groundwater are toxic, although some potential biodegradation by-products such as butyrate could adversely affect the taste and odor of drinking water sources. In addition, acetate and other volatile fatty acids could accumulate at high concentrations, causing a pH decrease in poorly buffered systems. It is unknown, however, whether the pH would decrease to a point that inhibits natural degradative processes.

Inhibition of microbial, activity near the source is likely to occur as a result of exposure to high alcohol concentrations, and bactericidal effects are likely to occur when cells are exposed to ethanol concentrations exceeding 10,000 mg/L. However, the maximum allowable ethanol content in gasoline is 10% by volume in the United States. Thus, such high ethanol concentrations are unlikely to be encountered at sites contaminated with ethanol-gasoline blends, except near the fuel/water interfaces or in the case of neat ethanol releases. Downgradient of the source area, biodegradation is unlikely to be inhibited by alcohol toxicity as concentrations decrease exponentially with distance.

The preferential degradation of fuel alcohols by indigenous microorganisms and the accompanying depletion of oxygen and other electron acceptors suggest that ethanol could hinder BTEX bioremediation. This is particularly important for the fate of benzene, which is the most toxic BTEX compound and the most recalcitrant under anaerobic conditions. Alternatively, ethanol represents a carbon and energy source that is likely to stimulate the growth of a variety of aerobic and anaerobic microbial populations, including those that can degrade BTEX compounds. A higher concentration of BTEX degraders would be conducive to faster BTEX degradation rates under carbon-limiting conditions. Nevertheless, controlled studies that assess the overall effect of ethanol on BTEX bioremediation are lacking.

RESUMEN. El reemplazo de la gasolina oxigenada que contiene MTBE por etanol representa un potencial económico y beneficio ambiental. Sin embargo, dichos beneficios pueden ser inapropiados, debido a un efecto del detrimento potencial de la calidad del agua subterránea y de los productos del petróleo liberados durante la atenuación natural. El objetivo de esta revisión es enlazar a algún nivel a los cuales dichos impactos que puedan ocurrir, resumir la información disponible sobre la biodegradación de etanol en el medio ambiente, ensayar los efectos potenciales que los procesos de biodegradación puedan tener sobre la velocidad y transporte de los BTEX y proveer las recomendaciones para realizar la investigación que mejoren la evaluación de riesgo y el manejo de decisiones.

El etanol que llega al acuífero del agua subterránea es similarmente degradado a velocidades más elevadas que otros constituyentes de la gasolina. Si la fuente de carbono no es limitante, se puede observar una degradación preferencial del BTEX bajo ambas condiciones aeróbicas y no aeróbicas. Dependiendo del nivel de liberación, el etanol puede ejercer una alta demanda bioquímica del oxígeno que pudiera contribuir al rápido agotamiento de oxígeno disuelto en aguas subterráneas. Así, el etanol probablemente será degradado predominantemente bajo condiciones anaeróbicas. Ninguno de los metabolitos potenciales de etanol que pudieran acumularse en las aguas subterráneas es tóxico, a pesar de que algunos sub-productos potenciales tóxicos, tales como butiratos pudieran afectar adversamente el sabor y el olor de fuentes de agua bebible. Además, el acetato y otros ácidos grasos volátiles pudieran acumularse a altas concentraciones, causando un decrecimiento en el pH en sistemas pobremente amortiguados. Sin embargo, se sabe, que el pH pudiera disminuir a un punto que inhibiera los procesos degradativos naturales.

Es probable que ocurra la inhibición de la actividad microbiana cerca del origen (manantial) como resultado de la exposición a altas concentraciones de alcohol, y el efecto bactericida probablemente ocurra cuando las células son expuestas a una concentración excedida de etanol de 10,000 mg/L. Sin embargo, en Estados Unidos las concentraciones máximas permitidas de etanol en gasolina es de 10% por volumen. De este modo, tales concentraciones altas de etanol son poco probables que puedan ser encontradas en sitios contaminados con mezclas de etanol-gasolina, excepto cerca de las interfaces combustible/agua o en el caso de la liberación de etanol puro. La pendiente de un gradiente del área de origen, es improbable la biodegradación por ser inhibida por la toxicidad del alcohol, cuando las concentraciones decrecen exponencialmente con la distancia.

La degradación preferencial de combustibles alcohólicos por microorganismos autóctonos y la disminución del oxígeno y otros aceptores de electrones, sugieren que el etanol pudiera impedir la biorremediación de BTEX. Esto es particularmente importante por el destino del benceno, el cual es el compuesto más tóxico de los BTEX's y el más recalcitrante bajo condiciones anaeróbicas. Alternativamente, el etanol representa una fuente de carbono y energía que probablemente estimula el crecimiento de una variedad de poblaciones

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Rev Latinoam Microbiol 2002; 44 (2): 83-104

In theory, ethanol could also contribute to longer BTEX plumes by enhancing BTEX solubilization from the fuel phase and by decreasing sorption-related retardation during transport. The overall effect of ethanol on BTEX plume length and treatment end points is likely to be system specific, and will depend largely on the release scenario and on the buffering and dilution capacity of the aquifer.
Additional research is needed to understand the effect of ethanol on the stability and dimensions of co-occurring and pre-existing BTEX plumes. Future laboratory and field studies should also address response variability as a function of release scenario and site specificity, to facilitate risk assessment and remedial action decisions.

Key words: Fuel alcohol, monoaromatic hydrocarbon, environmental.

microbianas aeróbica y anaeróbica, incluyendo aquellos que pueden degradar compuestos del BTEX. A altas concentraciones de los microorganismos degradadores de BTEX, se tendrían velocidades de degradación de BTEX más rápidas bajo condiciones de carbón limitante. No obstante, estudios controlados que evalúen el efecto global del etanol en la bioremediación de BTEX son carentes.
En teoría, el etanol pudiera también contribuir a plumas más largas de BTEX por el engrandecimiento de la solubilización de BTEX de la fase del combustible y por disminución de la adsorción, relacionada al retardamiento durante el transporte. El efecto global del etanol en la longitud y tratamiento del punto final de la pluma del BTEX es probablemente un sistema especifico, y dependerá grandemente del escenario de la liberación y en la amortiguación y de la capacidad de dilución del acuífero.
Son necesarias investigaciones adicionales para entender el efecto de etanol en la estabilidad y dimensiones de plumas co-ocurriendo y pre-existiendo los BTEX. Estudios futuros en laboratorio y campo deberían también dirigirse a la variabilidad de respuestas como una función del escenario liberado y la especificidad el sitio, para facilitar el impuesto riesgo y decisiones en la acción de remedición.

Palabras clave: Etanol, productos del petróleo, medio ambiente.

INTRODUCTION AND OBJECTIVES

Problem statement

Monoaromatic hydrocarbons such as benzene, toluene, ethylbenzene, and the three isomers of xylene (BTEX) are ubiquitous groundwater pollutants commonly associated with petroleum product releases. All six BTEX compounds can depress the central nervous system, and chronic benzene exposure can cause leukemia (Federal register, 1985). Thus, BTEX contamination of potential drinking water sources represents a threat to public health.

Understanding the factors that affect the fate and transport of BTEX compounds in aquifers is of paramount importance for risk assessment and corrective action purposes. Although considerable progress has been made towards understanding many hydrogeochemical factors that affect BTEX migration and biodegradation, little attention has been given to how differences in gasoline formulation affect natural attenuation processes. In this regard, there is a recent initiative being considered to phase out methyl tertiary butyl ether (MTBE) as a gasoline oxygenate, due to its recalcitrance, ability to rapidly impact drinking water sources, and low taste and odor thresholds. For example, Governor Gray Davis recently ordered all MTBE to be removed from gasoline sold in California by December 2002. However, before any lasting changes in reformulated gasoline are implemented, it is prudent to evaluate the potential environmental impacts resulting from different alternatives. The most likely candidates to replace MTBE (which accounts for 80% of current oxygenate use) are biodegradable fuel alcohols such as ethanol (accounting for 15%) and methanol. Therefore, a better understanding of the effects of ethanol on BTEX migration and natural attenuation is warranted.

General scope and purpose of this literature review

The use of ethanol as gasoline an additive is increasing worldwide, both as a substitute fuel for imported oil, and as an oxygenate to minimize air pollution from combustion. In Brazil, for example, approximately one-half of all automobiles run on gasoline containing 22% ethanol, with the remainder operating on hydrated ethanol (Petrobrás, 1995). In the U.S., gasoline containing 10% ethanol is already available in many states. A recent effort by some members of the House of Representatives to repeal the 5.4¢/gallon tax subsidy for gasoline with ethanol earlier than its original (year-2000) end date was defeated. Instead, the tax subsidy was extended (Chemical Market Reporter, 1998). In addition, ongoing advances in biotechnology will continue to lower ethanol production costs (Lugar and Woolsey, 1999; Carver, 1996).

Given the increasing financial and political incentives for expanding its use as an automotive fuel oxygenate, ethanol appears likely to be encountered more frequently in groundwater plumes containing BTEX. Consequently, a comprehensive understanding of the effects of ethanol on the fate and transport of BTEX compounds is needed to determine if the economic and air-quality benefits of adding ethanol to gasoline outweigh its potential detrimental effects on groundwater quality, environmental and human health.

This review aims to characterize potential environmental impacts associated with a possible widespread replacement

Rev Latinoam Microbiol 2002; 44 (2): 83-104

of MTBE with ethanol as a gasoline oxygenate. This will be accomplished by summarizing and critically analyzing the available information on the fate of ethanol in the environment, assessing the potential environmental impacts associated with ethanol releases, and evaluating their potential effect on natural attenuation of BTEX compounds.

Following a general review of subsurface requirements for biodegradation of organic pollutants (Section 2), available information on ethanol biodegradation pathways and kinetics under aerobic and anaerobic conditions is summarized (Section 3). The potential effects of ethanol on cellular and environmental processes that affect the rate and extent of BTEX biodegradation are covered subsequently (Section 4). Potential effects of by-products from fuel production and/or biodegradation also are addressed (Section 5). This literature review also identifies critical knowledge gaps about the effects of ethanol on environmental pollution and remediation and provides recommendations for future research that would enhance related risk assessment and management decisions (Section 6).

REQUIREMENTS FOR BIODEGRADATION OF ORGANIC POLLUTANTS

Bioremediation, which involves the use of indigenous microorganisms (or the catalysts that they produce) to degrade the target pollutants within the aquifer, is receiving increasing attention due to its potential cost-effectiveness. Advantages of bioremediation include potential savings in the duration and cost of cleanup operations, minimum land and environmental disturbance, and elimination of liability from transportation and disposal of hazardous wastes (Lee *et al.*, 1988). In addition, bioremediation has gained considerable public acceptance because it is environmentally sound and it ultimately transforms the target pollutants into harmless products such as carbon dioxide and water.

The common approach to engineered *in situ* bioremediation is to provide environmental conditions that overcome limitations and foster natural degradative processes. For example, fertilizers and oxygen can be injected into gasoline-contaminated aquifers to add limiting nutrients and electron acceptors. In some cases, however, natural conditions at contaminated sites meet all the essential environmental requirements so that bioremediation can occur at high rates without human interference. This approach is called intrinsic bioremediation, and differs from no-action alternatives in that it requires thorough documentation of the role of microorganisms in eliminating the target contaminants at a sufficiently high rate to provide adequate risk protection.

Resource allocation problems have motivated a recent paradigm shift in the U.S. towards risk-based corrective action and intrinsic bioremediation. It should be emphasized, however, that this approach is not a panacea that is applicable to all situations (National Research Council, 1993). For intrinsic bioremediation to be effective, the biodegradation rate of a given pollutant in the subsurface should be fast relative to its rate of introduction and migration to ensure plume stabilization and mitigation. Otherwise, the plume will expand and potentially reach groundwater users (Corseuil and Alvarez, 1996). These relative rates depend on the type and concentration of the contaminants, the indigenous microbial community, and the subsurface hydrogeochemical conditions. Extensive biodegradation of gasoline pollutants requires the fulfillment of several conditions, which are discussed below.

Occurrence of microorganisms with potential to degrade the target compounds

Organic pollutants will be degraded to an appreciable extent only if microorganisms exist that can catalyze their conversion to a product that is an intermediate or a substrate to common metabolic pathways. Only a few central metabolic pathways exist, and some structural features in organic compounds, called "xenophores" (e.g., Cl, NO_2, CN, and SO_3), make the molecule difficult to be recognized by these pathways (Alexander, 1994). Thus, such xenobiotics tend to be recalcitrant to microbial degradation. Nevertheless, ethanol and BTEX compounds have a natural origin and have been in contact with microorganisms throughout evolutionary periods of time (Dagley, 1984). Thus, it is not surprising that many microorganisms have developed mechanisms to feed on these compounds and utilize them as fuel molecules to obtain energy and building blocks for the synthesis of new cell material.

The ability of microorganisms to utilize BTEX compounds as sole carbon sources has been established since 1908, when Stormer isolated the bacterium *Bacillus hexavarbovorum* by virtue of its ability to grow with toluene and xylene aerobically (Gibson and Subramanian, 1984). The ubiquitous distribution of soil bacteria capable of metabolizing aromatic compounds under aerobic conditions was demonstrated in 1928 by Gray and Thornton, who reported that 146 out of 245 uncontaminated soil samples contained bacteria capable of metabolizing naphthalene, phenol, or cresol (Gibson and Subramanian, 1984). Many bacterial pure cultures have been reported to grow aerobically on BTEX compounds as sole carbon sources, including the following genera: *Pseudomonas, Burkholderia, Arthrobacter, Alcaligens, Corynebacterium, Flavobacterium, Norcadia, Achromobacter, Micrococcus,* and *Mycobacterium* (Atlas, 1984; Bayly and Barbour, 1984; Brown, 1989; Button and Robertson, 1986; Gibson and Subramanian, 1984; Kukor and Olsen, 1989; Oldenhuis *et al.*, 1989; Shields *et al.*, 1989; Schraa *et al.*, 1987).

