Editor's note: Traditionally, wastewater treatment systems removed biochemical oxygen demands (BODs). Then they also began to remove nutrients such as nitrogen and phosphorus. More recently, treatment systems have begun to remove toxic organic chemicals from wastewater. Pollution control engineers and scientists have found that exposure of microorganisms to sequential environments enhances the removal of pollutants and at the same time produces sludge with good settling characteristics.

In a review article by Richard Speece and Daniel Zitomer of Vanderbilt University, the authors describe the sequential exposure of microorganisms and the biological transformations that occur in aerobic environments, anoxic environments (no dissolved oxygen but nitrite or nitrate can be present), and anaerobic environments (no dissolved oxygen and no nitrite or nitrate are present). Now, it is possible to design a single-sludge process that relies on these sequential environments. Also, anaerobic processes are feasible for treatment of high-strength industrial wastewaters. Other possible applications of sequential environments are for treatment of hazardous waste, soil, and groundwater contaminated with chlorinated solvents such as pesticides and PCBs. The authors, however, caution that before sequential environments are used to their fullest potential, more work and research need to be done.

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ES&T CRITICAL REVIEW

SEQUENTIAL ENVIRONMENTS FOR ENHANCED BIOTRANSFORMATION OF AQUEOUS CONTAMINANTS

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Initially, aerobic biological processes were employed as cost-effective, reliable systems for removal of biochemical oxygen demand (BOD) only. As effluent nitrogen levels began to be more strictly regulated, modified systems comprised of sequential anoxic and aerobic environments were used to remove nitrogen and BOD from wastewater. Today, regulation of phosphorus levels has been imposed for industrial and municipal treatment plant effluents discharged to Lake Erie, Lake Ontario, the Chesapeake Bay, Susquehanna River, Potomac River, and other receiving waters deemed highly susceptible to eutrophication (*1*, *2*).

Again, aerobic processes have been modified, by including anaerobic and aerobic sequential environments that facilitate biological phosphorus removal. As regulation of inorganic pollutants in wastewater effluents has developed, so has biological technology. It is now possible to design a single-sludge process that relies on sequential anaerobic, anoxic, and aerobic envi-

ronments for removal of phosphorus, nitrogen, and BOD. Indeed, these technological developments have been so successful that some have claimed biological nutrient removal abilities can be incorporated into facility design at a cost consistent with the range of costs for conventional secondary treatment (*3, 4*).

Recently, pollution control engineers and scientists have been challenged with another task: the removal of toxic organic chemicals from wastewater. Strict toxicity reduction standards have been promulgated, and new toxicity standards are being considered (*5*). Can biological treatment processes be modified yet again to remove a broader range of toxic chemicals? Significant proof indicates they can. The sequential exposure of microorganisms to anaerobic (no nitrite or nitrate and no dissolved oxygen), anoxic (nitrite or nitrate present and no dissolved oxygen), and aerobic environments that facilitate biological nitrogen and phosphorus removal shows promise as an effective process for removing a broad range of chemicals.

This review describes sequential environments commonly encountered in wastewater treatment; biological transformations that occur in aerobic, anoxic, and anaerobic environments; and research on the degradation of specific organic compounds in sequential environments.

Nature of sewerage systems

Many treatment schemes incorporate sequential anaerobic, anoxic, and aerobic environments. Perhaps the most simple are conventional aerobic activated sludge plants, which receive wastewater conveyed through extensive gravity sewerage systems or force mains. An anaerobic environment may predominate within the conveyance system, whereas the activated sludge process at "the end of the pipe" constitutes an aerobic environment. In sewers, extensive biological reduction of sulfate to sulfide and anaerobic fermentation may occur within slime attached to submerged surfaces (*6*). Other researchers have observed that "particle hydrolysis and substrate production for sulfate reducers from fermentative bacteria alone or in syntrophic association with sulfate reducers make the system very complex" (*7*). Longer sewer detention times have been shown to greatly increase the fraction of fermentation endproducts in domestic wastewater, giving proof of extensive anaerobic biodegradation. Researchers have found that acetic acid concentrations in raw wastewater from Haifa, Israel, increased as much as 100% in 24 h at a temperature of 25 °C. They concluded that extensive fermentation of carbohydrates, proteins, and fats was taking place during the 6–12 h residence time in the sewers (*8*).

In fact, many have proposed to optimize the biological processes that occur in sewerage systems (*9, 10*). A conceptual model has been developed that includes return sludge pumping to increase biomass in the sewers of Tel Aviv, Israel. It indicates that using the sewers for biological treatment will produce effluent BOD concentrations below 25 mg/L and will save 50% of the cost of a required treatment plant expansion (*11*). Although these investigations include provisions for injecting air or oxygen into the sewerage system, they do reveal the subliminal theory that biological degradation—both anaerobic and aerobic—is an important process in conveyance systems that should not be ignored.

This review describes sequential environments commonly encountered in wastewater treatment; biological transformations that occur in aerobic, anoxic, and anaerobic environments; and research on the degradation of specific organic compounds in sequential environments.

Anaerobic pretreatment

Interest in anaerobic biotechnology for industrial wastewater treatment has greatly increased during the past decade. Today, anaerobic processes are recognized as feasible unit operations for treatment of many high-strength industrial wastewaters (*12*). Benefits of anaerobic pretreatment often cited include lower electrical power requirements; production of methane, which may be used for heating or power generation; and lower sludge generation than that of aerobic systems (*13*). Often, anaerobic effluents are discharged to public sewer systems or are followed by aerobic treatment. Therefore, in many cases where anaerobic treatment is employed, a sequential anaerobic–aerobic system is the overall process.

Anaerobic pretreatment has been successfully used to treat wastewater from Aspartame (a synthetic sweetener) production (*14*). A hybrid anaerobic upflow filter with a hydraulic detention time between 1.4 and 4 days removed 80–95% of the chemical oxygen demand (COD) from wastewater that had a COD between 10,000–30,000 mg/L. Subsequent treatment of the anaerobic effluent was completed at a publicly owned treatment plant. Other wastewaters amenable to anaerobic pretreatment include pharmaceutical fermentation, ethanol production, coal conversion, septic tank effluents, food processing, landfill leachates, dairy, distillery, and many other waste streams (*15, 16*). Recently, much work has focused on the application of anaerobic–aerobic systems for treatment of pulp and paper industry wastewaters. A review of anaerobic treatment indicates that 55% of the waste streams from kraft, sulfite, mechanical, and semi-chemical mills are amenable to anaerobic treatment (*17*). Furthermore, 37 full-scale anaerobic installations that treat pulp and paper wastes were in operation, in start-up, or under construction at the time of the review's preparation (*17*). Most of these systems include subsequent aerobic treatment. For example, some workers describe an upflow anaerobic sludge blanket reactor followed by an aerobic activated sludge process for the treatment of pulp and paper wastewater (*18*). Design objectives were to achieve a 75–80% overall reduction

in BOD and to produce a final effluent that is not acutely toxic to fish. Evidence that these objectives have been met has not been published; however, preliminary results demonstrate that these goals are achievable.

Anaerobic pretreatment of pulp and paper wastewater has resulted in lower sludge generation and methane gas production. Overall sludge yield from an anaerobic–aerobic system that treats white water from thermochemical pulp production has been found to be about one-third of that in a single-stage, activated sludge treatment unit (*19*). In addition, the aerobic post-treatment resulted in stable effluent quality during temporary failure of the anaerobic system.

Sequences for enhanced sludge settling

Many researchers have reported that under specific environmental conditions filamentous organism growth is greater than that of most floc-forming microorganisms. This is most notable when low substrate or dissolved oxygen predominates (*20*). The proliferation of filamentous bacteria results in a bulking sludge—which settles slowly, compacts poorly, and hinders treatment plant operations—causing a deterioration in effluent quality (*21*). One solution to activated sludge bulking, first proposed in 1973, is to include a tank or series of tanks in which return sludge and wastewater are mixed before they enter the aeration basin (*22, 23*). The growth of undesirable filamentous organisms is discouraged by exposing return sludge to an initially high concentration of substrate and maintaining a substrate concentration gradient (*24*).

Interestingly, the aeration of mixed liquor within the initial tank is not a prerequisite for optimum operation. In fact, some researchers report that anaerobic contact zones are the best design choice (*25*). One pilot plant included an anaerobic mixing zone in front of the aeration tank for treatment of a pulp and paper mill's wastewater (*26*). The resulting sludge possessed superior bioflocculation characteristics when compared with sludge produced in a plant without the anaerobic zone. In addition, the Massachusetts Water Resource Authority's treatment plant, currently under construction, includes 11 anaerobic tanks in which mixed liquor and primary effluent are blended before entering pure oxygen reactors (*27*).