Rev Latinoam Microbiol 2002; 44 (2): 83-104

BTEX compounds can also be degraded in the absence of molecular oxygen, with toluene being the most commonly reported BTEX compound to degrade under anaerobic conditions (Alvarez and Vogel, 1995; Anderson *et al.*, 1998; Beller and Spormann, 1997; Edwards and Grbic-Galic, 1992; Heider and Fuchs, 1997; Heider *et al.*, 1999; Hutchins *et al.*, 1991; Meckenstock, 1999; Phelps and Young, 1999; Zeyer *et al.*, 1990). Benzene, which is the most toxic of the BTEX compounds, is relatively difficult to degrade under anaerobic conditions. There are reports of benzene mineralization under iron-reducing (Lovley *et al.*, 1996; Rooney-Varga *et al.*, 1999), sulfate-reducing (Edwards and Grbic-Galic, 1992; Phelps *et al.*, 1998), nitrate-reducing (Burland and Edwards, 1999), and methanogenic conditions (Grbic-Galic and Vogel, 1987; Weiner and Lovley, 1998b), with acclimation periods often exceeding one year (Kazumi *et al.*, 1997). Nevertheless, research suggests that even with the appropriate environmental conditions, anaerobic benzene degradation will not occur in some contaminated aquifer sediments due the absence of microorganisms capable of performing the degradation (Anderson *et al*., 1998; Weiner and Lovley, 1998a).

The high biodegradability of ethanol is well established in the literature. Short-chain alcohols such as ethanol can be easily degraded under both aerobic and anaerobic conditions by microbial enzymes associated with central microbial metabolic pathways (Chapelle, 1993; Hunt *et al.*, 1997a; Hunt *et al.*, 1997b; Madigan *et al.*, 1997). In addition, ethanol is highly bioavailable to microorganisms in aquifer material, due to its miscibility with water. Thus, a wide distribution of ethanol-degraders in the environment can be expected.

Bioavailability of target pollutants

A common limitation of natural degradative processes is the lack of adequate contact between pollutants and microorganisms. The availability of many target pollutants to microorganisms can be affected by a series of ill-defined, often uncharacterized processes (Alexander, 1994). In a physicochemical context, adsorption of a compound or complexation onto aquifer solid surfaces, sequestration in soil nanopores, and partitioning into NAPLs are common mechanisms that reduce contaminant bioavailability. In such cases, the rate of biodegradation can be controlled by the rate desorption or dissolution (Alexander, 1994). If biodegradation is mediated by extracellular enzymes, the bonds requiring cleavage must be exposed and not occluded by sorption to solid surfaces, or sterically blocked by large atoms such as chlorine. Most priority pollutants, however, are degraded by intracellular enzymes. Therefore, bioavailability also implies the ability of the pollutant to pass through the cellular membrane. In regards to ethanol and BTEX compounds, all of these bioavailability requirements are generally met easily because of their relatively high aqueous solubility. However, when (hydrophobic) polycyclic aromatic hydrocarbons are a contaminant of concern, the characteristically high sorption to soil with PAHs makes bioavailability a significant factor limiting the success of bioremediation.

Induction of appropriate degradative enzymes

This process involves activation of specific regions of the bacterial genome. Some enzymes, such as those participating in central metabolic pathways, are always produced (at some level) regardless of environmental conditions. These are known as constitutive enzymes. The enzymes that initiate BTEX degradation, however, are generally inducible. Such enzymes are only produced when an inducer (e.g., toluene) is present at a higher concentration than the minimum threshold for induction (Linkfield *et al.*, 1989). In general, this threshold is very low, on the order of a few micrograms per liter (Robertson and Button, 1987). Toluene is generally a good inducer of oxygenase enzymes with relaxed specificity, and its presence has been reported to enhance the degradation of other BTEX compounds (Arvin *et al.,* 1989; Alvarez and Vogel 1991, 1995; Chang *et al.,* 1993; Gülensoy and Alvarez, 1999). On the other hand, the presence of easily degradable substrates such as ethanol could repress the production of BTEX-degrading enzymes and result in the preferential degradation of the substrate (Corseuil *et al.*, 1998; Duetz *et al.*, 1994).

It should be pointed out that enzyme induction could be hindered by a lack of bioavailability. Specifically, the presence of a compound in a NAPL or its sequestration in nanopores might result in sub-threshold concentrations in the aqueous phase that are insufficient to trigger enzyme induction and/or sustain a viable microbial population.

Environmental conditions conducive to the growth of specific degraders and the functioning of their enzymes

Recalcitrance to biodegradation of a given compound is a consequence not only of chemical structure and physiological limitations, but also of environmental properties. To function properly, microorganisms need "recognizable" substrate(s) that can serve as energy and carbon source(s) (e.g., the target organic pollutants), and favorable environmental conditions to sustain life functions.

Availability of electron acceptors

Ethanol and BTEX compounds are in a reduced state, and their oxidation is thermodynamically favorable. Oxidative biodegradation requires the presence of electron acceptors that

Rev Latinoam Microbiol 2002; 44 (2): 83-104

microbes use to "respire" the electrons removed from the target contaminants. This transfer of electrons releases energy to drive microbial life functions. Under aerobic conditions, molecular oxygen is utilized for this purpose. Under anaerobic conditions, nitrate, sulfate, ferric iron, sulfate, and carbon dioxide can serve as electron acceptors. Often, a sequential utilization of electron acceptors is observed in contaminated sites, in preferential order of oxidation potential (Fig. 1).

The most energetically favored mechanism by which microorganisms oxidize organic compounds is aerobic metabolism. Therefore, oxygen is preferentially utilized over anaerobic electron acceptors because this yields more energy to the microbial community and results in faster contaminant oxidation rates. In intrinsic BTEX bioremediation, the rate-limiting attenuation mechanism is frequently the influx of oxygen, which in turn limits aerobic BTEX degradation kinetics (National Research Council, 1993).

The redox potential in subsurface environments is highly site-dependent. Oxygen is usually present in and around groundwater recharge areas as a result of infiltrating rainwater. Nevertheless, the available oxygen within the contaminant plume is often exceeded by the biochemical oxygen demand exerted by the contaminants, and anaerobic conditions often develop in highly contaminated areas. The depletion of oxygen results in much slower BTEX degradation rates, and sometimes, in the persistence of benzene, which is the most toxic of the BTEX compounds (Alvarez and Vogel, 1995; Anderson *et al.*, 1998).

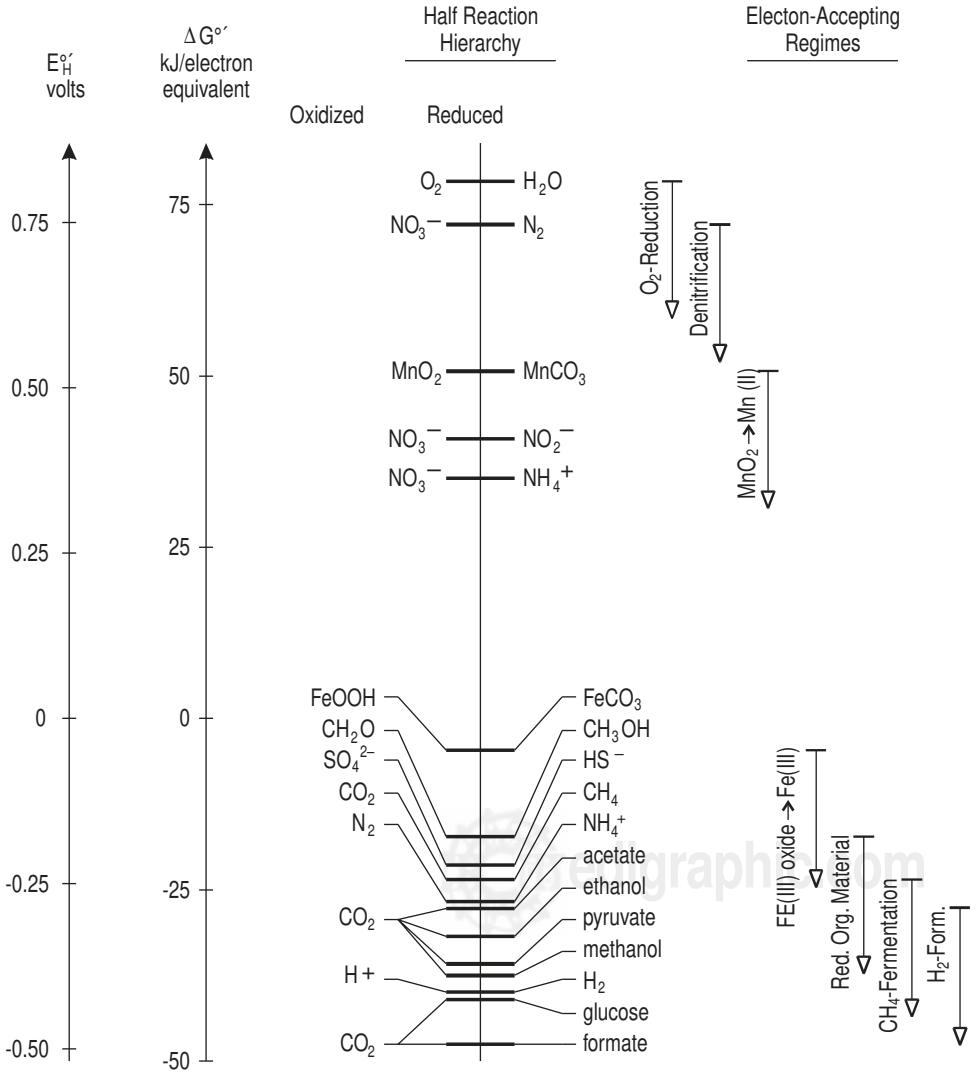

Figure 1. *Free energy diagram for common electron acceptors and donors. The hierarchy of electron acceptors provides a simple means to integrate thermodynamics, microbiology, and physiology of oxidation-reduction reactions. $E_H°'$ is the equilibrium redox potential and DG°' is the half-reaction free energy. These values are for unit activities of oxidant and reductant in water with a pH of 7.0 (adapted from Zehnder and Stumm, 1988).*

Rev Latinoam Microbiol 2002; 44 (2): 83-104

Availability of inorganic nutrients

Microorganisms need macronutrients to synthesize cellular components, such as nitrogen for amino acids and enzymes, phosphorus for ATP and DNA, sulfur for some coenzymes, calcium for stabilizing the cell wall, and magnesium for stabilizing ribosomes. In general, microbial growth in sub-soils is not limited by nitrogen and phosphorus as long as the contaminant concentrations are in the sub part per million (mg/l) range (Tiedje, 1993). A carbon:nitrogen:phosphorus ratio of 30:5:1 is generally sufficient to ensure unrestricted growth in aquifers (Paul and Clarck, 1989). Microbes also need micronutrients to perform certain metabolic functions. For example, trace metals such as iron, nickel, cobalt, molybdenum, and zinc are needed for some enzymatic activities. Nevertheless, geochemical analyses and laboratory biodegradation assays should be performed to verify the presence of inorganic nutrients is sufficient for the success of natural bioremediation.

Buffering capacity

Most microorganisms grow best in a relatively narrow range of pH around neutrality (6 to 8). Enzymes are polymers of amino acids, and their activity requires the proper degree of amino acid protonation. This is controlled by pH. The optimum groundwater pH is usually near neutral (pH 7), but most aquifer microorganisms can perform well between pH values of 5 and 9. This range generally reflects the buffering capacity of the carbonate or silicate minerals present in aquifers (Chapelle, 1993; King *et al.*, 1992). Groundwater is typically well buffered within this range, so microbial pH requirements are generally met in aquifers (Chapelle, 1993). Nevertheless, aquifers contaminated by municipal Landfill Leachates may contain elevated concentrations of volatile fatty acids (e.g., acetic acid) resulting in pH values as low as 3. In these cases, acidity may suppress microbial activity. As discussed later in this review, the potential accumulation of volatile fatty acids during anaerobic degradation of ethanol is a potential mechanism that could decrease the pH below the optimum range of common bacteria that degrade BTEX.

Temperature

Temperature is one of the most important environmental factors influencing the activity and survival of microorganisms. Microbial metabolism accelerates with increasing temperatures up to an optimum value at which growth is maximal. Most of the bacteria present in subsurface environments operate most effectively at 20 to 40°C, which is a little higher than typical groundwater temperatures in the USA (Chapelle, 1993). Low temperatures reduce the fluidity and permeability of the cellular membrane, which hinders nutrient (and contaminant) uptake. Higher temperatures are associated with higher enzymatic activity and faster biodegradation rates, up to an optimum value that is species specific. BTEX degradation rates can double or triple due to a temperature increase of 10°C (Corseuil and Weber, 1994). If the temperature rises much beyond the optimum value, proteins enzymes, and nucleic acids become denatured and inactive. The temperature of the upper 10 m of the subsurface may vary seasonally; however, that between 10 and 100 m approximates the mean annual air temperature of a particular region (Lee *et al.*, 1988).

Absence of inhibitory substances

It is possible for aquifer microorganisms to encounter potentially toxic heavy metals such as lead, mercury, cadmium, and chromium. While heavy metals are required in trace quantities for nutritional purposes, they can be bacteriostatic or bactericidal if present in soluble form at concentrations greater than about 1 mg/l. High pollutant concentrations can also have toxic effects, such as gross physical disruption (e.g., membrane dissolution) or competitive binding of critical enzyme (Alexander, 1994). In addition, the presence of easily degradable substrates that are preferentially utilized commonly hinders the degradation of the target contaminants.

Other environmental factors

While moisture is not a limiting factor in the saturated zone, it can be an important factor in the Vadose zone. A moisture content of about 80% of soil field capacity, or 15% H_2O on a weight basis, is optimum for Vadose zone remediation (English and Loehr, 1991). Inadequate moisture (less than 40%) can significantly reduce biodegradation rates. High salinity can also exert osmotic stress on microorganisms, which would hinder biodegradative processes.

BIODEGRADATION OF ETHANOL

One of the most undesirable aspects of microbial degradation of organic pollutants is the potential formation of toxic metabolites. A large number of non-toxic chemicals can be converted to products that may be harmful to humans, animals, plants, or microorganisms. This process is a major reason to study the pathways and products of breakdown of organic molecules. This chapter summarizes the diversity of aerobic and anaerobic transformation pathways for ethanol. Emphasis was placed on addressing the potential accumulation of metabolites that may have adverse im-

Rev Latinoam Microbiol 2002; 44 (2): 83-104

pacts to water quality, or that may hinder intrinsic bioremediation of BTEX compounds. The kinetics of ethanol biodegradation under aerobic and anaerobic conditions are also discussed.

Ethanol degradation pathways

Aerobic degradation

Most common aerobic bacteria can mineralize ethanol through Kreb's cycle. In this process, ethanol is first oxidized to acetaldehyde by an alcohol dehydrogenase enzyme. Acetaldehyde is converted to acetyl-CoA either directly by an acetylating acetaldehyde dehydrogenase or through acetate by an acetaldehyde dehydrogenase and an acetate-CoA ligase. The acetyl-CoA is oxidized to CO_2 in Krebs cycle (Fig. 2). Many bacteria are also capable of operating a modified Krebs cycle, known as the glyoxylate shunt (Fig. 3). This shunt enables bacteria to grow on compounds with two carbon atoms (C2-compounds), such as acetate, by facilitating the synthesis of C4-building blocks, such as malate and oxaloacetate (Madigan *et al.*, 1997).

None of the intermediates in these common metabolic pathways are toxic. In addition, these intermediates are me-

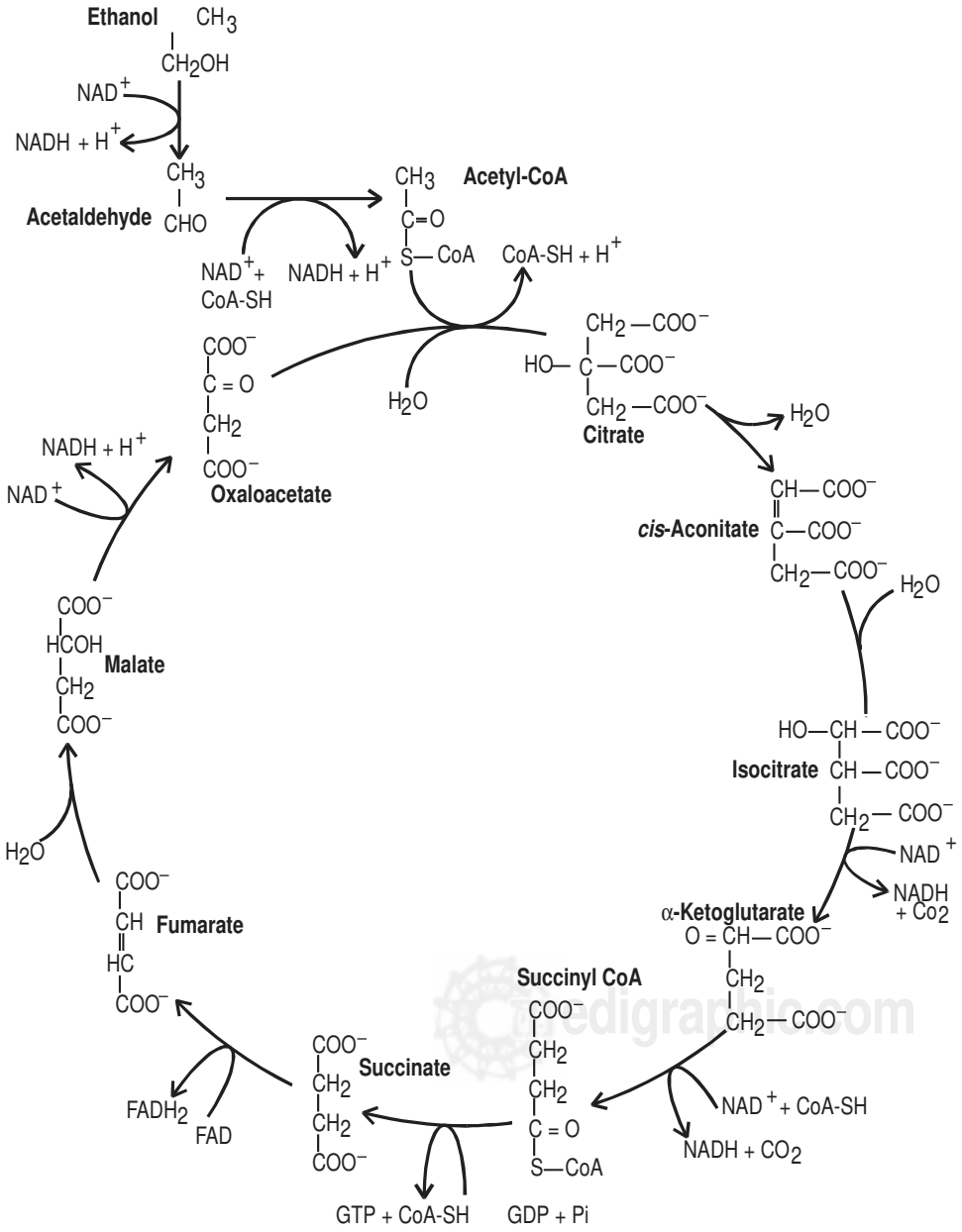

Figure 2. *Ethanol degradation through Kreb's cycle (adapted from Stryer, 1988).*

Rev Latinoam Microbiol 2002; 44 (2): 83-104

tabolized rapidly intracellularly and are rarely excreted in significant amounts, so their accumulation in groundwater is highly unlikely. One exception, however, are the acetic acid bacteria which excrete acetate (Gottschalk, 1986, Xia *et al.*, 1999).

Acetic acid bacteria excrete acetate because they lack the necessary enzymes to rapidly metabolize it. For example, *Gluconobacter* cannot oxidize the activated form of acetate (i.e., acetyl-CoA) in Krebs cycle because it lacks a key enzyme, succinate dehydrogenase (Gottschalk, 1986). *Acetobacter* species can operate Krebs cycle, but still produce large amounts of acetic acid in the presence of ethanol (Gottschalk, 1986). These bacteria are unlikely to significantly contribute acidity to ethanol-contaminated groundwater, however, because they are obligate aerobes that typically live on the surfaces of plants and fruits (Gottschalk, 1986). Therefore, they are unlikely to thrive in aquifers contaminated with gasoline-ethanol mixtures, where the high biochemical oxygen demand often depletes available oxygen.

Anaerobic pathways

The anaerobic food chain

Microorganisms that can ferment ethanol are ubiquitous (Eichler and Schink, 1984; Wu and Hickey, 1996). Ethanol is a common intermediate in the anaerobic food chain, where labile organic matter is degraded to non-toxic prod-

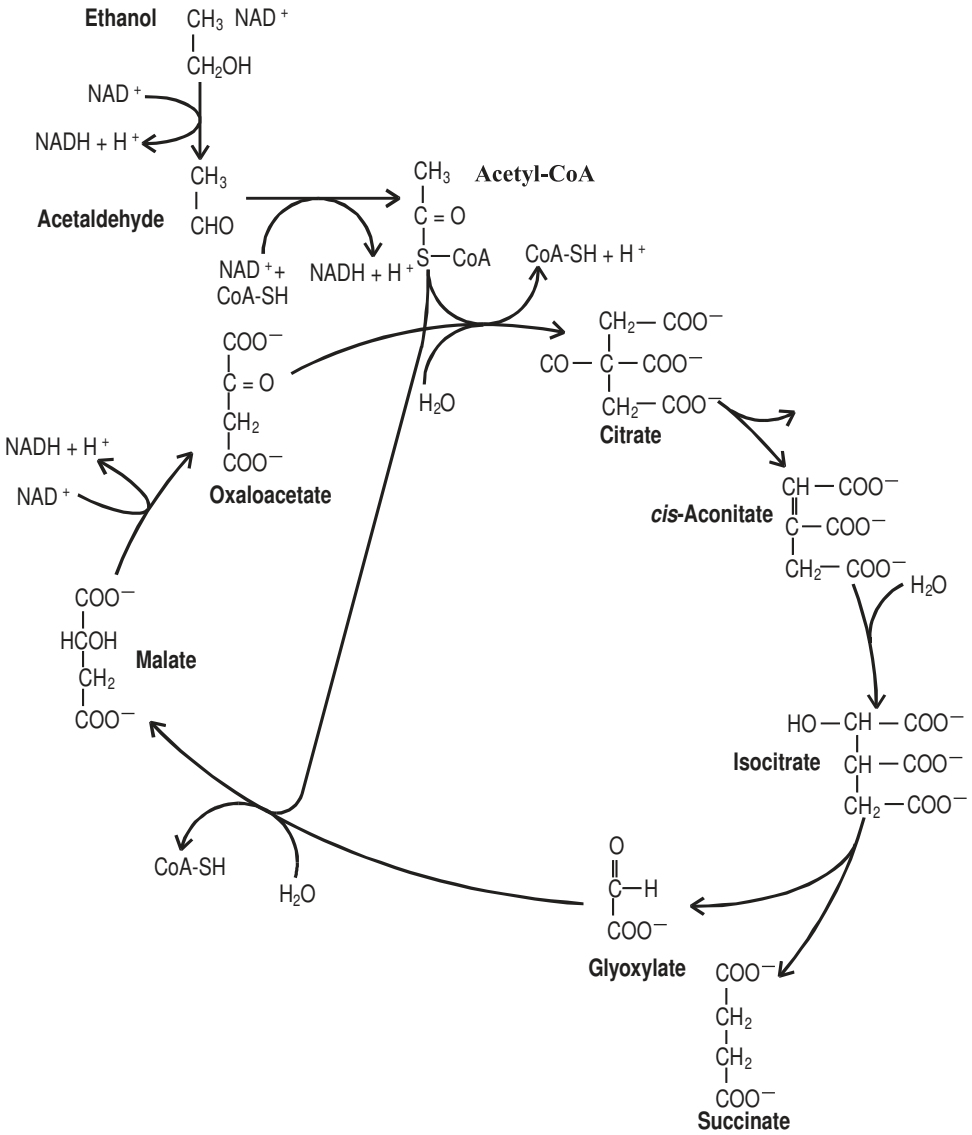

Figure 3. *Ethanol degradation by the glyoxylate shunt (adapted from Stryer, 1988).*

Rev Latinoam Microbiol 2002; 44 (2): 83-104

ucts such as acetate, CO_2, CH_4 and H_2 by the combined action of several different types of bacteria (White, 1995). As illustrated in Figure 4, the anaerobic food chain consists of three stages. In the first stage, fermenters produce simple organic acids, alcohols, hydrogen gas, and carbon dioxide. Other members of the consortium oxidize these fermentation products in the second stage to CO_2, H_2, and acetate, such as sulfate reducers and organisms that use water-derived protons as the major or sole electron sink. The latter include the obligate proton-reducing acetogens, which oxidize butyrate, propionate, ethanol, and other compounds to acetate, H_2 and CO_2. Acetate can also be produced by homoacetogens, which are bacteria that utilize CO_2 and H_2 for this purpose (Madigan *et al.*, 1997). Mineralization occurs in the third stage. This is accomplished by acetoclastic methanogens, which break down acetate into CO_2 and CH_4. Some sulfate reducers and other anaerobic microorganisms can also mineralize acetate and participate in the final stabilization stage (Atlas and Bartha, 1997).

Interspecies hydrogen transfer is a critical link in the anaerobic food chain. Hydrogen-producing fermentative and acetogenic bacteria are at a thermodynamic disadvantage if hydrogen accumulates (Conrad *et al*., 1985; Wolin and Miller, 1982). For example, the fermentation of ethanol to acetate and propionate by *Desulfobulbus* is strongly inhibited by high hydrogen concentrations (Schink *et al.*, 1987; Wu, and Hickey, 1996). Therefore, fermenters and acetogens live syntrophically with hydrogen consumers that keep the H_2 levels low (Fig. 5).

When sulfate is not limiting, sulfate reducers compete favorably for H_2 and predominate over methanogens (Phelps *et al*., 1985). Incomplete oxidizers (a.k.a. Type I sulfate reducers) can oxidize ethanol, lactate, and other organic acids to acetate, while complete oxidizers (a.k.a. Type II sulfate reducers) can use either a carbon monoxide dehydrogenase pathway or a modified Kreb's cycle to oxidize acetate further to CO_2 (Madigan *et al.*, 1997; Postgate and Campbell, 1966; Thauer *et al*., 1989; Wu and Hickey, 1996).

Fermentative microorganisms can also transform ethanol by condensation reactions to form propionate (Braun *et al.*, 1981; Wu and Hickey, 1996) or butyrate (Bornstein and Barker, 1948). These compounds are not toxic either, but could adversely affect groundwater quality by impacting its taste and odor. Examples of such condensation transformations are given below.

Pelobacter propionicus Ethanol metabolism

Propionate forming bacteria are believed to contribute significantly to the anaerobic degradation of ethanol (Wu, and Hickey, 1996). *Pelobacter propionicus* can produce propionate and acetate from its metabolism of ethanol. If sulfate is present, *P. propionicus* will oxidize ethanol to acetate, using sulfate as the terminal electron acceptor. If sulfate is not present, ethanol will be condensed with bicarbonate to form just propionate if hydrogen is available. With insufficient hydrogen, both propionate and acetate will be formed. Ethanol utilization by *P. propionicus* is diagrammed in Figure 6. The different stoichiometric balances for ethanol utilization are:

$$CH_3CH_2OH + HCO_3^- + H_2 \rightarrow CH_3CH_2COO^- + 2\ H_2O$$
$$3\ CH_3CH_2OH + 2\ HCO_3^- \rightarrow CH_3COO^- + 2\ CH_3CH_2COO^- + H^+ + 3\ H_2O$$
$$2\ CH_3CH_2OH + SO_4^{2-} \rightarrow 2\ CH_3COO^- + HS^- + H^+ + 2\ H_2O$$

$$\text{i.e., ethanol} + HCO_3^- + H_2 \rightarrow \text{propionate}^- + 2\ H_2O$$
$$3\ \text{ethanol} + 2\ HCO_3^- \rightarrow \text{acetate}^- + 2\ \text{propionate}^- + H^+ + 3\ H_2O$$
$$2\ \text{ethanol} + SO_4^{2-} \rightarrow 2\ \text{acetate}^- + HS^- + H^+ + 2\ H_2O$$

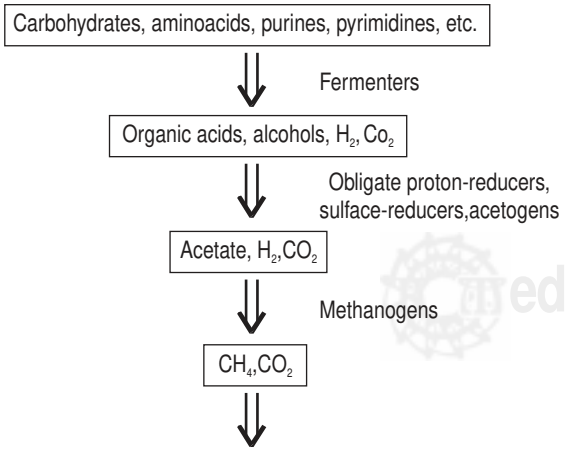

Figure 4. *The anaerobic food chain (modified from White, 1995).*

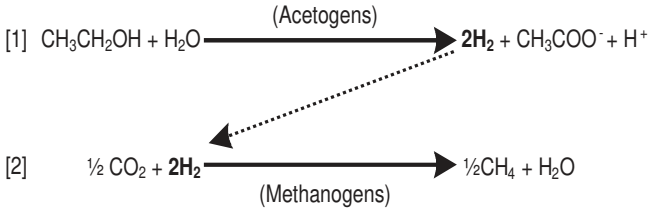

Figure 5. *Interspecies hydrogen transfer. Anaerobic oxidation of ethanol to acetate [1] is not thermodynamically feasible under standard conditions (DG'_0 = +9.6 kJ). This reaction can proceed only if the H_2 produced by acetogens and other fermenters is removed (law of mass action). The removal of H_2 by hydrogenotrophic methanogens [2] or sulfate reducers enhances the thermodynamic feasibility of acetogenesis and the subsequent mineralization of acetate by acetoclastic methanogens and (Type II) sulfate reducers. Thus, interspecies H_2 transfer prevents the accumulation of fermentation products and enhances anaerobic mineralization.*

Rev Latinoam Microbiol 2002; 44 (2): 83-104

Table 1. Metabolites and end products of ethanol biodegradation.