A comparison of the performance of aerobic, microaerophilic (low-dissolved-oxygen zone designed to avoid patent infringements), anoxic, and anaerobic mixing zones (*28*) has shown that anaerobic, anoxic, and aerobic zones could all be used to control filamentous bulking. However, the microaerophilic zone did not cure the bulking sludge problem. This failure was attributed to dissolved oxygen gradients, caused by the use of mechanical aerators, within the main aeration tank; however, no proof of oxygen gradients within the aeration tank was presented.

Sequences for nitrogen removal

Many biological and physicochemical processes have been employed to remove nitrogen from wastewaters (*29, 30*). Biological nitrogen removal methods include the use of artificial wetlands in which aquatic plants absorb nitrogen from wastewater, as well as sequential anoxic–aerobic processes that result in nitrogen removal by bacteria (*31–33*). Often, plant uptake of nitrogen cannot account for the high nitrogen removal efficiencies observed in artificial wetlands. Some have suggested that a major role of aquatic plants is to provide oxygen rich microzones in the otherwise reduced environment. These microzones establish anoxic–aerobic interfaces that stimulate bacterial nitrogen removal (*34*).

In anoxic–aerobic nitrogen removal systems, ammonia ($NH_3NH_4^+$) is oxidized in aerobic zones to nitrite (NO_2^-), and nitrite is then oxidized to nitrate (NO_3^-) by bacteria from the family *Nitrobacteraceae*:

$$NH_4^+ + 1.5O_2 + 2HCO_3^- \rightarrow NO_2^- + 2H_2CO_3 + H_2O$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^-$$

This process is referred to as nitrification. Commonly, bacteria from the genus *Nitrosomonas* accomplish the first step, and *Nitrobacter* perform the second oxidation. In addition, bacteria of the genus *Nitrosospira*, *Nitrococcus*, *Nitrosolobus*, and *Nitrosovibrio* oxidize ammonia to nitrite, and *Nitrospina* and *Nitrococcus* oxidize nitrite to nitrate (*35*).

In anoxic environments, many bacteria can reduce nitrate to nitrogen gas (N_2) when electron donors are present. This process is referred to as denitrification. The most common denitrification reactions are accomplished by heterotrophic bacteria (*Pseudomonas*, *Micrococcus*, *Bacillus*, and *Alcaligenes*), which rely on organic compounds such as methanol, as electron donors:

$$NO_3^- + 0.83CH_3OH \rightarrow 0.5N_2 + 0.83CO_2 + 1.17H_2O + OH^-$$

The nitrogen gas produced is released to the atmosphere; therefore, it is removed from the wastewater.

Heterotrophic denitrification is often accomplished in wastewater treatment by including anoxic zones in the activated sludge process. Single, double, and triple sludge systems have been employed (*30, 33, 36*). However, the single sludge system is often considered to be the best activated sludge modification for biological nitrogen removal. Single sludge systems require minimal clarifier construction. In addition, the external carbon source required for double and triple sludge systems is not needed for single sludge nitrogen removal (*37*). A single sludge nitrogen removal system is comprised of an initial anoxic stage, which facilitates nitrate reduction to nitrogen gas and BOD removal, followed by an aerobic tank for continued removal of BOD and oxidation of ammonia to nitrate. A portion of the mixed liquor is recycled to the anoxic tank, which provides nitrate to maintain anoxic conditions.

Denitrification may also be accomplished by the autotrophic bacteria *Paracoccus denitrificans* and *Thiobacillus denitrificans*, which use hydrogen or sulfur compounds as electron donors, and reduce nitrate to nitrogen gas (*38*):

Hydrogen: $2NO_3^- + 5H_2 \rightarrow N_2 + 4H_2O + 2OH^-$

Thiosulfate: $8NO_3^- + 5S_2O_3^{-2} + H_2O \rightarrow 4N_2 + 10SO_4^{-2} + 2H^+$

Sulfide: $8NO_3^- + 5S^{-2} + 8H^+ \rightarrow 4N_2 + 5SO_4^{-2} + 4H_2O$

Also, filamentous *Beggiatoa* spp., which often form mats in freshwater and marine environments, have recently been found to oxidize sulfite while denitrifying (*39*). In addition, *Gallioneila ferruginea*, which is often found in iron-bearing waters, can oxidize iron while denitrifying, but this process is relatively slow (*40*).

Autotrophic denitrification has not been applied to full-scale wastewater treatment. In bench-scale studies, nitrified wastewater was denitrified by *Thiobacillus denitrificans* in packed-bed reactors con-

taining elemental sulfur (*41*). The feasibility of autotrophic denitrification using elemental sulfur was investigated; it was concluded that the applicability of autotrophic denitrification depends on the prices of sulfur and methanol (*42*). Also, it was noted that autotrophic denitrification may be more stable than some heterotrophic systems during transient nitrate loadings. For instance, the addition of methanol or other electron donors must be precisely controlled to prevent overdosing and underdosing heterotrophic systems. On the other hand, elemental sulfur is relatively insoluble and an excess may remain within an autotrophic reactor in the solid form without causing problems.

Sequences for phosphorus removal

Phosphate is often removed from wastewater by the addition of aluminum or iron salts, which combine with phosphate to form insoluble metal–phosphate precipitates that can be eliminated by clarification (*43, 44*). The metal salts used are relatively expensive and the disposal of the large amounts of precipitate sludge generated is costly. Operators at a few U.S. treatment plants—most notably: San Antonio, TX; Baltimore, MD; Los Angeles, CA; Milwaukee, WI; Amarillo, TX; and Fort Worth, TX—and treatment plant operators in South Africa observed serendipitous phosphorus removal of 70% or greater without chemical addition (*45–49*). Important questions were then raised. Is the serendipitous phosphorus removal biological? How can a treatment plant be designed to achieve phosphorus removal without chemical addition?

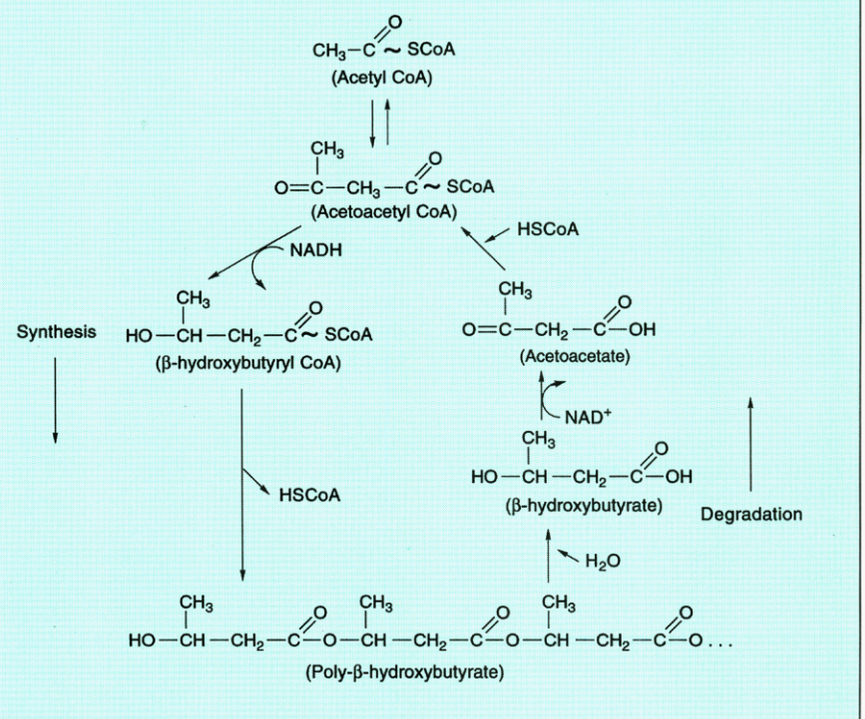
The conditions at Baltimore's Back River Treatment Plant are typical of facilities that remove phosphorus without chemical addition. Early investigators noted certain factors that appear to contribute to the phenomena. First, the Baltimore activated sludge system is an extreme example of plug-flow aeration; other plants that remove phosphorus in the same manner also have plug-flow aeration systems (*46*). Second, at the Baltimore plant, air was supplied by fixed diffuser plates, which were not cleaned or replaced often. This resulted in detectable dissolved oxygen concentrations at the aeration tank head and concentrations between 2 and 3 mg/L at the final aeration tank (*46*). The anaerobic–aerobic configuration existed at other similar plants (*50*). Third, the pH of mixed liquor was often found to rise from an influent value of approximately 7 to an effluent value of approximately 8 pH units (*50*).

Some investigators argued that significant removal of calcium phosphate precipitate would occur in hard water sewages as the pH of mixed liquor increased. The increase in pH was attributed to high aeration rates, which tend to strip carbon dioxide out of the mixed liquor. Therefore, enhanced phosphate removal was not considered a biological process. However, it was found that under certain anaerobic–aerobic conditions, activated sludge microorganisms can contain more than the normal 2–3% phosphorus. In fact, some forms of bacteria, most notably *Acinetobacter calcoaceticus*, can store as much as 25% of their weight as phosphorus (*51*). Therefore, precipitation of calcium phosphate is not considered to be a major phosphorus removal mechanism, and the biological phosphorus removal (bio-P) hypothesis is now accepted as the predominant mechanism of enhanced removal. Some fundamental biological models have been developed to explain this phenomenon, and a comprehensive understanding of the process has emerged (*52–59*). A biochemical model offers an insightful description of the fundamental process (*60*).

A hypothetical biochemical model lends order to the current description of bio-P processes. Therefore, the Comeau model is described in the following paragraphs (*60*). However, it should be noted that further research is necessary before a conclusive, detailed biochemical model can be described (*61*). The Comeau model is based on the necessary maintenance of a proton motive force across the cell membrane. In the anaerobic reactions, carbonaceous substrates are transported across the cell wall and stored as poly-β-hydroxybutyrate (PHB), a biologically synthesized polyester that serves as an energy reserve. The pathway for PHB synthesis and degradation is a branch of the fatty acid synthesis pathway. The final portion of the transformation of substrates into PHB is represented in Figure 1.

Bio-P systems operate most effectively when a major portion of carbonaceous substrate is in the form of volatile fatty acids such as acetic and propionic acid (*63*). These acids are transported into the cell in the neutral form and shed a proton in the slightly basic environment inside the cell. This may lower the

FIGURE 1
Poly-β-hydroxybutyrate metabolic pathways



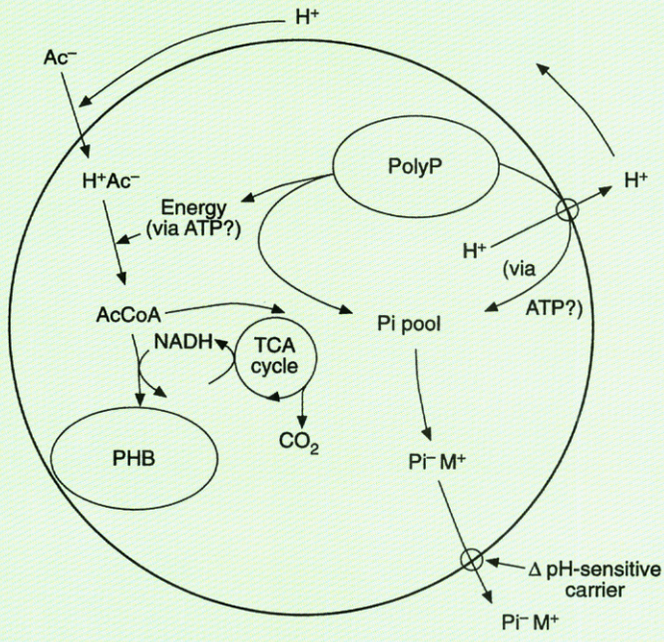
Source: Reference 62.

internal cell pH and destroy the proton motive force, which is essential to cell metabolism. To maintain a negative charge distribution on the inner cell membrane, a proton may be exported from the cell in the form of some protonated phosphate anions complexed with magnesium or calcium. Alternatively, adenosine triphosphate (ATP) may be synthesized from polyphosphate and employed as an energy source for a translocating enzyme that expels protons and phosphate. Both mechanistic theories are partially supported by the increase in dissolved phosphate in anaerobic stages of bio-P systems. In addition, other chemicals that disrupt the proton motive force induce phosphate release, even under aerobic conditions. For example, the addition of the electron transport chain uncoupler 2,4-dinitrophenol induces phosphorus release. This demonstrates that a link between proton motive force and phosphorus release exists. Comeau's hypothesized anaerobic model concurs with observed phenomena and is outlined in Figure 2. Acetate is shown as the carbonaceous substrate for simplicity.

It is evident that bacteria with large reserves of phosphate, such as bio-P bacteria, have a distinct advantage when anaerobic storage of substrate is necessary. The proposed anaerobic biochemical model is presented in Figure 2. It has been suggested that PHB is oxidized, yielding energy for cell maintenance and new cells (*60*). At the same time, excess phosphate is removed by the bacteria. This process is schematically presented in Figure 3. Before aerobic respiration, it is advantageous for substrate to be stored during anaerobic feeding because aerobic respiration produces much more biologically useful energy than fermentation or other anaerobic respiration processes (*64*). The bio-P bacteria can sorb substrate in an anaerobic, high substrate environment and then aerobically respire their purloined stores, creating increased amounts of energy. This results in preferential selection of bio-P bacteria under suitable anaerobic–aerobic conditions.

A typical bio-P system is composed of an anaerobic stage (all nitrate and dissolved oxygen excluded) in which substrate is sorbed by bacteria and phosphate, calcium, and magnesium are released. This is followed by an aerobic stage in which stored PHB is oxidized and large quantities of phosphate are removed from the wastewater.

FIGURE 2
Postulated model for bio-P bacteria anaerobic metabolism[a]



[a] Ac^-, acetate; AcCoA, acetyl CoA; H^+, proton; H^+Ac^-, acetic acid; M^+, metallic cation; NADH, reduced nicotinamide adenine dinucleotide; PHB, poly-β-hydroxybutyrate; Pi, phosphate; PolyP, polyphosphate; TCA, tricarboxylic acid. Under anaerobic conditions, substrates (such as acetate) diffusion as neutral molecules will decrease the pH gradient of bacteria. Bio-P bacteria will be able to utilize their polyphosphate reserves to reestablish the pH gradient possibly directly or via the production of ATP. Polyphosphate can also provide energy for the formation of acetyl CoA. Storage of acetyl CoA as PHB requires NADH, which the TCA cycle can produce anaerobically. Phosphate expulsion takes place because of excess phosphate molecules accumulating in the cell. A pH gradient-sensitive carrier could "sense" that phosphate cannot be used for synthesis. Metallic cations are co-transported with phosphate.
Source: Reference 60.

Sequences for nitrogen and phosphorus removal

Investigators have found that nitrification, denitrification, and bio-P processes can be accomplished by a single bacterial mixed culture. In fact, some early bio-P plants were designed to only remove nitrogen, but because of spontaneous anaerobic conditions at the beginning of the treatment process, removal of phosphorus also occurred (*45*). The important mechanisms of nitrification, denitrification, and bio-P removal occur in this single sludge system.

The first North American facility specifically designed to facilitate a sequence of anaerobic, anoxic, and aerobic environments for the removal of BOD, nitrogen, and phosphorus was constructed in Palmetto, Florida, and began operation in October 1989. The city of Palmetto has National Pollutant Discharge Elimination System (NPDES) permit requirements of a maximum of 8 mg/L suspended solids (SS), 8 mg/L BOD_5, 5 mg/L total nitrogen, and 2 mg/L total phosphorus. These standards are implemented because treated effluent is released to the biologically sensitive Terra Ceia Bay, a small embayment on Tampa Bay on Florida's west coast (*65*).

A typical activated sludge plant designed for phosphorus and nitrogen removal is composed of an anaerobic tank followed by an anoxic tank, which is followed by an aerobic tank; this layout is represented in Figure 4. Many similar proprietary configurations are also used (*66*). Initially, influent wastewater is mixed with return activated sludge in the anaerobic zone where bio-P organisms store BOD and release phosphorus. Subsequently, mixed liquor enters the anoxic reactor where nitrate (which is continuously recycled from the aerobic stage) is reduced to nitrogen gas and some remaining BOD is oxidized. Finally, mixed liquor enters the aerobic tank where any remaining BOD in the bulk liquid is oxidized, ammonia is oxidized to nitrate, and bio-P bacteria oxidize stored substrate while removing large amounts of phosphate from the wastewater.