Aerobic	Anaerobic
Acetaldehyde	Acetate
Acetate	Butyric acid
Acetyl-CoA	Propionic acid
Carbon dioxide	Hydrogen gas
	n-propanol
	Acetone
	Carbon dioxide
	Methane

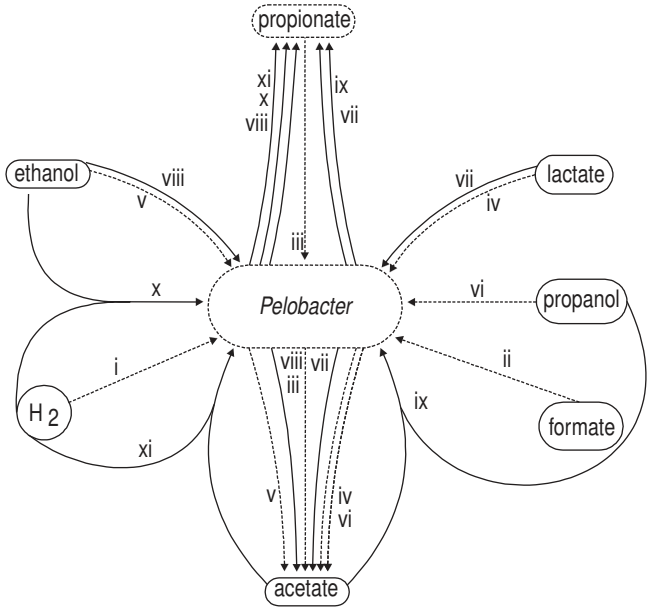

Figure 6. *Propionate formation during ethanol fermentation by* P. propionicus *(Adapted from Laanbroek* et al.*, 1982).* Broken lines: *conversions in the presence of sulfate;* solid lines: *conversions in the absence of sulfate. The numbers correspond with the following reactions:*

i. $H_2 + \frac{1}{4}H^+ + \frac{1}{4}SO_4^{2-} \rightarrow \frac{1}{4}HS^- + H_2O$

ii. $formate^- + \frac{1}{4}H^+ + \frac{1}{4}SO_4^{2-} \rightarrow HCO_3^- + \frac{1}{4}HS^-$

iii. $Proprionate^- + \frac{3}{4}SO_4^{2-} \rightarrow acetate^- + HCO_3^- + \frac{3}{4}HS^- + \frac{1}{4}H^+$

iv. $lactate^- + \frac{1}{2}SO_4^{2-} \rightarrow acetate^- + HCO_3^- + \frac{1}{2}HS^- + \frac{1}{2}H^+$

v. $ethanol + \frac{1}{2}SO_4^{2-} \rightarrow acetate^- + \frac{1}{2}HS^- + H\frac{1}{2}^+ + H_2O$

vi. *l* - $propanol + \frac{5}{4}SO_4^{2-} \rightarrow acetate^- + HCO_3^- + \frac{5}{4}HS^- + \frac{3}{4}H^+ + H_2O$

vii. $lactate^- \rightarrow \frac{1}{3}acetate^- + \frac{2}{3}Proprionate^- + \frac{1}{3}HCO_3^- + \frac{1}{3}H^+$

viii. $ethanol + \frac{2}{3}HCO_3^- \rightarrow \frac{1}{3}acetate^- + \frac{2}{3}proprionate^- + \frac{1}{3}H^+ + H_2O$

ix. *l* - $propanol + \frac{2}{3}acetate^- + \frac{2}{3}HCO_3^- \rightarrow \frac{5}{3}Proprionate^- + \frac{1}{3}H^+ + H_2O$

x. $ethanol + HCO_3^- + H_2 \rightarrow Proprionate^- + 2H_2O$

xi. $acetate^- + HCO_3^- + H^+ + 3H_2 \rightarrow Proprionate^- + 3H_2O$

Clostridium kluyveri fermentation

Clostridium kluyveri produces butyrate, caproate, and hydrogen from ethanol and acetate. This strain cannot ferment ethanol alone, and can replace acetate with propionate as a cosubstrate for the condensation of ethanol. The ratio in which butyrate and caproate are formed can vary; an increase of the ethanol concentration of the medium favors caproate formation (Fig. 7) (Gottschalk, 1986).

A typical fermentation balance is for this pathway is:

$$6\ CH_3CH_2OH + 3\ CH_3COO^- \rightarrow$$
$$3\ CH_3CH_2CH_2COO^- + CH_3CH_2CH_2CH_2CH_2COO^- + 2\ H_2 + 4\ H_2O + H^+$$
i.e., 6 ethanol + 3 acetate → 3 butyrate + 1 caproate + 2 H_2 *+ 4* H_2O *+* H^+

Thus, approximately 0.3 moles of hydrogen are evolved per mole of ethanol fermented.

Summary of metabolic intermediates of importance in ethanol degradation

Potential metabolic intermediates and end products for microbial degradation of ethanol are listed in Table 1. Oxygen is often quickly depleted by microbial respiration in gasoline-contaminated aquifers (Lee *et al.*, 1988; National Research Council, 1993). Therefore, ethanol is likely to be degraded predominantly under anaerobic conditions, and some anaerobic metabolites are likely to be encountered in contaminated groundwater. None of these metabolites are toxic, although some anaerobic metabolites such as butyrate could adversely affect the taste and odor of groundwater supplies. In addition, acetate and other volatile fatty acids can cause a decrease in pH if they accumulate at high concentrations in poorly buffered systems. It is unknown whether the pH could decrease to a level that inhibits the further degradation of the ethanol. Such effects are likely to be system specific due to variability in buffering and dilution capacity among contaminated sites.

Aerobic and anaerobic biodegradation kinetics

General background

The degradation rate of BTEX and ethanol is often described by a first-order decay regime with respect to the contaminant concentration (C):

$$\frac{dC}{dt} = -\lambda C \qquad (3.1)$$

For a batch, completely mixed system, Equation (3.1) can be integrated to yield:

Rev Latinoam Microbiol 2002; 44 (2): 83-104

$$\frac{C}{Co} = e^{-\lambda t} \qquad (3.2)$$

where l is the first-order decay coefficient and Co is the initial concentration. Equation (3.2) can be rearranged as

$$t = \frac{1n\left[Co/C\right]}{\lambda} \qquad (3.3)$$

The half-life ($t_{1/2}$) of the contaminant, which is defined as the time required to reduce its concentration by one-half (i.e., Co/C = 2), is given by

$$t_{1/2} = \frac{1n\left[2\right]}{\lambda} \qquad (3.4)$$

It should be emphasized that these equations apply only to batch, completely mixed systems, where dilution and advection are not factors that influence contaminant concentrations. Because aquifers are open systems subject to dilution and advection, other approaches that incorporate these processes must be used to determine λ (ASTM, 1998)

The first-order kinetic assumption is often appropriate to describe the kinetics of organic pollutant biodegradation in aquifers. This is mainly due to mass transfer limitations in porous media as the contaminants diffuse from the bulk liquid to the microorganisms, which are predominantly attached to the aquifer material (Simoni *et al.*, 1999). In addition, a decrease in BTEX concentrations to levels that are below the corresponding Monod half saturation coefficient (K_S) contributes to first order kinetics (Alvarez *et al.*, 1991). It should be pointed out that when mass transport is not rate limiting, λ can be explained in terms of Monod parameters. Specifically, when the contaminant concentration is relatively low, we can ignore C in the denominator and the Monod equation reduces to a linear equation:

$$\frac{dC}{dt} = -\frac{kXC}{K_s + C} = -\left(\frac{kX}{K_s}\right)C \quad (\text{When } C << K_S) \qquad (3.5)$$

A comparison of equations 3.1 and 3.5 therefore reveals that

$$\lambda = \frac{kX}{K_s} \qquad (3.6)$$

This theoretical analysis indicates that the value of λ depends on:

a) **k** (the maximum specific substrate utilization rate) which in turn depends primarily on the prevailing electron acceptor conditions, and on the type of microbe present;
b) $\mathbf{K_S}$ (the half-saturation coefficient), which is related to enzyme affinity, bioavailability, and mass transport limitations (Merchuk and Ansejo, 1995); and
c) **X** (the active biomass concentration) which may not be constant, and depends on environmental conditions and aquifer chemistry, including available substrates.

Therefore, λ is not necessarily a constant, but a coefficient that can vary in time and space due to microbial population shifts resulting from changes in aquifer chemistry. This can explain the wide range of λ values that have been observed for benzene at different sites, ranging over orders of magnitude from less than 0.0001 to 0.0870 day^{-1} (Alvarez *et al.*, 1991; Aronson and Howard, 1997; Howard, 1991; Rifai *et al.*, 1995). Therefore, for risk-assessment purposes, λ should not be extrapolated from the literature. Rather, considerable care must be exercised in its determination to avoid over-predicting or under-predicting actual biodegradation rates and plume behavior.

Ethanol degradation rates in aquifers

Ethanol can be degraded in both aerobic and anaerobic environments, faster than other gasoline constituents and

Table 2. First-order rate coefficients (λ) for anaerobic and aerobic degradation of ethanol by aquifer microorganisms (Estimated from laboratory experiments by Corseuil *et al.*, 1998).

Compound	Electron acceptor	λ (day^{-1})	Half-life (days)
Ethanol	O_2	0.23 - 0.35	2-3
	NO_3^-	0.53	1.3
	Fe^{3+}	0.17	4
	SO_4^{-2}	0.1	7
	CO_2	0.12	6

Table 3. Time Required to Biodegrade 80 to 100 mg/l of Ethanol in Microcosms Under Various Redox Conditions (Corseuil *et al.*, 1998).

Redox condition	Degradation (days)*
Aerobic	5
Denitrifying	3
Iron-reducing	12.5
Sulfidogenic	25
Methanogenic	12

* The sources of soil and groundwater in the microcosms were different for each set.

Rev Latinoam Microbiol 2002; 44 (2): 83-104

oxygenates (Chapelle, 1993; Malcom Pirnie, 1998). Ethanol (first-order) degradation rate coefficients have been measured in several aquifer microcosm studies (Table 2). Only large concentrations (> 100,000 mg/l) of alcohols are not biodegradable due to their toxicity to most microorganisms (Brusseau, 1993; Hunt *et al.*, 1997a). Such high concentrations could be encountered near the source of neat ethanol releases. However, since the maximum allowable ethanol content in gasoline is 10% by volume in the United States, such high concentrations are unlikely to be encountered at sites contaminated with ethanol-gasoline blends (except near the fuel/water interfaces).

Ethanol concentrations should become exponentially more dilute as the distance from the source increases but may inhibit microbial activity near the source. Thus, alcohol plumes should be degraded by indigenous microbes located a sufficient distance beyond the source. The only fuel alcohol field-scale studies performed have been with methanol and not ethanol. One field study investigated methanol biodegradation in soils from three different sites under various redox conditions. Methanol concentrations of 1,000 mg/l were removed in all soils in less than one year, at pH values of 4.5 to 7.8 and at temperatures of 10 to 11°C (Butler *et al.*, 1992). A similar study investigated the persistence and fate of M85 fuel (85% methanol, 15% gasoline) in a shallow sandy aquifer (Barker *et al.*, 1998). All of the methanol (approximately 2,400 l resulting in an initial concentration of 7,000 mg/l) was biodegraded below 1 mg/l in 476 days, yielding a methanol half-life of about 40 days. Because of the similar properties of methanol and ethanol, the biodegradation of ethanol is also expected to be relatively fast.

While there are no known field-scale studies of the fate and transport of ethanol, a few laboratory studies have focused on ethanol biodegradation. Acclimation periods (periods before

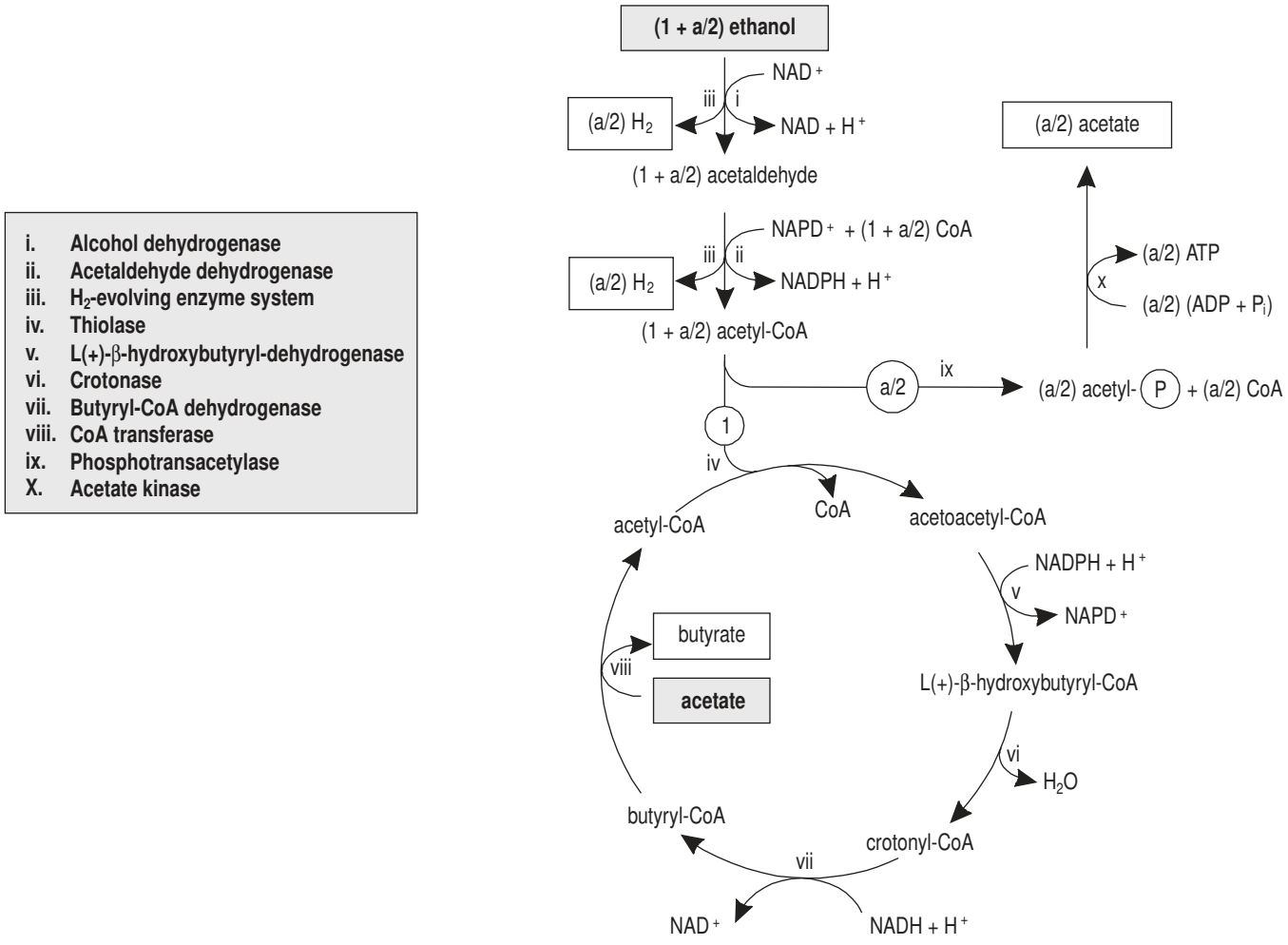

Figure 7. *The Ethanol-Acetate fermentation of* C. kluyveri *(From Gottschalk, 1986).*

Rev Latinoam Microbiol 2002; 44 (2): 83-104

degradation proceeded) and zero-order biodegradation rates of ethanol and other fuel oxygenates were measured in anaerobic aquifer slurries by Suflita and Mormile (1993). For an initial ethanol concentrations of 50 mg/l, an acclimation period of 25 to 30 days and an anaerobic biodegradation rate of 17.9 ± 0.6 mg/l/day were observed. Compared to ethanol, the observed acclimation period for methanol was shorter (5 days), but its biodegradation rate was slower (7.4 ± 0.7 mg/l/day). In a subsequent study, these authors illustrated that their initial results could be extrapolated to other redox conditions. They showed that various short chain alcohols were easily degraded in different sediments under a range of redox conditions (Mormile *et al.*, 1994).

Biodegradation of ethanol under various redox conditions was investigated in aquifer microcosms at 28ºC by Corseuil *et al.* (1998). The time required to degrade ethanol (80 to 100 mg/l) in this study is summarized in Table 3. Lower microbial concentrations, colder temperatures, and mass transfer limitations would likely result in longer degradation times *in situ* than those depicted in Table 3. Nevertheless, it is expected that regardless of the available electron acceptors, ethanol will undergo rapid biodegradation in the subsurface under typical pH, temperature, and nutrient conditions.

Surface water

In surface water bodies, the dominant process responsible for the removal of ethanol is also expected to be biodegradation (Malcolm Pirnie, Inc., 1998). Under aerobic conditions, the reported half-lives of ethanol in surface waters are short. Half-lives span 6.5 to 26 hours for (Howard, 1991). In moving water bodies, reaeration form the atmosphere generally ensures that oxygen will be available to support aerobic degradation processes, and oxygen is not expected as much of a limiting factor as in groundwater systems. Anaerobic biodegradation in oxygen-limited environments such as the bottom layers of stratified lakes is also expected to proceed at rapid rates. Reported half-lives for ethanol biodegradation under anaerobic conditions range from 1 to 7 days (Howard, 1991). The nutrient supply in rivers and lakes is generally not expected to restrict the rate of biochemical transformations because the required nutrient supplies are constantly recharged by rainfall (Alexander, 1994).

POTENTIAL EFFECTS OF ETHANOL ON BTEX BIODEGRADATION

Direct (Intracellular) Effects

Enzyme induction and repression

Often, target pollutants are degraded by inducible enzymes whose expression can be repressed when easily degradable substrates are present at high concentrations (Duetz *et al*., 1994; Monod, 1949). However, only indirect evidence has been presented in the literature about the potential effects of ethanol on the expression of enzymes involved in BTEX degradation.

Hunt *et al*. (1997a) reported that ethanol at 20 mg/l was preferentially degraded under aerobic conditions over benzene, presumably due to repression of the synthesis of enzymes needed to degrade benzene. This retarded the onset of benzene degradation. Additional microcosm studies also suggested that the preferential utilization of ethanol might increase the lag time before *in situ* BTEX biodegradation begins (Corseuil *et al*., 1998). Specifically, little or no BTEX degradation occurred in aerobic, denitrifying, iron-reducing, sulfate reducing, and methanogenic microcosms while ethanol was present (Corseuil *et al*., 1998). Therefore, ethanol may prevent the bacteria sub-population capable of degrading BTEX from fully expressing its catabolic potential, which would hinder BTEX degradation.

Numerous studies show that carbon-limiting conditions are conducive to simultaneous utilization of multiple substrates (for review, see Egli, 1995). This suggests that simultaneous ethanol and BTEX degradation is likely to occur when these compounds are present at low concentrations (e.g., in aquifers with low levels of contamination). Interestingly, a pure culture of *Pseudomonas putida* F1 was reported to simultaneously degrade ethanol and toluene with no apparent inhibitory effect up to 500 mg/l of ethanol (Hunt *et al.*, 1997a). This suggests that while high ethanol concentrations are likely to exert a diauxic effect that would inhibit *in situ* BTEX degradation, the metabolic diversity of microorganisms precludes generalizations about the concentration of ethanol that triggers enzyme repression. Such effects are probably species specific.

Stimulation of microbial growth

Ethanol represents a carbon and energy source that is likely to stimulate the growth of a variety of microbial populations, including species that can degrade BTEX compounds. A proliferation of BTEX degraders would be conducive to faster degradation rates, although this positive effect is likely to be offset by the preferential degradation of ethanol and the associated depletion of electron acceptors discussed later in this chapter.

As discussed earlier, ethanol can be degraded by constitutive enzymes associated with central metabolic pathways, and microorganisms that can degrade simple alcohols are more common in nature than microorganisms that degrade BTEX compounds. Therefore, many species that cannot degrade BTEX are likely to proliferate when etha-

Rev Latinoam Microbiol 2002; 44 (2): 83-104

nol is present. In fact, microbial growth is generally faster on ethanol than on BTEX, due to more favorable thermodynamics. Using a thermodynamic model by McCarty (1969), the predicted maximum specific growth rate on ethanol is 45% greater than the predicted maximum specific growth rate with benzene (Hunt, 1999). Nevertheless, BTEX degraders are also likely to grow faster on ethanol than on BTEX under a given set of conditions, and the effect of ethanol on the relative abundance of BTEX degraders has not been investigated.

Corseuil *et al*. (1998) pointed out that there may be some exceptions to the detrimental effect of ethanol on BTEX degradation, and hypothesized that this may be related to ethanol-induced microbial population shifts. Specifically, although ethanol was preferentially degraded under all electron acceptor conditions tested, ethanol enhanced toluene degradation in all three sulfate-reducing microcosms used in this study. The reason for this enhancement was unclear, but the possibility that this enhancement was due to an incidental growth of toluene degraders during ethanol degradation could not be ruled out. This untested hypothesis does not imply that ethanol would select for BTEX degraders, which is highly unlikely. Rather, the concentration of some BTEX degraders could increase after growth on ethanol, although their fraction of the total heterotrophic consortium would likely decrease.

In summary, little is known about the effect of ethanol on microbial population shifts and the resulting catabolic diversity. Considering that the efficiency of bioremediation depends, in part, on the presence and expression of appropriate biodegradative capacities, studying the microbial ecology of aquifers contaminated with gasoline-alcohol mixtures might be a fruitful avenue of research.

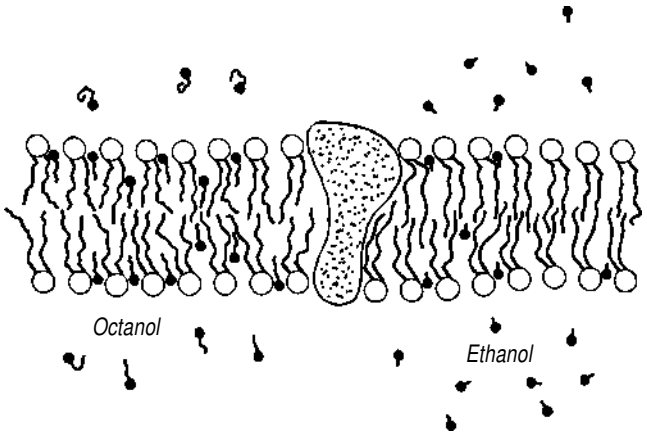

Figure 8. *Model showing interactions of octanol and ethanol with a cell membrane (adapted from Widdel, 1986).*

Toxicity of ethanol

The toxicity of alcohols to microorganisms has received considerable attention in the literature, although only a few studies have evaluated the effect of ethanol on subsurface microbial populations. Hunt *et al.* (1997a) reported that ethanol concentrations in microcosm experiments higher than 40,000 mg/l were toxic to the microorganisms, as shown by complete lack of oxygen consumption. Other studies have found that some soil microbial activity can occur at 100,000 mg/l ethanol, but not at 200,000 mg/l (Araujo *et al.*, 1998).

Ingram and Buttke (1984) conducted a thorough literature review on the effects of alcohol on microorganisms. Disruption of the cellular permeability barrier is thought to be the basis of bacterial killing by high concentrations of alcohols (Brusseau, 1993; Ingram and Buttke, 1984; Harold, 1970). Ethanol concentrations above 100,000 mg/l result in the immediate inactivation of most vegetative organisms, although spore-forming organisms are more resistant (Dagley *et al.*, 1950; Hugo, 1967). Most bacteria exhibit a dose-dependent inhibition of growth over the range of 10,000 to 100,000 mg/l and very few species can grow at ethanol concentrations higher than 100,000 mg/l (Ingram and Buttke, 1984).

The toxicity of alcohols is related to their chain length and hydrophobicity (Harold, 1970; Hugo, 1967). Longer chain alcohols, up to a chain length of around 10 carbon atoms, are much more potent inhibitors than are the shorter-chain alcohols. This is attributed to the fact that alcohols have two basic functional groups, namely, a hydroxyl function and a hydrocarbon tail. Ethanol and methanol are very polar and partition poorly into the hydrophobic cell mem-

Table 4. Toxicity thresholds for a *Pseudomonas putida* (From Bringmann and Kuhn, 1980).

Compound	Concentration (mg/l)
Ethanol	6500
Methanol	6600
1-propanol	2700
2-propanol	1050
1-butanol	650
2-butanol	500
Tertiary amyl alcohol	410
Methyl ethyl ketone	1150
Acetic acid	2850
n-butyric acid	875
Benzene	92
Toluene	29
Ethylbenzene	12

Rev Latinoam Microbiol 2002; 44 (2): 83-104

brane (Fig. 8). In contrast, the longer (hydrophobic) hydrocarbon tail of octanol favors its concentration within the membrane, which increases its toxicity. Thus, relatively high ethanol concentrations are required to cause lethal effects on biological systems (Ingram and Buttke, 1984).

Ethanol can exert a variety of biophysical effects on microorganisms. The basic actions of alcohols on prokaryotic organisms appear to be dominated by the physicochemical properties of alcohols rather than involving specific receptors. All hydrophobic and electrostatic interactions in the cytosolic and envelope components of cells can potentially be affected. These include cell membranes, conformations of enzymes and macromolecules, activity coefficients of metabolites, ionization potentials, pKa values of functional groups, and intracellular pH (Franks and Ives, 1966; Ingram and Buttke, 1984; Jukes and Schmidt, 1934; Yaacobi, 1974). High ethanol concentrations can also inhibit the synthesis of various organelles, including the cell wall (Blumberg and Strominger, 1974), RNA (Mitchell and Lucas-Leonard, 1980) DNA (Osztovics *et al.*, 1981), and proteins (Haseltine *et al.*, 1972). Ethanol it self is not mutagenic. However, acetaldehyde, which is a metabolite of aerobic ethanol degradation, increases cell mutation rates (Igali and Gazsó, 1980).

Ethanol has also been reported to adversely affect the activity of some critical enzymes. Addition of low ethanol at 3,350 mg/l did not cause a significant inhibition of the Na^+, K^+-dependent ATPase, NADH oxidase or D-lactate oxidase (Eaton *et al.*, 1982). However, 8,500 mg/l ethanol inhibited these enzymes, with ATPase being the most resistant enzyme examined (Eaton *et al*., 1982). In contrast, succinate dehydrogenase, part of the Kreb's cycle, is more sensitive, showing 20% inhibition with 3,350 mg/l ethanol and 50% inhibition with 8,500 mg/l ethanol. Transport systems are uniformly more sensitive to inhibition by ethanol. The lactose permease system exhibits a dose-dependent inhibition with increasing concentrations of ethanol (Ingram *et al.*, 1980). Uptake of glutamate, proline, leucine and the lactose permease was reduced by 10-30% with 3,350 mg/l ethanol and by 60-80% with 8,500 mg/l ethanol (Eaton *et al.*, 1982). However, inhibition of both the membrane-bound enzymes and transport systems was substantially relieved after removal of alcohol by washing.

Bringmann and Kuhn (1980) developed a cell multiplication test to characterize the inhibitory effect of common water pollutants. This turbidimetric test estimates the concentration at which the inhibitory action of a pollutant starts. The toxicity threshold is taken as the pollutant concentration that yields a biomass concentration that is at least 3% below the mean value of extinction for non-toxic dilutions of the same test culture. This test was applied to the model organism *P. putida,* which is a common BTEX degrader in the subsurface environment. Table 4 compares the toxicity thresholds for several pollutants that could be a involved in a gasoline spill. Based on this study, it can be concluded that indigenous microorganisms are more resistant to high ethanol concentrations than to high BTEX and other fuel constituent concentrations.

Indirect (environmental) effects

Depletion of nutrients and electron acceptors (e.g., oxygen)

Ethanol in groundwater constitutes a significant biochemical oxygen demand compared to that exerted by other soluble components of gasoline, and is likely to accelerate the depletion of dissolved oxygen (Corseuil *et al.*, 1998). This would decrease the extent of aerobic BTEX degradation in oxygen limited aquifers. Such an effect is particularly important for the fate of benzene, which is the most toxic of the BTEX and degrades slowly under anaerobic conditions or not at all (Alvarez and Vogel, 1995; E; Anderson *et al.*, 1998;Weiner and Lovley, 1998a).