Biodegradation of toxic and hazardous compounds

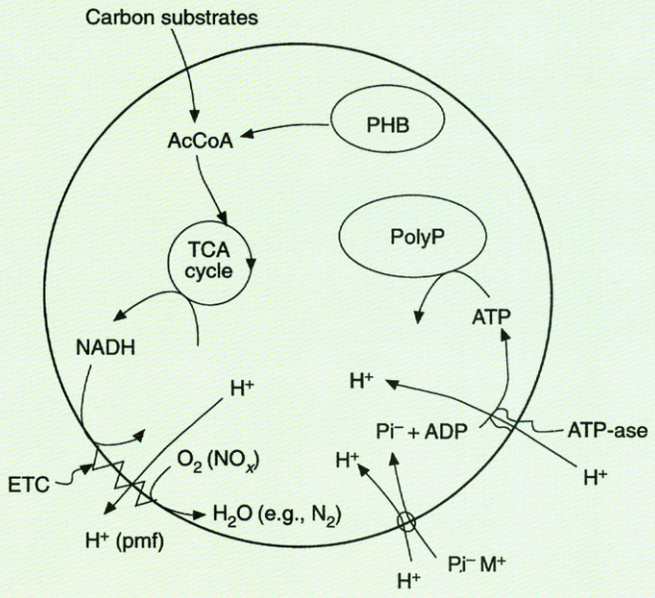
The sequential environments previously reviewed include schemes

for industrial pretreatment, enhanced sludge settling, and nutrient removal. In addition, sequential environments are sometimes the best alternative for the detoxification of organic compounds. For example, compounds that degrade through a series of reductive and oxidative steps may be most efficiently biodegraded by sequential anaerobic–aerobic processes. Other benefits relate to the detoxification of a broad range of chemicals; aerobic and anaerobic environments each have limitations in their biodegrading abilities, but they often compliment each other when they are combined. One limitation of aerobic processes involves the recalcitrance of highly chlorinated chemicals, such as hexachlorobenzene, tetrachloroethylene, and carbon tetrachloride, which appreciably degrade only under anaerobic conditions. In contrast, conventionally cultured aerobic bacteria are efficient degraders of aromatic compounds that are anaerobically recalcitrant.

Notable exceptions to the these generalizations exist. For example, highly chlorinated compounds such as trichloroethylene, 1,1,1-trichloroethane, and chloroform will biotransform under aerobic conditions if methane, phenol, or toluene is provided as a primary source of carbon and energy for biological growth. However, these reactions are co-metabolic rather than conventional. Therefore, it is important to define exact conditions when discussing biodegradation results. To this end, transformations that occur under conventional aerobic, co-metabolic aerobic, anoxic, and anaerobic conditions are described next.

Conventional aerobic biodegradation of toxic compounds. Conventional aerobic biodegradation involves the oxidation of organic chemicals, which are used as carbon and energy sources for biological growth. Typically, the major oxidized product is carbon dioxide, whereas water is produced from oxygen reduction. The conventional aerobic degradability of some compounds is presented in Table 1. The extent of biodegradation can be related to bacterial oxygen consumption. This is done by comparing the BOD and theoretical oxygen demand (TOD) of organic compounds. TOD is calculated from reaction stoichiometry and is the theoretical amount of oxygen required to totally oxidize a compound to carbon dioxide and other inorganic products. Actual BOD is lower than theoretical values because of bacterial synthesis and because some compounds are only partially oxidized or not oxidized at all by bacteria. Although it has been reported that trichloroethylene is readily biodegradable, others have found that it is not appreciably degraded under conventional aerobic conditions (*67, 68*).

FIGURE 3
Postulated model for bio-P bacteria aerobic (and anoxic) metabolism[a]



[a] AcCoA, acetyl CoA; ETC, electron transport chain; H^+, proton; M^+, metallic cation; NADH, reduced nicotinamide adenine dinucleotide; NO_x, oxidized nitrogen; PHB, Poly-β-hydroxybutyrate; pmf, proton motive force; PolyP, polyphosphate; TCA, tricarboxylic acid. Consumption of external or stored substrate in the presence of oxygen (or oxidized nitrogen under anoxic conditions) will allow bio-P bacteria to produce a pmf. The pmf can be used, in particular, for phosphate transport and ATP production. ATP is then utilized for growth but also for polyphosphate storage as a result of the availability of soluble phosphate and of energy. Metallic cations are co-transported with phosphate molecules.
Source: Reference 60.

In fact, the majority of highly chlorinated compounds—such as 1,2,4-trichlorobenzene, 1,2,4,5-tetrachlorobenzene, and hexachlorobenzene—are refractory under conventional aerobic conditions. For example, chlorinated compounds used as carbon and energy sources for bacterial growth were studied, and actively growing enrichment cultures were obtained only when mono- and dichlorinated compounds were provided as substrates; all 1-monohalo-*n*-alkanes and many dichloroalkanes tested were found to be aerobically degradable (*68*). None of the cultures tested was able to employ chloroform, 1,1,1-trichloroethane, trichloroethylene, tetrachloroethylene, or hexachlorobutadiene as substrates under conventional aerobic conditions (see box).

In contrast to highly chlorinated aliphatic compounds, aromatic compounds are more successfully degraded under aerobic, rather than anaerobic, conditions. Conventionally cultured aerobic microorganisms are considered particularly successful degraders of aromatic compounds because they often produce mixed function oxidase enzymes, which initiate aromatic ring cleavage (*69*). Early evidence of aerobic ring opening was reported by Ludzack and Ettinger (*70*), who observed that "Certain aromatics added to river water . . . tend to show decreased aromaticity with a concurrent increase in aliphatics, probably as a result of ring opening." They also reported that alcohols; phenols; some mono- and dichlorophenols; aldehydes; some compounds containing a vinyl group; and nonsulfonated, low molecular weight surfactants are biodegradable. The distinction between co-metabolic and conventional biodegradation was not investigated.

In addition to chemical structure, stripability, and operating parameters—such as solids retention time (SRT), mixed liquor suspended solids (MLSS) concentration, SS removal, and overall BOD removal—influence aerobic biological degradation in engineered systems. In the activated sludge process, aerobic biodegradation and fate of the following were studied: tetrachloro-

ethane, nitrobenzene, dichlorophenol, acrolein, acrylonitrile, 1,2-dichloropropane, methylene chloride, ethyl acetate, benzene, 1,2-dichloroethane, phenol, and 1,2-dichlorobenzene (*71*). Stripping of tetrachloroethane, 1,2-dichloropropane, and 1,2-dichloroethane was a significant mechanism accounting for 93%, 99%, and 98% removal, respectively. Also, the benefit of increased SRT was demonstrated. This was most notable for nitrobenzene, which had 76% removal overall at an SRT of 2 days, but had 98% removal overall at an SRT of 6 days. The majority of removal efficiencies for the tested compounds was 99%, but these high efficiencies could be caused by judicious selection of organic chemicals; most of the compounds studied were not highly chlorinated, and co-metabolic reactions were not described.

Process investigators compared the removal of toxic pollutants by eight wastewater treatment processes: primary clarification, primary clarification and filtration, chemical clarification, high-rate trickling filter, standard-rate trickling filter, aerated lagoon, facultative lagoon, and activated sludge (*72, 73*). Influent wastewater was spiked with 21 priority pollutants dissolved in toluene. The activated sludge system provided the best removal of both conventional pollutants and priority pollutants, but the standard-rate trickling filter gave comparable results when air stripping was a principal removal mechanism. It was concluded that "the chemical clarification system and the primary plus filtration system removed only those toxics associated with wastewater solids; these two systems would not be good choices for the removal of toxic priority pollutants . . . in general, the alternative processes do not produce overall toxics removals comparable to activated sludge treatment" (*73*).