Anaerobic processes are believed to play a major role in containing and removing petroleum product releases at sites undergoing natural attenuation, where engineered oxygen addition is uncommon (Rifai *et al.*, 1995; Corseuil *et al*., 1998). Because ethanol can be degraded under all common electron acceptor conditions, they can also contribute to the consumption of dissolved electron acceptors needed for anaerobic BTEX biodegradation (e.g., nitrate, ferric iron, and sulfate). Therefore, depending on aquifer chemistry and the rate of natural replenishment of electron acceptors, ethanol could impede natural attenuation of BTEX compounds by contributing to the depletion of the electron acceptor pool.

The extent to which ethanol is likely to cause the depletion of nutrients and electron acceptors has not been evaluated at the field scale. Nevertheless, a relevant field study was conducted with methanol, which is likely to cause similar effects as ethanol. Barker *et al*. (1992) conducted experiments involving controlled releases of BTEX and methanol mixtures at the Borden site, Canada. At the end of the 476-day experiment, they observed that a greater mass of BTEX remained in the plume from the gasoline with methanol than in the plume from just gasoline. They attributed this effect to oxygen removal by methanol biodegradation as well as to microbial inhibition due to high methanol concentrations.

Accumulation of volatile fatty acids

As discussed previously, the degradation of ethanol by mixed anaerobic cultures can result in the production of

Rev Latinoam Microbiol 2002; 44 (2): 83-104

volatile fatty acids (VFAs) such as acetic, propionic, and butyric acid. In the absence of adequate interspecies H_2 transfer, such VFAs can accumulate and decrease the pH (Lasko *et al.*, 1997; Speece, 1983). This could inhibit some microbial populations and would be particularly detrimental to methanogens, which are usually the most sensitive group of anaerobic consortia. Methanogens are generally inhibited when the pH decreases below 6 (McCarty, 1964). Because methanogens often mediate the final pollutant-stabilization step in the absence of nitrate- and sulfate-based respiration (Section 3.1.2.1), an inhibition of methanogens could adversely affect anaerobic BTEX mineralization.

It should be pointed out that methanogens are not significantly inhibited by VFAs in well-buffered systems. For example, methanogens are often exposed up to 2,000 mg/l VFAs in anaerobic digesters (McCarty, 1964). Other bacteria, however, might be inhibited by high VFA concentrations, even if the pH does not decrease significantly. For example, protein production by *E. coli* at pH 7 is inhibited by acetate at about 2,400 mg/l, especially in the case of expression of recombinant proteins, and growth is retarded at 6,000 mg/l total acetate (Lasko *et al.*, 1997; Sun *et al.*, 1993).

It is unknown whether VFAs would accumulate in aquifers contaminated with alcohol-amended gasoline at sufficiently high concentrations to significantly decrease the pH and inhibit BTEX degradation. Such effects are likely to be system-specific due to variability in buffering and dilution capacity among contaminated sites. It should be kept in mind, however, that VFAs are easily degraded and should not accumulate at high concentrations when alternative electron acceptors such as nitrate, sulfate, and ferric iron are present.

Bioavailability

BTEX bioavailability is rarely a limiting factor. However, ethanol might affect the availability of critical nutrients and co-substrates needed for BTEX bioremediation. As discussed in Section 4.1.3, ethanol exerts a significant biochemical demand for nutrients and electron acceptors. In addition, BTEX migration is often retarded by sorption to aquifer solids. If significant retardation is occurring, dissolved oxygen and other nutrients and electron acceptors traveling at the groundwater velocity can sweep over the contaminant plume from the upgradient margin. This can replenish nutrients and electron acceptors needed for *in situ* BTEX biodegradation. In theory, ethanol could decrease the extent to which BTEX compounds are retarded by sorption. Indeed, evidence suggests that ethanol can affect the sorptive properties of soil organic matter (Brusseau *et al.*, 1991; Kimble and Chin, 1994). A decrease in BTEX retardation would hinder the ability of essential nutrients and electron acceptors transported by bulk flow to catch up with the migrating BTEX compounds. In addition, adsorption of a contaminant to the aquifer matrix increases dilution of the dissolved contaminant plume, which is a process that might also be affected. The extent to which ethanol might hinder these processes, however, is unknown.

Impact of microbial processes on aquifer permeability

Depending on aquifer chemistry and redox conditions, ethanol could stimulate microbial processes that affect the hydrodynamic properties of the aquifer. For example, fuel ethanol would stimulate microbial growth. Therefore the formation of cell aggregates and biofilms that reduce the available pore space is a potential clogging mechanism of concern (Taylor and Jaffe, 1990; Vandevivere and Baveye, 1992). In theory, microorganisms could also affect aquifer permeability by contributing to mineral dissolution (e.g., $CaCO_3$) or precipitation (e.g., FeS). A combination of excessive microbial growth and mineral precipitation could result in a significant reduction in porosity and permeability over a longer period.

An important mechanism by which microorganisms could reduce the effective porosity is the production of gas bubbles that increase the pressure and restrict water flow (Soares *et al.*, 1988, 1989, and 1991). Controlled experiments that address the significance and extent of such phenomena for ethanol contamination are lacking. Therefore, their potential impact is discussed below from a theoretical point of view.

The overall stoichiometry of methanogenesis from ethanol is given by

$$CH_3CH_2OH \rightarrow 1.5\ CH_4 + 0.5\ CO_2$$

Thus,

$$\textit{Potential methane production} = 1.5 \times \frac{14g - CH_4/mol}{46g - ethanol/mol} = 0.5217\ \frac{g - CH_4}{g - ethanol}$$

Based on the ideal gas law, and assuming a typical groundwater temperature of 15°C, the volume of methane produced at 1 ATM from one gram of ethanol is:

$$0.5217\ \frac{g - CH_4}{g - ethanol} \times \frac{1\ mol\ CH_4}{gram} \times \frac{22.4\ liters}{mole\ (at\ STP)} \times \frac{273 + 15K}{273K} = \mathbf{0.73}\ \frac{\mathbf{liters\ CH_4}}{\mathbf{gram - ethanol}}$$

As discussed previously, a 1,000 mg/l ethanol concentration is generally not toxic to methanogenic consortia. This concentration could produce up to 0.73 l of methane within a

one-liter pore volume. This is likely to increase the pressure and could result in some bubble formation that could restrict groundwater flow. Such a reduction in aquifer permeability could also hinder the replenishment of nutrients and electron acceptors by natural or engineered processes into the contaminated zone. Whether sufficient methane would accumulate to create an explosion hazard is unknown.

POTENTIAL EFFECTS OF TRACE COMPOUNDS INTRODUCED DURING PRODUCTION OF FUEL-GRADE ETHANOL

The majority of fuel ethanol in the market is processed by large corn wet millers. These processors generally use highly purified starch as the feedstock. Small processors however still use whole grain fermentation processing where the whole corn grain processed without first separating out starch through wet milling. When purified starch is used, the recovered fuel ethanol is relatively free of contaminants. Beside ethanol, there is glycerol as a major by-product of fermentation (about 5-10% of the quantity of ethanol). However, glycerol has a boiling point higher than 200 °C and is therefore an unlikely contaminant in the distilled final ethanol product. Besides glycerol, another byproduct of the fermentation process for industrial ethanol production is what is called "fusel oil". It is an alcohol mixture having a boiling range of 80-132°C. The amount and composition of fusel oil produced in the fermentation process depend on the raw materials used. A typical fusel oil production ratio during fermentation process is 0.2-0.7% (wt) on the basis of pure ethanol (Karaosmanoglu *et al.*, 1996). Although 50 different compounds have been identified in fusel oil, the major components of fusel oil are fermentation amyl alcohols such as 2-methyl-1-butanol and 3-methyl-1-butanol (Karaosmanoglu *et al.*, 1996). In addition, there are also compounds such as acetaldehydes and ethyl acetate. These contaminants are only in the mg/l range when purified starch is used as the feedstock.

Ethanol fuel from whole grain process will contain substantially more impurities that are derived from the non-starch portions of the whole grains. For instance, in hemicellulose and pectin there are methyl and acetyl groups. Upon hydrolysis, methanol and acetic acid are formed. Subsequently, these are distilled over together with ethanol. The quantity of fusel oil will also be higher in fuel ethanol from whole grain processes. In the case of fuel ethanol, these by-products are not separated out and come with the final product. Methanol is another common process impurity in fuel grade ethanol. Typically, fuel grade ethanol consists of the following: 95.1 wt% ethanol, 4.8 wt% water, and 0.1 wt% higher alcohols (Paul, 1978). At the time of blending, fuel alcohol must meet the criteria in Table 5.

Denaturants are used in fuel ethanol directed for storage. These are toxic or noxious materials added to make the ethanol unfit for oral human consumption. Denaturants used for fuel ethanol are generally unleaded gasoline or rubber hydrocarbon solvent. These are added to ethanol at a minimum of two parts by volume per 100 parts by volume of fuel ethanol, as defined by formula CDA 20 of the Bureau of Alcohol Tobacco and Firearms of the U.S. Treasury Department (American Society of Testing and Materials (ASTM), 1995). This specification prohibits the use of hydrocarbons such as kerosene with an end boiling point higher than 225°C. Thus, only hydrocarbons in the gasoline boiling range can be used as denaturants (ASTM, 1995).

Denatured fuel ethanol may contain additives such as corrosion inhibitors and detergents (ASTM, 1995). Various blending agents also have been used in fuel alcohol gaso-

Table 5. Fuel grade alcohol at time of blending as specified by ASTM specification D4806-95a.

Water content, max, mass %	1.25
Existent gum, max, mg/100 ml	5
Chloride ion content, max, mass ppm	40
Copper content, max, mg/kg	0.1
Acidity (as acetic acid), max, mass %	0.007
Appearance	Visibly free of suspended or precipitated contaminants (clear and bright)

Table 6. Trace compounds introduced during production of fuel ethanol*.

Impurities	Denaturants	Blending agents	Additives
Methanol Fusel oil: amyl and isoamyl alcohols	Unleaded gasoline Rubber hydrocarbon solvent Hydrocarbons with end boiling point < 225 °C (437°F)	Aromatic compounds Aromatic alcohols Higher aliphatic alcohols	Corrosion inhibitors Detergents

* The total content of impurities, denaturants, blending agents, water, and additives must be less than 5% (by volume) of fuel alcohol (ASTM, 1995).

Rev Latinoam Microbiol 2002; 44 (2): 83-104

line mixtures to lower the phase separation temperature of the blends below ambient temperatures experienced during the winter season (Karaosmanoglu *et al.*, 1996). These blending agents can be grouped as aromatic compounds, higher aliphatic alcohols, and aromatic alcohols. The addition of other materials is prohibited. In the final analysis, the ethanol content of denatured fuel ethanol must be at least 95% by volume (ASTM, 1995).

A summary of the trace compounds created or introduced during production and processing of fuel ethanol is shown in Table 6. This table shows no additional trace elements that are noteworthy of concern due to their solubility and associated toxicity compared to other common gasoline components. Neither are any of these trace compounds substantially less biodegradable than other common gasoline components.

CRITICAL KNOWLEDGE GAPS AND RECOMMENDATIONS FOR FUTURE RESEARCH

The proposed replacement of the fuel oxygenate MTBE with ethanol represents potential economic and environmental quality benefits. However, fuel releases that contaminate the subsurface are likely to continue well into the future. Thus, a comprehensive understanding of how ethanol affects the fate and transport of BTEX in aquifers (and related remediation activities) is needed before a widespread changeover can occur. This section outlines critical knowledge gaps and recommendations for future research to enhance evaluating the potential environmental impacts associated with replacing MTBE with ethanol.

The exploitation of natural attenuation processes, within the context of a carefully controlled and monitored site cleanup program, is often the preferred approach to deal with petroleum product releases (National Research Council, 1993). However, current bioremediation and risk management practices may have to be adapted to the increasing possibility of encountering ethanol as a co-contaminant. As a first step, additional laboratory and field research is needed to delineate the applicability and limitations of natural attenuation for different release scenarios of BTEX-alcohol mixtures.

Little is known about the effect of ethanol on microbial population shifts and the resulting catabolic diversity. Considering that the efficiency of bioremediation depends, in part, on the presence and expression of appropriate biodegradative capacities, studying the microbial ecology of aquifers contaminated with gasoline-alcohol mixtures might be a fruitful avenue of research. Such studies should address response variability as a function of release scenario and site specificity, to facilitate risk assessment and remedial action decisions.

Ethanol stimulates microbial processes that may affect aquifer porosity and hydraulic conductivity (e.g., biofilm growth, mineral precipitation or dissolution, and N_2 or CH_4 gas generation). Therefore, it is important to study how the additional presence of ethanol influences the dynamics of anaerobic microbial communities and related processes that affect the hydraulic and chemical properties of the aquifer. Such research should delineate the conditions that lead to a significant accumulation of volatile fatty acids, which could decrease the pH to levels that inhibit bioremediation. Emphasis should be placed on evaluating the potential for alcohol-induced methane production to restrict groundwater flow (thus hindering the replenishment of nutrients and electron acceptors) and pose an explosion hazard (which raises the possibility of requiring unique corrective action measures).

Much of the relevant research to date reflects a reductionist approach to study the effect of ethanol on natural attenuation. For example, to study the effect of ethanol on specific biodegradation activities, batch studies have often been used that eliminate confounding effects from other variables such as BTEX and electron-acceptor concentration gradients, sorption-related retardation, and some mass transport limitations. Similarly, pure cultures have been used to eliminate confounding effects of microbial population shifts. Reductionism generally facilitates hypothesis testing and yields results that are easier to interpret, possibly at the expense of oversimplifying the complex conditions encountered in the field. To determine how ethanol affects BTEX plume dimensions and treatment end points, future research should take on a more holistic approach that considers transport and degradation processes interactively.

Based on laboratory studies and theoretical considerations, it is expected that ethanol will increase BTEX plume length by hindering BTEX biodegradation, enhancing LNAPL dissolution, and facilitating BTEX migration due to a decrease in sorption-related retardation during transport. Nevertheless, there is no information about the subsurface characteristics of ethanol plumes or about the variability of their effect on BTEX fate and transport. Therefore, ethanol and volatile fatty acids data should be collected from sites contaminated with BTEX-ethanol mixtures and used to interpret how the release scenario affects ethanol plume characteristics. Emphasis should be placed on statistically analyzing BTEX data to determine how ethanol affects the stability and dimensions of individual BTEX plumes. Such a survey would provide an integrated picture of the overall effects of ethanol on groundwater pollution and natural attenuation. This information would also provide a stronger basis for risk assessment and for the selection and operation of appropriate remedial systems.

Natural attenuation of BTEX contamination relies heavily on anaerobic biodegradation processes (Rifai *et al.*,

Rev Latinoam Microbiol 2002; 44 (2): 83-104

1995). In such cases, indigenous microorganisms degrade BTEX using electron acceptors preferentially in order of decreasing oxidation potential (Chapelle, 1993). Sequential depletion of electron acceptors often leads to successive transitions from aerobic to denitrifying, iron-reducing, sulfate reducing, and methanogenic conditions. Fuel alcohols contribute to the depletion of electron acceptor pools during their microbial degradation, which is likely to affect temporal and spatial transitions in electron acceptor conditions during natural attenuation of petroleum product releases. Such geochemical transitions are important to study because they affect both BTEX degradation and migration rates. For example, both the changes in electron acceptor availability and the presence of easily degradable ethanol could affect catabolic diversity and the relative abundance of specific BTEX degraders.

Regarding biochemical effects, the need to understand substrate interactions between BTEX and ethanol is very recent, and little research has been conducted on the potential effects of ethanol on BTEX degradation. Often, target pollutants are degraded by inducible enzymes whose expression can be repressed when easily degradable substrates (e.g., ethanol) are present at high concentrations. Although biodegradation of contaminant mixtures is not very well understood at the biochemical level, preferential substrate degradation appears to be a concentration-dependent phenomenon related to repression of the enzymes needed to degrade the target compounds (Egli *et al.*, 1993). Currently, little is known about the conditions leading to sequential or simultaneous degradation of BTEX in the presence of ethanol. This suggests the need to investigate the concentration-dependent effect that ethanol may have on the induction or repression of enzymes that degrade BTEX. In particular, we need to obtain a better understanding of the regulation of specific enzyme activity as a function of the metabolic flux of ethanol under different electron acceptor conditions (e.g., How does ethanol affect the induction or repression of BTEX degradation activity? What ethanol concentrations or metabolic fluxes repress the synthesis of BTEX degrading enzymes? Are the energy state of the cells and the specific ethanol utilization rate critical variables in regulating catabolic gene expression?)

Regarding transport effects, ethanol is completely miscible in water and is often present in gasoline at much higher concentrations than BTEX. Therefore, groundwater impacted by ethanol-amended gasoline is likely to have much higher alcohol than BTEX concentrations. As discussed earlier, high ethanol concentrations in groundwater can enhance the solubilization of BTEX from the free product. Nevertheless, it is unknown if alcohols can enhance BTEX migration by decreasing BTEX adsorption onto aquifer material, which would decrease retardation factors. Lower BTEX retardation factors would contribute to faster migration and longer plumes. In addition, a decrease in BTEX retardation would hinder the ability of dissolved oxygen and other electron acceptors transported by bulk flow to catch up with the migrating BTEX compounds. This implies that an effect of BTEX retardation can indirectly affect biodegradation kinetics. Thus, knowledge of how ethanol affects retardation factors will be useful to formulate more accurate fate-and transport models and for setting site-specific cleanup levels in risk-based corrective action efforts.

Unlike MTBE, which is blended at the refinery and then shipped through pipelines or tankers/barges, ethanol must be blended at the distribution terminal just prior to delivery to the end user. This is because the presence of as little as 1 percent of water can cause "phase separation" of an ethanol-gasoline mixture into alcohol-rich and a hydrocarbon-rich phase (Bauman, 1999). This means that pure ethanol must be stored at terminals in separate tankage, which could also have a release and require remediation at some time. These neat ethanol releases could magnify the negative effects of many of the issues discussed above. In addition, such releases could occur at sites that are already contaminated. Therefore, the effect of neat alcohol releases on natural attenuation of pre-existing BTEX and MTBE contamination should also be investigated.

In summary, additional research is needed to determine if the economic and air-quality benefits of adding ethanol to gasoline outweigh the potential detrimental effects on groundwater pollution and related health risks. Perhaps, the implementation of BTEX bioremediation and natural attenuation strategies will need to be adapted to the increasing possibility of encountering ethanol as a co-contaminant. Clearly, a comprehensive understanding of process mechanisms, microbial ecology, and of response variability as a function of site specificity, should lead to unifying principles that facilitate risk assessment and remedial action decisions.

REFERENCES

1. Alexander, M. (1994). Biodegradation and Bioremediation. Academic Press, Inc., San Diego, CA.
2. Alvarez, P.J.J., P.J. Anid, and T.M. Vogel (1991). Kinetics of aerobic biodegradation of benzene and toluene in sandy aquifer material. Biodegradation 2: 43-51.
3. Alvarez, P.J.J., and T.M. Vogel (1991). Substrate interactions of benzene, toluene, and p-xylene during microbial degradation by pure cultures and mixed culture aquifer slurries. Appl. Environ. Microbiol. **57**: 2981-2985.
4. Alvarez, P.J.J., and T.M. Vogel (1995). Degradation of BTEX and their aerobic metabolites by indigenous microorganisms under nitrate reducing conditions. Wat. Sci. Tech. 31 (1):15-28.
5. Anderson, R.T., J.N. Rooney-Varga, C.V. Gaw, and D.R. Lovley (1998). Anaerobic benzene oxidation in the Fe(III) reduction zone of petroleum-contaminated aquifers. Environ. Sci. Technol. 32 (9): 1222-1229.

6. Araujo, D.B., B. Butler, and C. Mayfield (1998). Effects of gasoline and ethanol mixtures on aquifer microorganisms. Poster presented at the 48th Annual Meeting of the Canadian Society of Microbiologists, June 14-17. Guelph, Ontario.
7. Aronson, D., and P.H. Howard (1997). Anaerobic biodegradation of organic chemicals in groundwater: A summary of field and laboratory studies. Report prepared for the American Petroleum Institute, Chemical Manufacturer's Association, National Council of the Paper Industry for Air and Stream Improvement, Edison Electric Institute, and American Forest and Paper Association. pp. 16 and 224-228.
8. Arvin, E., B.K. Jensen, and A.T. Gundersen (1989). Substrate interactions during aerobic biodegradation of benzene. Appl. Environ. Microbiol. **55** (12): 3221-3225.
9. ASTM (American Society of Testing and Materials) (1995). Standard specification for denatured fuel ethanol for blending with gasolines for use as automotive spark-ignition engine fuel. D 4806-95a. Philadelphia, PA.
10. ASTM (American Society of Testing and Materials) (1998). Standard guide for remediation of ground water by natural attenuation at petroleum release sites. E 1943-98. Philadelphia, PA, pp. 875-916.
11. Atlas, R.M. (ed.) (1984). Petroleum Microbiology. Macmillan Publishing Company, New York, NY.
12. Atlas, R.M., and R. Bartha (1997). Microbial Ecology, 4th ed. Benjamin/Cummings, Menlo Park, CA.
13. Barker, J.F., C.E. Hubbard, L.A. Lemon, and K.A. Vooro (1992). The influence of methanol in gasoline fuels on the formation of dissolved plumes, and the fate and natural remediation of methanol and BTEX dissolved in groundwater. In: E.J. Calabrese and P.T. Kostecki (eds.), Hydrocarbon Contaminated Soils and Groundwater. Lewis Publishers, New York, NY, pp. 103-113.
14. Barker, J.F., M. Schirmer, and B.J. Butler (1998). Fate and transport of MTBE in groundwater - results of a controlled field experiment in light of other experience. Presented at The Southwest Focused Ground Water Conference: Discussing the Issue of MTBE and Perchlorate in Ground Water, Anaheim, CA.
15. Bauman, B. (1999). Personal Communication.
16. Bayly, R.C., and M.G. Barbour (1984). The degradation of aromatic compounds by the meta and gentisate pathways. In: D.T. Gibson (ed.), Microbial Degradation of Organic Compounds. Marcel Dekker, Inc., New York, NY, pp. 253-294.
17. Beller, H.R., and A.M. Spormann (1997). Anaerobic activation of toluene and o-xylene by addition to fumarate in denitrifying strain T. J. Bacteriol. 179 (3): 670-676.
18. Blumberg, P., and J. Strominger (1974). Interaction of penicillin with the bacterial cell: penicillin-binding proteins and penicillin-sensitive enzymes. Bacteriol. Rev. 38: 291.
19. Bornstein, B., and H. Barker (1948). The energy metabolism of Clostridium kluyveri and the synthesis of fatty acids. J. Biol. Chem. 172: 659-669.
20. Braun, M., F. Mayer, and G. Gottschalk (1981). Clostridium aceticum (Wieringa), a microorganism producing acetic acid from molecular hydrogen and carbon dioxide. Arch. Microbiol. 128: 288-293.
21. Bringmann, G., and R. Kuhn (1980). Comparison of the toxicity thresholds of water pollutants to bacteria, algae, and protozoa in the cell multiplication inhibition test. Wat. Res. 14: 231-241.
22. Brown, M. (1989). Biodegradation of oil in freshwaters. In: J. Green and M.W. Trett (eds.), The Fate and Effects of Oil in Freshwater. Elsevier Applied Science, New York, NY.
23. Brusseau, M.L. (1993). Complex mixtures and water quality. EPA/600/S-93/004, United States Environmental Protection Agency, Washington, D.C.
24. Brusseau, M., A. Wood, and P. Rao (1991). Influence of organic solvents on the sorption kinetics of hydrophobic organic chemicals. Environ. Sci. Technol. 25: 903.
25. Butler, B.J., M. VanderGriendt, and J.F. Barker (1992). Impact of methanol on the biodegradative activity of aquifer microorganisms. Paper presented at the 13th Annual Meeting of the Society of Environmental Toxicology and Chemistry, November 8 - 12. Cincinnati, OH.
26. Burland, S.M., and E.A. Edwards (1999). Anaerobic benzene biodegradation linked to nitrate reduction. Appl. Environ. Microbiol. 65 (2): 529-533.
27. Button, D.K., and B.R. Robertson (1986). Dissolved hydrocarbon metabolism: the concentration-dependent kinetics of toluene oxidation in some North American estuaries. Limnol. Oceanogr. 31: 101-111.
28. Carver, M. (1996). Renewable fuels and the future security of U. S. energy supplies. Report to Senate Committee on Agriculture, Nutrition and Forestry, October 2.
29. Chang, M.K., T.C. Voice, and C.S. Criddle (1993). Kinetics of competitive inhibition and cometabolism in the biodegradation of benzene, toluene, and p-xylene by two *Pseudomonas* isolates. Biotechnol. Bioeng. 41: 1057-1065.
30. Chapelle, F.H. (1993). Ground-Water Microbiology and Geochemistry. John Wiley & Sons, Inc., New York, NY.
31. Chemical Market Reporter (1998). Ethanol lobbyists display political clout in renewing the fuel's tax subsidy. November 30, 1998: 25.
32. Conrad, R., T.J. Phelps, and J.G. Zeikus (1985). Gas metabolism evidence in support of the juxtaposition of hydrogen-producing and methanogenic bacteria in sewage sludge and lake sediments. Appl. Environ. Microbiol. 50: 595-601.
33. Corseuil, H., and P.J.J. Alvarez (1996). Natural bioremediation perspective for BTX-contaminated groundwater in Brazil: effect of ethanol. Wat. Sci. Tech. 34 (7-8): 311-318.
34. Corseuil, H., C. Hunt, R. dos Santos Ferreira, and P. Alvarez (1998). The influence of the gasoline oxygenate ethanol on aerobic and anaerobic BTX biodegradation. Wat. Res. 32 (7): 2065-2072.
35. Corseuil, H., and W. Weber (1994). Potential biomass limitations on rates of degradation of monoaromatic hydrocarbons by indigenous microbes in subsurface soils. Wat. Res. 28: 1415-1423.
36. Dagley, S. (1984). Microbial degradation of aromatic compounds. Develop. Indust. Microbiol. 25: 53-65.
37. Dagley, S., E.A. Dawes, and G. Morrison (1950). Inhibition of growth of Aerobacter aerogenes: the mode of action of phenols, alcohols, acetone, and ethyl acetate. J. Bacteriol. 60: 369.
38. Duetz, W.A., S. Marques, C. de Jong , J.L. Ramos, and J-G. Van Andel (1994). Inducibility of the TOL catabolic pathway in *Pseudomonas putida* (pWWO) growing on succinate in continuous culture: evidence of carbon catabolite repression. J. Bacteriol. 176: 2354-2361.
39. Eaton, L., T. Tedder, and L. Ingram (1982). Effects of fatty acid composition on the sensitivity of membrane functions to ethanol in *Escherichia coli*. Subst. Alcohol Actions Misuse 3: 77.
40. Edwards, E.A., and D. Grbic-Galic (1992). Complete mineralization of benzene by aquifer microorganisms under strictly anaerobic conditions. Appl. Environ. Microbiol. 58 (8): 2663-2666.
41. Egli T. (1995). The ecological and physiological significance of the growth of heterotrophic microorganisms with mixtures of substrates. Adv. Microb. Ecol. 14: 305-386.
42. Egli, T., U. Lendenmann, and M. Snozzi (1993). Kinetics of microbial growth with mixtures of carbon sources. Antonie van Leeuwenhoek 63: 289-298.
43. Eichler, B., and B. Schink (1984). Oxidation of primary aliphatic alcohols by Acetobacterium carbinolicum sp. nov., a homoacetogenic anaerobe. Arch. Microbiol. 140: 147-152.
44. English, C.W., and R.C. Loehr (1991). Degradation of organic vapors in unsaturated soil. In: Bioremediation Fundamentals and Effective Application, Proceedings of the 3rd Annual Symposium at the Gulf Coast Hazardous Substance research center, pp. 65-74. February, 1991.