Benefits of efficient BOD and SS removal were recorded by other researchers. A toxicity reduction evaluation of the Patapsco Wastewater Treatment Plant in Baltimore, Maryland, revealed that "decreased plant performance as measured by low BOD and COD removal (i.e., BOD removal < 80% and COD removal < 75%) appear to be associated with events of effluent toxicity" (*74*). It was not known whether poor COD removal resulted in effluent toxicity or whether effluent toxicity caused poor COD removal, but it was evident that high effluent COD indicated effluent toxicity. As far as SS removal is concerned, "individual semi-volatiles [semi-volatile organic chemicals] associated with the wastewater solids were removed effectively by those treatment processes that produced effluents with low suspended solids concentrations" (*72*). Moreover, researchers found that one-half of the hydrocarbons in integrated oil refinery wastewater effluent were incorporated in the bioflocs that were carried over in the effluent (*75*). Therefore, just as high effluent SS concentrations can contribute to high effluent BOD—because a fraction of SS can be oxidized by bacte-

FIGURE 4
Typical activated sludge scheme for removal of nitrogen, phosphorus, and BOD

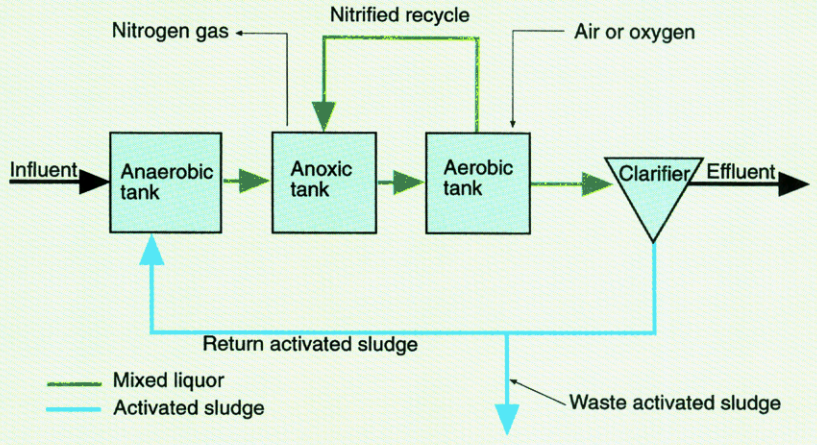


TABLE 1
Aerobic biodegradability of organic compounds

Readily biodegradable[a]	Moderately biodegradable[b]	Slightly biodegradable under studied conditions[c]	Refractory under studied conditions[d]
Cyclohexane	1-Decanol	Decane	Dodecane
Octane	1-Dodecanol	1,3-Dichloropropane	Dichloromethane
Phenol	Acetone	Ethyl ether	Chloroform
Methanol	Ethylbenzene	Phenanthrene	1-Chloropropane
1-Propanol	2-Furaldehyde		1-Chlorobutane
1-Butanol	Benzonitrile		1-Chloropentane
1-Pentanol	4-Bromophenol		1-Chlorohexane
1-Hexanol	Hydroquinone		1-Chlorodecane
1-Octanol			1,2-Dichloroethylene
2-Chloropropionic acid			3-Chloro-1,2-propane
Diethanol amine			Isopropylether
Acetronitrile			Trichloroacetic acid
Acrylonitrile			Chlorobenzene
Benzene			1,3-Dichlorobenzene
Toluene			1,4-Dichlorobenzene
Xylene			1,2,4-Trichlorobenzene
Benzyl alcohol			1,2,4,5-Tetrachlorobenzene
Nitrobenzene			Hexachlorobenzene
Naphthalene			Benzidine
Pyridine			
Quinoline			
m-Cresol			
p-Cresol			
4-Chlorophenol			
4-Nitrophenol			

[a]Biochemical oxygen demand (BOD)/theoretical biochemical oxygen demand (TOD), > 50%.
[b]BOD/TOD, 25–50%.
[c]BOD/TOD, 10–25%.
[d]BOD/TOD, < 10%.
Source: Reference 67.

Chlorinated compounds that support aerobic growth of pure bacterial isolates

Enrichment cultures were developed on a mineral medium supplemented with 10 mg/L of yeast extract and 2 mM of the chlorinated compound tested. Inocula were obtained from activated sludge and soils contaminated with chlorinated compounds.

Dichloromethane	2,3-Dichloro-1-propanol
1,2-Dichloroethane	1,3-Dichloro-2-propanol
2-Chloroethanol	1-Chloro-2,3-propanediol
Chloroacetic acid	Epichlorohydrin
cis-3-Chloroacrylic acid	Chloroethylvinylether
trans-3-Chloroacrylic acid	1,6-Dichlorohexane
2-Chloro-1-propanol	1,9-Dichlorononane

Source: Reference 68.

ria—they can also contribute to high concentrations of semi-volatile compounds in effluents. Furthermore, toxic chemicals that tend to sorb to SS could desorb when they escape to a receiving water with very low ambient concentrations of these toxic compounds. In addition, biomonitoring test organisms, such as *Daphnia* and shrimp, which tend to consume effluent suspended solids, could conceivably be much more sensitive to toxic organics that concentrate on solid surfaces. Again, high effluent SS concentrations would result in greater toxicity values.

Finally, high SRTs (SRT > 15 days) and MLSS concentrations (MLSS > 5000 mg/L) have resulted in better removal of many toxic chemicals (*71, 74, 76, 77*). The benefits of high MLSS concentrations include: (1) a low food to microorganism ratio (F:M), which results in a starvation environment causing microorganisms to consume potentially toxic, less energetically favorable substrates; and (2) high biomass concentrations, which promote partitioning of high K_{ow} (octanol–water partition coefficient) organics to biomass.

Co-metabolic aerobic biodegradation of toxic compounds. Co-metabolic transformations are fortuitous biological degradation reactions involving the transformation of compounds that do not provide carbon or energy for biological growth. Most reported co-metabolic reactions involve the aerobic transformation of one- and two-carbon chlorinated aliphatics when methane is provided as a primary substrate (*68*). This process is catalyzed by a methane monooxygenase enzyme (MMO) produced by bacteria that employ methane as a primary substrate (methanotrophic bacteria). In addition, the MMO enzyme catalyzes the transformation of alkanes, alkenes, and alkylbenzenes to alcohols, epoxides, and phenols, respectively (*78, 79*). Other studies report MMO transformations of *cis*-1,2-dichloroethylene, *trans*-1,2-dichloroethylene, vinyl chloride, chloroform, and dichloromethane, whereas tetrachloroethylene and carbon tetrachloride are resistant (*80, 81*). Some chlorinated compounds that degrade under aerobic–methanotrophic conditions are presented (see box). Other reported co-metabolic oxidations describe bacteria that use propane; toluene, phenol, or cresol; dichlorophenoxyacetic acid; and ammonia as primary substrates (*83–86*).

The unique potential of methanotrophic reactions has encouraged the investigation of strategies for the enhancement of co-metabolic processes. The mechanisms unique to co-metabolism and a proposal for a co-metabolic degradation model and a two-stage methanotrophic system for biodegradation of trichloroethylene have been described (*87, 88*). In addition, trichloroethylene, dichloroethylene, and vinyl chloride degradation have been accomplished in an aquifer that received injections of water containing dissolved oxygen and methane (*89*). In situ degradation has been documented at 20, 40, 85, and 95% for trichloroethylene, *cis*-1,2-dichloroethylene, *trans*-1,2-dichloroethylene, and vinyl chloride, respectively (*89*). Others have proposed using the same methanotrophic approach for remediation of similarly contaminated aquifers (*90*).

Anoxic biodegradation of toxic compounds. Toxic pollutant biotransformation by nitrate-reducing bacteria has not been as extensively investigated as aerobic biotransformation processes. However, many toxic aromatic and aliphatic organics do degrade under denitrifying conditions. Table 2 presents some organic compounds that degrade anoxically. The importance of anoxic conditions is more fully appreciated when dealing with groundwater pollution because soil–water systems are often anoxic (*97*).

It has been suggested that restoration of hydrocarbon-contaminated groundwater can be most quickly and economically accomplished by providing nitrate, as opposed to oxygen or hydrogen peroxide, as an electron acceptor because nitrate is more soluble. Therefore, nitrate may be more precisely directed to the contamination zone (*91*). Unfortunately, this creates a potential problem because nitrate is a regulated pollutant that may cause adverse health effects when present in excessive amounts in well water.

Other applications of denitrifying processes involve the treatment of steel mill coke-oven wastewater, which often contains phenol, cresols, catechols, and cyanides, as well as high nitrate concentrations. The anoxic degradation of phenol and toluene compounds is encouraging because less aeration may be required when treating coke-oven and other similar wastewaters anoxically (*93*). Although oxygenated aromatic compounds such as phenol can be metabolized under anoxic conditions, other aromatics such as benzene do not anoxically degrade, or they degrade extremely slowly. For example, chlorinated benzenes, ethyl benzene, and naphthalene do not significantly degrade under anoxic conditions, suggesting that molecular oxygen is probably required for ring cleavage (*96*). In contrast, researchers studying bioremediation of a contaminated aquifer report that toluene, ethylbenzene, xylenes, and 1,2,4-trimethyl benzene (JP-4 jet fuel contamination) significantly degrade under anoxic conditions (*91*). Also, acenaphthene and naphthalene may degrade when in the presence of acclimated denitrifying bacteria, whereas no degradation occurs under strict anaerobic conditions (*92*). Nevertheless, many aromatic compounds are more quickly degraded under aerobic conditions.