45. EPA (U.S. Environmental Protection Agency) (1998). Corrective action measures for 2nd half FY98. URL: http://www.epa.gov/swerust1/cat/cam1198.htm.
46. Federal Register (1985). U. S. Government Printing Office, Washington D.C. November 13.
47. Franks, F., and D. Ives (1966). The structural properties of alcohol-water mixtures, Quarterly Reviews (Chemical Society of London) 20: 1-44.
48. Gibson, D.T., and V. Subramanian (1984). Microbial degradation of aromatic hydrocarbons. In: D.T. Gibson (ed.), Microbial Degradation of Organic Compounds. Marcel Dekker, Inc. New York, NY. pp 181-252.
49. Gottschalk, G. (1986). Bacterial Metabolism. Springer-Verlag, New York, NY, pp. 162-171.
50. Grbic-Galic, D., and T.M. Vogel (1987). Transformation of toluene and benzene by mixed methanogenic cultures. Appl. Environ. Microbiol. 53 (2): 254-260.
51. Gülensoy, N., and P.J.J. Alvarez (1999). Diversity and correlation of aromatic hydrocarbon biodegradation capabilities. Biodegradation (in press).
52. Harold, F. (1970). Antimicrobial agents and membrane function. Adv. Microbial Physiol. 4: 45.
53. Haseltine, W.A., R. Block, W. Gilbert, and K. Weber (1972). MSI and MSII made on ribosome in idling step of protein synthesis. Nature 238: 381.
54. Heider, J., and G. Fuchs (1997). Anaerobic metabolism of aromatic compounds. Eur. J. Biochem. 243: 577-596.
55. Heider, J., A.M. Spormann, H.R. Bellar, and F. Widdel (1999). Anaerobic bacterial metabolism of hydrocarbons. FEMS Microbiol. Rev. 22: 459-473.
56. Howard, P.H. (ed.) (1991). Handbook of Environmental Degradation Rates. Lewis Publishers, Chelsea, MI.
57. Hugo, W.B. (1967). The mode of action of antibacterial agents. J. Appl. Bacteriol. 30: 17.
58. Hunt, C.S. (1999). Effect of alternative substrates on the biodegradation of monoaromatic hydrocarbons. Doctoral dissertation, The University of Iowa, Iowa City, Iowa.
59. Hunt, C., P. Alvarez, R. dos Santos Ferreira, and H. Corseuil (1997a). Effect of ethanol on aerobic BTEX degradation. In: B.C. Alleman and A. L. Leeson (eds.), *In situ* and Onsite Bioremediation, 4 (1). Batelle Press, Columbus, OH, pp. 49-54.
60. Hunt, C.S., L.A. Cronkhite, H.X. Corseuil, and P.J. Alvarez (1997b). Effect of ethanol on anaerobic toluene degradation in aquifer microcosms. In: Preprints of Extended Abstracts 37 (1): 424-426. Proceedings of the 213th ACS National Meeting. San Francisco, CA.
61. Hutchins, S.R., W.C. Downs, J.T. Wilson, G.B. Smith, D.A. Kovacs, D.D. Fine, R.H. Douglas, and D.J. Hendrix (1991). Effect of nitrate addition on biorestoration of fuel-contaminated aquifer: field demonstration. Groundwater 29: 572-580.
62. Igali, S., and L. Gazsó (1980). Mutagenic effect of alcohol and acetaldehyde on *Escherichia coli*, Mutat. Res. 74: 209.
63. Ingram, L., and T. Buttke (1984). Effects of alcohols on micro-organisms. Advances in Microbial Physiology 25: 253-303.
64. Ingram, L., B. Dickens, and T. Buttke (1980). Reversible effects of ethanol on *E. coli*. Adv. Exp. Med. Biol. 126: 299.
65. Jukes, T., and C. Schmidt (1934). The apparent dissociation constants of certain amino acids and related substances in water-ethanol mixtures. J. Biol. Chem. 105: 359.
66. Karaosmanoglu, F., A. Isigigur, and H. Ayse Aksoy (1996). Effects of a new blending agent on ethanol-gasoline fuels. Energy & Fuels 10: 816-820.
67. Kazumi, J., M.E. Caldwell, J.M. Suflita, D.R. Lovley, and L.Y. Young (1997). Anaerobic degradation of benzene in diverse anoxic environments. Environ. Sci. Technol. 31 (3): 813-818.
68. Kimble, K., and Y. Chin (1994). The sorption of polycyclic aromatic hydrocarbons by soils in low-methanol/water mixtures. J. Contam. Hydrol. 17: 129-143.
69. King, R.B., G.M. Long, and J.K. Sheldon (1992). Practical Environmental Bioremediation. Lewis Publishers, Boca Raton, FL.
70. Kukor, J.J., and R.H. Olsen (1989). Diversity of toluene degradation following long term exposure to BTEX *in situ*. In: D. Kamely, A. Chakabarty, and G. Omenn (eds.), Biotechnology and Biodegradation. Portfolio Publishing Company, The Woodlands, TX, pp. 405-421.
71. Laanbroek, H., T. Abee, and J. Voogd (1982). Alcohol conversion by Desulfolobus propionicus Lindhorst in the presence and absence of sulfate and hydrogen. Arch. Microbiol. 133: 178-184.
72. Lasko, D., C. Schwerdel, J. Bailey, and U. Sauer. (1997). Acetate-specific stress response in acetate-resistance bacteria: an analysis of protein patterns. Biotechnol. Prog. 13: 519-523.
73. Lee, M., J. Thomas, R. Borden, P. Bedient, C. Ward, and J. Wilson (1988). Biorestoration of aquifers contaminated with organic compounds. CRC Crit. Rev. Environ. Control 1: 29-89.
74. Linkfield, T., J. Suflita, and J. Tiedje (1989). Characterization of the acclimation period before anaerobic dehalogenation of chlorobenzoates. Appl. Environ. Microbiol. 55: 2773-2778.
75. Lovley, D.R., J.C. Woodward, and F.H. Chapelle (1996). Rapid anaerobic benzene oxidation with a variety of chelated Fe(III) forms. Appl. Environ. Microbiol. 62 (1): 288-291.
76. Lugar, R.G., and R.J. Woolsey (1999). The new petroleum. Foreign Affairs 78: 88-102.
77. Madigan, J.T., J.M. Martinko, and J. Parker (1997). Brock Biology of Microorganisms, 8th ed. Prentice Hall, Upper Saddle River, NJ.
78. Malcolm Pirnie, Inc. (1998). Evaluation of the fate and transport of ethanol in the environment. Report Prepared for the American Methanol Institute, Washington, D.C.
79. McCarty, P.L. (1964). Anaerobic waste treatment fundamentals, part three, toxic materials and their control. Public Works 95: 91-94.
80. McCarty, P. L. (1969). Energetics and bacterial growth. Fifth Rudolf Research Conference, Rutgers, The State University, New Brunswick, New Jersey.
81. Meckenstock, R.U. (1999). Fermentative toluene degradation in anaerobic defined syntrophic cocultures. Fems Microbiol. Lett. 177: 67-73.
82. Merchuk, J.C., and J.A. Ansejo (1995). The Monod equation and mass transfer. Biotechnol. Bioeng. 45: 91-94.
83. Mitchell, J., and J. Lucas-Leonard (1980). The effect of alcohols on guanosine 5'-diphosphate-3'-diphosphate metabolism in stringent and relaxed *Escherichia coli*. J. Biol. Chem. 255: 6307.
84. Monod, J. (1949). The growth of bacterial cultures. Ann. Rev. Microbiol. 3: 371-394.
85. Mormile, M., S. Liu, and J. Suflita (1994). Anaerobic biodegradation of gasoline oxygenates: extrapolation of information to multiple sites and redox conditions. Environ. Sci. Technol. 28 (9): 1727-1732.
86. National Research Council (1993). *In situ* Bioremediation: When Does it Work? National Academy Press, Washington, DC.
87. Oldenhuis, R., L. Kuijk, A. Lammers, D.B. Janssen, and B. Witholt (1989). Degradation of chlorinated and non-chlorinated aromatic solvents in soil suspensions by pure bacterial cultures. Appl. Environ. Microbiol. 30: 211-217.
88. Osztovics, M., S. Igali, A. Antal, and P. Veghelyi (1981). Alcohol is not mutagenic. Mutat. Res. 74: 247.
89. Paul, E., and F. Clarck (1989). Soil Microbiology and Biochemistry. Academic Press, San Diego, CA.
90. Paul, J. (1978). Methanol Technology and Application in Motor Fuel. Noyes Data, Park Ridge, NJ.
91. Petrobrás (1995). Relatório Anual de Atividades (Annual Report). Rio de Janeiro, RJ, Brazil.

Rev Latinoam Microbiol 2002; 44 (2): 83-104

92. Phelps, T.J., R. Conrad, and J.G. Zeikus (1985). Sulfate-dependent interspecies H_2 transfer between Methanosarcina barkeri and Desulfovibrio vulgaris during coculture metabolism of acetate or methanol. Appl. Environ. Microbiol. 50: 589-594.
93. Phelps, C.D., L.J. Kerkhof, L.Y. Young (1998). Molecular characterization of a sulfate-reducing consortium which mineralizes benzene. FEMS Microbiol. Ecol. 27: 269-279.
94. Phelps, C.D., and L.Y. Young (1999). Anaerobic biodegradation of BTEX and gasoline in various aquatic sediments. Biodegradation 10: 15-25.
95. Postgate, J., and L. Campbell (1966). Classification of Desulfovibrio species, the nonsporulating sulfate-reducing bacteria. Bacteriol. Rev. 30: 732-738.
96. Rifai, H.S., R.C. Borden, J.T. Wilson, and C.H. Ward (1995). Intrinsic bioattenuation for subsurface restoration. In: R.E. Hinchee, F.J. Brockman, and C.M. Vogel (eds.), Intrinsic Bioremediation. Battelle Press, Columbus, OH.
97. Robertson, B.R., and D.K. Button (1987). Toluene induction and uptake kinetics and their inclusion in the specific-affinity relationship for describing rates of hydrocarbon metabolism. Appl. Environ. Microbiol. 53 (9): 2193-2205.
98. Rooney-Varga, J.N., R.T. Anderson, J.L. Fraga, D. Ringelberg, and D.R. Lovley (1999). Microbial communities associated with anaerobic benzene degradation in a petroleum-contaminated aquifer. Appl. Environ. Microbiol. 65 (7): 3056-3063.
99. Schink, B., D. Kremer, and T. Hansen (1987). Pathway of propionate formation from ethanol in *Pelobacter propionicus*. Arch. Microbiol. 147: 321-327.
100. Schraa, G., B.M. Bethe, A.R.W. Van Neerven, W.J.J. Can Den Tweel, E. Van Der Wende and A.J.B. Zehnder (1987). Degradation of 1,2-dimethylbenzene by *Corynebacterium* strain C125. Antoine van Leeuwenhoek 53: 159-170.
101. Shields, M.S., S.O. Montgomery, P.J. Chapman, S.M. Cuskey and P.H. Pritchard (1989). Novel pathway of toluene catabolism in trichloroethylene-degrading bacterium G4. Appl. Environ. Microbiol. 55: 1624-1629.
102. Simoni, S.F., A. Schafer, H. Harms, and A.J.B. Zehnder (1999). Factors affecting mass transfer limitation of biodegradation in saturated porous media. Environ. Sci. Technol. (submitted).
103. Soares, M.I.M., S. Belkin, and A. Abeliovich (1988). Biological groundwater denitrification: laboratory studies. Wat. Sci. Technol. 20 (3): 189-195.
104. Soares, M.I.M., S. Belkin, and A. Abeliovich (1989). Clogging of microbial denitrification sand columns: gas bubbles or biomass accumulation? Z. Wasser Abwass. For. 22: 20-24.
105. Soares, M.I.M., C. Braester, S. Belkin, and A. Abeliovich (1991). Denitrification in laboratory sand columns: carbon regime, gas accumulation and hydraulic properties. Wat. Res. 25 (3): 325-332.
106. Speece, R.E. (1983). Anaerobic biotechnology for industrial wastewater treatment. Environ. Sci. Technol. 17 (9): 416A-426A.
107. Stryer, L. (1988). Biochemistry, 3rd ed. W.H. Freeman and Company. New York.
108. Suflita, J.M., and M.R. Mormile (1993). Anaerobic biodegradation of known and potential gasoline oxygenates in the terrestrial subsurface. Environ. Sci. Technol. 27 (6): 976-978.
109. Sun, W., C. Lee, H. George, A. Powell, M. Dahlgren, and C. Park (1993). Acetate inhibition on growth of recombinant *E. coli* and expression of fusion protein TGF-a-PE40. Biotechnol. Lett. 25: 809-814.
110. Taylor, S.W., and P.R. Jaffe (1990). Biofilm growth and the related change in the physical properties of a porous medium – 1. Experimental investigation. Wat. Res. 26 (9): 2153-2159.
111. Thauer, R.K., D. Möller-Zinkhan, and A.M. Spormann (1989). Biochemistry of acetate catabolism in anaerobic chemotrophic bacteria. Annu. Rev. Microbiol. 43: 43-67.
112. Tiedje, J.M. (1993). Bioremediation from an ecological perspective. In: *In situ* Bioremediation: When Does it Work? National Research Council, National Academy Press, Washington, D.C.
113. Vandevivere, P., and P. Baveye. (1992). Relationship between transport of bacteria and their clogging efficiency in sand columns. Appl. Env. Microbiol. 58 (8): 2523-2530.
114. Weiner, J.M., and D.R. Lovley (1998a). Anaerobic benzene degradation in petroleum-contaminated aquifer sediments after inoculation with a benzene-oxidizing enrichment. Appl. Env. Microbiol. 64 (2): 775-778.
115. Weiner, J.M., and D.R. Lovley (1998b). Rapid benzene degradation in methanogenic sediments from a petroleum-contaminated aquifer. Appl. Env. Microbiol. 64 (5): 1937-1939.
116. White, D. (1995). The Physiology and Biochemistry of Prokaryotes. Oxford University Press, New York, NY, pp. 101-102.
117. Widdel, F. (1986). Growth of methanogenic bacteria in pure culture with 2-propanol and other alcohols as hydrogen donors. Appl. Environ. Microbiol. 51: 1056-1062.
118. Wolin, M.J., and T.L. Miller (1982). Interspecies hydrogen transfer: 15 years later. ASM News 48: 561-565.
119. Wu, M., and R. Hickey (1996). n-Propanol production during ethanol degradation using anaerobic granules. Wat.Res. 30 (7): 1686-1694.
120. Xia, Z., Y. He, W. Dai, S. White, G. Botd, and F. Matthews (1999). Detailed active site configuration of a new crystal form of methanol dehydrogenase from Methylophilus W3A1 at 1.9 A resolution. Biochemistry 38: 1214-1220.
121. Yaacobi, M., and A. Ben-Naim (1974). Solvophobic interaction. J. Phys. Chem. 78: 175.
122. Zehnder, A.J.B., and W. Stumm (1988). Geochemistry and biogeochemistry of anaerobic habitats. In: A.J.B. Zehnder (ed.), Biology of Anaerobic Organisms. Wiley-Liss, New York, NY.
123. Zeyer, J., P. Eicher, J. Dolfing, and R.P. Schwarzenbach (1990). Anaerobic degradation of aromatic hydrocarbons. In: D. Kamely, A. Chakrabarty, and G.S. Omenn (eds.), Biotechnology and Biodegradation. Portfolio Publishing Company, The Woodlands, TX, pp. 33-40.

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