Interestingly, some chlorinated aliphatics are anoxically degradable. Furthermore, reductive dehalogenation may occur in anoxic systems even though their conditions are not considered highly reduced. For instance, chloroform was de-

Co-metabolic degradation of chlorinated compounds in aerobic–methanotrophic environments

The bacteria employed for biodegradation experiments were *Methylosinus trichosporium* OB3b, which were grown in a medium without copper and contained 20 mM of formate and 0.2 mM of halogenated compound.

Compounds that appreciably degrade

Dichloromethane
Chloroform
1,1-Dichloroethane
1,2-Dichloroethane
1,1,1-Trichloroethane
1,1-Dichloroethylene
trans-1,2-Dichloroethylene
cis-1,2-Dichloroethylene
1,2-Dichloropropane
trans-1,3-Dichloropropylene
Vinyl chloride[a]

Compounds that do not degrade

Carbon tetrachloride
Tetrachloroethylene

[a] Reference 82.
Source: Reference 81 unless otherwise stated.

TABLE 2
Anoxic biodegradability of organic compounds[a]

Compound	Intermediates	Products	Initial inoculum	Primary substrate	Reference
Toluene *o*-xylene *m*-xylene *p*-xylene 1,2,4-Trimethylbenzene	ND	ND	Soil	—	91
Naphthol Naphthalene Acenaphthene	ND	ND	Soil	Soil organic matter	92
Phenol Catechol *o*-cresol *m*-cresol *p*-cresol	ND	ND	Municipal activated sludge	—	93
Benzoic acid 3-Hydroxybenzoate 3,4-Dihydroxybenzoic acid 4-Hydroxybenzoic acid *o*-cresol *m*-cresol *p*-cresol phenol	*n*-Caproic acid	ND	Soil Manure Municipal activated sludge	—	94
Carbon tetrachloride	ND	Carbon dioxide and a nonvolatile fraction	Soil	Acetate	95
Carbon tetrachloride Bromodichloromethane Dibromochloromethane Bromoform	Chloroform	Carbon dioxide	Primary sewage	Ethanol	96

[a] ND, not determined.

tected in anoxic mixed cultures spiked with carbon tetrachloride; however, this product may have been formed by organisms other than denitrifying bacteria (*96*). Similarly, the biodegradation of trihalomethanes, 1,1,1-trichloroethane, and 1,2-dibromomethane was observed in a mixed anoxic culture, but denitrifying bacteria may not have initiated the transformation (*96*). More important, pure culture studies have demonstrated that a denitrifying bacterium will transform carbon tetrachloride to carbon dioxide; chloroform is not produced as an intermediate. Even so, the transformation of carbon tetrachloride to carbon dioxide by a denitrifying bacterium is extremely rare and slow (*95, 98*).

Conventional anaerobic biodegradation of toxic compounds. Conventional anaerobic biodegradation involves the conversion of organic compounds to methane, carbon dioxide, and other inorganic products. This process is accomplished by a consortium of bacteria, which use the organic compound as a source of carbon and energy. In the most simplistic model, the consortium is composed of acidogenic bacteria, which transform complex organic compounds into acetate, carbon dioxide, and hydrogen and methanogenic bacteria, which convert the intermediates into methane. Theoretical methane and carbon dioxide production is calculated from reaction stoichiometry; this quantity is referred to as the theoretical gas production (TGP). However, actual gas production (AGP) is lower than theoretical values because of bacterial synthesis and because some compounds are only partially converted or not converted at all. For example, measurements of the gas produced by anaerobic consortia degrading various organic chemicals determined that many chloro- and nitrophenols did not support anaerobic gas production (*99*). Table 3 presents the results. It was concluded that chloro- and nitro-substituents inhibited anaerobic gas formation, whereas carboxyl and hydroxyl groups resulted in enhanced gas formation.

The biodegradability of carboxylated and hydroxylated aromatic compounds is reported in studies of the anaerobic treatability of coal conversion wastewater (CCWW). Benzoic acid, hexanoic acid, phenol, resorcinol, catechol, *p*-cresol, and 4-methylcatechol can be significantly biodegraded under methanogenic conditions (*100*). More recent reports on CCWW also describe the anaerobic degradation of these compounds and the treatment of full strength CCWW using attached anaerobic growth on granular activated carbon (*101*).

In contrast to aromatics that contain oxygenated substituents, other aromatics are relatively resistant to conventional anaerobic degradation. Accordingly, it was found that benzene and naphthalene were not degraded by diluted (2.5% solids) anaerobic cultures (*99*). Nonetheless, some have found that toluene, ethylbenzene, and *o*-xylene will degrade extremely slowly under methanogenic conditions (*102*). These

TABLE 3
Anaerobic biodegradability of organic compounds[a]

Readily biodegradable[b]	Moderately biodegradable[c]	Not biodegradable under studied conditions[d]		Inhibitory under studied conditions[e]
Phenol	3-Cresol	3-Aminophenol	*cis*-permethrin	3-Chlorophenol
2-Aminophenol	4-Chlorobenzoic acid	4-Aminophenol	*trans*-permethrin	4-Chlorophenol
3-Cresol	Dimethyl phthalate	2-Chlorophenol	Benzene	2,4-Dichlorophenol
Sodium benzoate	Pyridine	2-Cresol	Chlorobenzene	2,6-Dichlorophenol
4-Aminobenzoic acid		2-Nitrophenol	Cumene	3,5-Dichlorophenol
3-Chlorobenzoic acid		3-Aminobenzoic acid	Aniline	2,4,6-Trichlorophenol
Phthalic acid		2-Chlorobenzoic acid	*N*-methylaniline	Pentachlorophenol
Ethylene glycol		Di-*n*-butyl phthalate	Sodium benzenesulfonate	3-Nitrophenol
Diethylene glycol		bis(2-Ethylhexyl) phthalate	1-Naphthoic acid	4-Nitrophenol
Triethylene glycol		Hexylene glycol	Tetrahydrofuran	2,4-Dinitrophenol
Sodium stearate		Neopentyl glycol	Furan	2,5-Dinitrophenol
Catechol		*n*-undecane	Pyrrole	4-Nonylphenol
Quinoline		*n*-hexane	N-Methylpyrrole	2-Phenyl phenol
		2,4-Dichlorophenoxy acetic acid	Thiophene	2-Nitrobenzoic acid
		4-Chloro-2-methyl phenoxyacetic acid	Pyrimidine	3-Nitrobenzoic acid
		2-(4-Chloro-2-methylphenoxy) propionic acid	Naphthalene	4-Nitrobenzoic acid
		Dieldrin		Anthraquinone
				1-Naphthol
				2-Naphthol

[a] Anaerobic biodegradation potential was determined by incubating 2 to 3 g (dry) of municipal digester sludge with 50 mg of carbon/L of tested chemical. Tests were done at 35 °C and lasted at least 60 days. Theoretical gas production (TGP) was calculated from the stoichiometry of test chemical degradation to CH_4 and CO_2.
[b] Actual gas production (AGP)/theoretical gas production (TGP), ≥ 80%.
[c] AGP/TGP, 30–80%.
[d] BOD/TOD, ≥ 30%.
[e] Gas production initially negative and continuously decreasing.
Source: Reference 99.

positive results may stem from the microorganisms used—soil microorganisms from an aquifer that had been exposed for decades to landfill leachate containing alkylbenzenes. In addition, it is possible that microaerophilic conditions occurred during the experiments. Low concentrations of oxygen may have initiated ring cleavage. More recently, benzene has been found to be slowly mineralized by aquifer organisms under strictly anaerobic conditions (*103*). A lag time of at least 30 days was observed. More important, toluene inhibited benzene mineralization, and it is probable that sulfate served as a terminal electron acceptor. It is now evident that the fastidiousness of the responsible organisms has made it difficult to document the anaerobic degradation of benzene.

The anaerobic degradation of chlorinated organic compounds has also been investigated. Chloroform and trichloroethylene biodegradation in propionate, acetate, and formate-enriched anaerobic cultures were studied (*104*). The major objective of the study was to determine the maximum chlorinated compound biodegradation rates that would be sustained without causing excessive deterioration of primary substrate removal efficiency. With this in mind, the maximal loading rate was defined as the daily loading rate (mg/L) of chloroform or trichloroethylene, which resulted in a 50% reduction in primary substrate removal efficiency. The methanogenic cultures were acclimated for approximately one month, and pollutant losses caused by abiotic degradation, adsorption to biosolids, and volatilization were determined and corrected for. The maximum daily loading rates of chloroform achieved when formate, acetate, and propionate individually served as primary substrates were 15, 61, and 98 mg/L of reactor, respectively. The maximum daily loading rates of trichloroethylene achieved when formate, acetate, and propionate served as primary substrates were 15, 109, and 104 mg/L of reactor, respectively. Propionate utilizing bacteria were the least affected class of microorganisms, whereas bacteria, which utilize formate or its abiotic breakdown products, hydrogen and carbon dioxide, were the most sensitive microorganisms.

In an extensive study, it was found that a methanogenic consortium would degrade dichloromethane (DCM) (*105*). The predominant pathway for DCM degradation was oxidation to carbon dioxide; therefore, the methane produced resulted from carbon dioxide reduction and not directly from DCM. In addition, selective inhibition of methanogens did not affect the rate of DCM degradation. Consequently, it was concluded that acetogenic bacteria accomplished DCM degradation.

Anaerobic reductive dehalogenation of toxic compounds. Many anaerobic biodegradation studies have focused on reductive dehalogenation, the successive shedding of halogen atoms under reduced, anaerobic conditions. These reactions are usually catalyzed biologically because they have not occurred in sterile sediment or in acclimated cultures incubated aerobically (*106*). The benefits of reductive dehalogenation are clear: highly chlorinated compounds, which are often toxic, can be dehalogenated, yielding less halogenated compounds. The less halogenated products are less toxic and more amenable to further aerobic and anoxic biodegradation (*96, 107*). For these reasons, the importance of reductive dehalogenation has been acknowledged: "At present, anaerobic reductive dehalogenation, either biologically or nonbiologically, is . . . recognized as the critical factor in the transformation or biodegradation of certain classes of compounds" (*108*). Table 4 presents some compounds that undergo reductive dehalogenation.

Compounds involved in the most significant dechlorinations do not normally transform under conventional aerobic, anoxic, and anaerobic conditions, but they more readily undergo reductive dechlorination. Insecticides, polychlorinated biphenyls (PCBs), chlorinated benzenes, tetrachloroethylene, and carbon tetrachloride are included in this category. For example, the in-

TABLE 4
Anaerobic reductive dehalogenation of organic compounds

Compound	Intermediates	Products	Initial inoculum	Primary substrates	Reference
Hexachlorobenzene	Pentachlorobenzene 1,2,3,5-Tetrachlorobenzene	1,3,5-Trichlorobenzene	Municipal digester sludge	—	109
Hexachlorobenzene	Pentachlorobenzene 1,2,4,5-Tetrachlorobenzene 1,2,4-Trichlorobenzene	1,2,-Dichlorobenzene 1,3-Dichlorobenzene 1,4-Dichlorobenzene	Municipal digester sludge	—	109
1,2,3,-Trichlorobenzene	1,3,-Dichlorobenzene	Chlorobenzene	Anaerobic river sediment	—	110
1,3,5-Trichlorobenzene	1,3-Dichlorobenzene	Chlorobenzene	Anaerobic river sediment	—	110
1,2,4-Trichlorobenzene	1,4-Dichlorobenzene	Chlorobenzene	Anaerobic river sediment	—	110
2,4,6-Trichlorophenol	2,4-Dichlorophenol	4-Chlorophenol	Municipal digester sludge	Yeast extract Peptone Casamino acids Glucose Galactose Lactose	111
2,4,5-Trichlorophenol	—	3,4-Dichlorophenol	Municipal digester sludge	Yeast extract Peptone Casamino acids Glucose Galactose Lactose	111
2,4,5-Trichlorophenol	3,4-Dichlorophenol	3-Chlorophenol	Municipal digester sludge	—	112
3,4,5-Trichlorophenol	3,5-Dichlorophenol	3-Chlorophenol	Municipal digester sludge	Yeast extract Peptone Casamino acids Glucose Galactose Lactose	111
2,4-Dichlorophenol	4-Chlorophenol	Phenol	Methanogenic aquifer material Pond sediment	—	112
2,5-Dichlorophenol	3-Chlorophenol	Phenol	Methanogenic aquifer material Pond sediment	—	112
3-Chlorobenzoate	—	Benzoate	Methanogenic aquifer material Pond sediment Municipal digester sludge	—	112
3,4-Dichlorobenzoate	3-Chlorobenzoate	Benzoate 4-Chlorobenzoate	Methanogenic aquifer material Pond sediment Municipal digester sludge	—	112
3,5-Dichlorobenzoate	3-Chlorobenzoate	Benzoate	Methanogenic aquifer material Pond sediment	—	112
3-Chlorobenzoate	Benzoate Acetate Hydrogen	Methane	Municipal digester sludge	—	113
Tetrachloroethylene	Trichloroethylene 1,2-Dichloroethylene Vinyl chloride	Ethylene	Municipal digester sludge	Glucose Acetate Formate Hydrogen	114
Dichloromethane	Chloromethane	Methane Carbon dioxide	Municipal digestersludge	—	105
DDT	—	DDD,DDE	Municipal digester sludge	—	115
2,4-Dichlorophenoxy-acetate	2,4-Dichlorophenol 4-Chlorophenol	Phenol	Methanogenic aquifer material Pond sediment Municipal digester sludge	—	112
2,4,5-Trichlorophenoxy-acetate	2,4,5-Trichlorophenol	Phenol	Municipal digester sludge	—	112
2,4,5-Trichlorophenoxy-acetate	2,5-Dichlorophenoxyacetate 3-Chlorophenol 2,4-Dichlorophenoxyacetate 4-Chlorophenol	Phenol	Methanogenic aquifer material Pond sediment	—	112

secticide DDT, which is aerobically recalcitrant, will reductively dechlorinate in thick, anaerobically digested wastewater sludge (*115*). Unfortunately, the products of dechlorination are the priority pollutants DDD and DDE. However, the importance of reductive dechlorination as the first step in DDT mineralization is apparent (*116*).

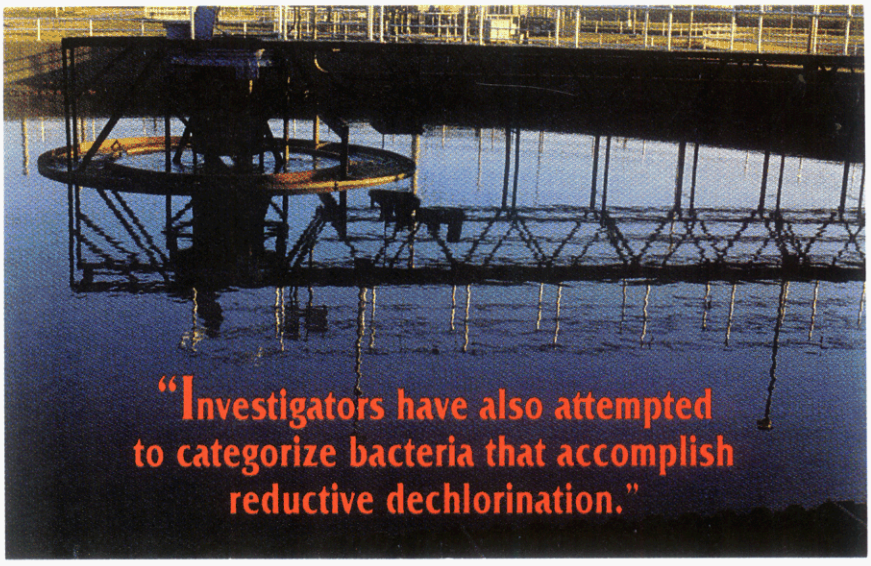
Similarly, highly chlorinated biphenyls are extremely resistant to conventional aerobic transformation, but they will undergo anaerobic reductive dechlorination. Studies of PCB contamination in Hudson River sediment demonstrate that anaerobic environments

yield markedly lower levels of tri-, tetra-, and pentachlorobiphenyls and higher levels of mono- and dichlorobiphenyls (*117*). Other reports indicate that reductive dechlorination of 2,3,4,5,6-pentachlorobiphenyl involves the release of chlorine as a chloride ion and the addition of a proton from water (*118*). Furthermore, many of the less chlorinated PCBs are aerobically biodegradable and generally less toxic than highly chlorinated PCBs. Therefore, reports indicate that "the dechlorination step alone . . . has significant [positive] toxicological consequences" (*117*).

Moreover, hexachlorobenzene dechlorinates to tri- and dichlorobenzenes in anaerobic sewage sludge, and the chlorine is sequentially removed from the aromatic ring (hexachlorobenzene reduces to pentachlorobenzene, then to 1,2,3,5-tetrachlorobenzene, and finally to 1,3,5-trichlorobenzene) (*109*). All three isomers of trichlorobenzene have been reductively dechlorinated to monochlorobenzene via dichlorobenzenes in anaerobic sediment from the Rhine River (*110*).

Other aromatic compounds that undergo reductive dechlorination include chlorophenols, chloroguaiacols, chloroveratroles, and chlorocatechols. These compounds were reductively dechlorinated in an upflow anaerobic sludge blanket reactor while glucose, methanol, and acetate were employed as primary substrates (*119*). Complete mineralization did not occur; however, the chlorinated compounds were transformed to less chlorinated homologs. Similarly, 2,4,6-trichlorophenol was reduced to 4-chlorophenol, 2,4,5- trichlorophenol was reduced to 3,4-dichlorophenol, and 3,4,5-trichlorophenol was reduced to 3-chlorophenol in a methanogenic enrichment culture receiving yeast extract, peptone, casamino acids, glucose, galactose, and lactose as primary substrates (*111*). Others found that pentachlorophenol (PCP) can be reductively dechlorinated in anaerobic digesting sewage sludge (*120*). After acclimation, 5.0 mg/L PCP was biotransformed to less than 5 μg/L.

In a study of 16 chlorinated aromatics, only 4-chlorobenzoate was not dehalogenated by at least one of four anaerobic cultures employed: sewage sludge, pond sediment, methanogenic aquifer material, and sulfate-reducing aquifer material (*112*). Interestingly, the source of the culture greatly influenced dehalogenating ability. For instance, 6% of 3,5-dichlorobenzoate disappeared in sewage sludge, whereas 100% disappeared in methanogenic aquifer seed from a site bordering a municipal landfill.



Chlorinated aliphatics also reductively dechlorinate under anaerobic conditions. It was found that 0.75 mg/L of tetrachloroethylene can be sequentially dechlorinated to ethylene in enrichment cultures that produce methane (*105*). In addition, the same methanogenic culture dechlorinated 91 mg/L of tetrachloroethylene to ethylene; however, methanogenesis ceased as the vinyl chloride conversion to ethylene increased. Therefore, it was determined that methanogenesis is not a necessary condition for tetrachloroethylene or vinyl chloride reduction; however, methanogenic bacteria may still play an essential role (*121*). In addition, carbon tetrachloride was shown to undergo reductive dechlorination, whereas chloroform and dichloromethane were identified as intermediates; carbon dioxide and acetate were the major products (*122*). Other reports indicate that 1,1,1-trichloroethane, chloroform, and carbon tetrachloride are reductively dechlorinated by anaerobic, acetogenic bacteria (*123*).

Although the majority of reductive dehalogenations are biologically catalyzed, some are spontaneous. Abiotic dehalogenation of 1,2-dichloroethane (1,2-DCA) and 1,2-dibromoethane (EDB) in the presence of sodium sulfide (Na_2S), which is usually present in reduced anaerobic environments, is reported to be "significant with respect to the time scales that are typical of groundwater remediation efforts" (*124*). The half-life of 1,2-DCA was reduced from 170 to 23 years when 1 mmol Na_2S was present. Similarly, the half-life of EDB was reduced from 16 years to 160 days. Others have found that many alkyl halides will undergo nucleophilic substitutions in highly reduced conditions when hydrogen sulfide is present, producing dialkyl sulfides and other volatile sulfur-containing compounds (*125*).

Sulfite reduces the mutagenic activity of chlorinated waters (probably by causing dehalogenation) and could possibly be used for treatment; many chlorinated compounds yield dehalogenated products at technically feasible rates (*126*). In fact, mutagenicity of softwood kraft chlorination effluent was diminished by addition of various nucleophiles, some of which are often present in anaerobic, reduced environments (*127*). Detoxifying effectiveness was comparable to decreasing nucleophile basicity (in order from best to worst mutagenicity reducers: hydroxide ion, sulfur dioxide, bisulfite ion, glutathione, pyrrolidine, thiosulfate ion). Factors that affect the rate of spontaneous dehalogenation reactions and indicate that bromine is shed more quickly than chlorine are described (*82*). In addition, the more halogenated a compound is, the faster the dehalogenation reaction (*82*).

Nevertheless, most reductive dehalogenations described were biologically catalyzed. But does an anaerobic organism obtain energy from reductive dehalogenation, or is the phenomenon simply co-metabolic? It has generally been assumed that reductive dehalogenation will not yield energy; therefore the co-metabolic definition applies (*128*). However, the molar growth

yield of a bacterial consortium was 4.9 grams of protein per mole of benzoate digested but increased to 6.8 grams of protein per mole of 3-chlorobenzoate degraded (*113*). In addition, the ATP content in initially starved sludge was twice as high when the bacteria were fed 3-chlorobenzoate rather than benzoate (*113*). Thermodynamic estimates predict that a dechlorinating organism will obtain about 16 times more energy for each mole of hydrogen oxidized than will a methanogen, and microscopic investigations suggest that dechlorinating bacteria are predominant in a methanogenic co-culture that degrades 3-chlorobenzoate (*113*). Therefore, dehalogenation does yield biologically useful energy in some cases but more study is needed to explain just how important this phenomenon is.

Recently, researchers presented more evidence to support the position that reductive dehalogenation is not simply an electrophilic or nucleophilic substitution (*129*). They found that the effects of aryl substituents on chlorobenzoate did not correlate with Hammett substituent constants. On the other hand, simple chemical reaction rates, such as those measured during electrophilic and nucleophilic substitutions, often correlate with Hammett constants. This suggests that the biologically catalyzed dehalogenation reaction may be more complicated than most abiotic substitutions. In fact, the increased growth rate of dehalogenating bacteria has stimulated some to refer to the process as "halide respiration," such as in the following: "Strong evidence for the occurrence of such a 'halide respiration' mechanism was obtained by Dolfing and Tiedje . . . they observed a growth yield increase coupled to reductive dechlorination" (*110*).

Investigators have also attempted to categorize bacteria that accomplish reductive dechlorination. It is most important to distinguish whether they are strict anaerobes, such as methanogens, or whether they are facultative anaerobes. Actually, obligate anaerobes and facultative bacteria have catalyzed reductive dehalogenation reactions (*130*). Obligately anaerobic bacteria that reductively dechlorinate include a non-spore-forming sulfate reducer that dehalogenates some halobenzoates and nonaromatic compounds (*131*). In addition, an obligate anaerobe that ferments pyruvate to acetate and reductively dechlorinates 2, 4, 6,-trichlorophenol to 4-chlorophenol has been described (*132*). Others have described a methanogen, *Methanosarcina* sp., that transforms tetrachloroethylene to trichloroethylene (*133*).

The ability of facultative bacteria, as opposed to strict anaerobes, to reductively dechlorinate hexachlorocyclohexane has been observed. Aerobically grown cells of *Citrobacter freundii* (a facultative bacteria) were washed and suspended in a complex glucose medium and then anaerobically incubated (*134*). Within four days, all gamma-hexachlorocyclohexane was removed and 90.4% of the bound chlorine was released into the bulk liquid as chloride ions. It was concluded that ". . . the observation of the decomposition of hexachlorocyclohexane isomers by facultative anaerobes under anaerobic conditions seems to be important because they can degrade these compounds also if propagated aerobically and subjected afterwards to anaerobic conditions" (*134*).

An aerobic enrichment culture's ability to reductively dechlorinate tetrachloroethylene to *cis*-1,2-dichloroethylene was shown to be dependent on a cyclic transition from aerobic to anaerobic conditions and limited oxygen supply (*135*). When the aerobic enrichment culture was subsequently maintained in a purely anaerobic mode, reductive dechlorination ceased. However, subcultures maintained at an air–liquid ratio of 1:4 (microaerophilic conditions) continued to reductively dechlorinate tetrachloroethylene. It was concluded that aerobic or aerotolerant bacteria can reductively dechlorinate.

Sequential biodegradation of toxic compounds

The ability of an aerobically activated sludge process to reduce toxicity is highly variable even when efficient SS and BOD removal is accomplished; some treatment plants exhibit little or no toxicity reduction at all (*136*). Occasionally, anaerobic–aerobic sequences are more successful at reducing toxicity, and they may be used to mineralize otherwise recalcitrant compounds. For example, tetrachloroethylene and carbon tetrachloride may be mineralized by a sequential anaerobic–aerobic process. Initial anaerobic stages may accomplish reductive dechlorination, producing trichloroethylene and chloroform. Subsequent aerobic–methanotrophic stages may convert trichloroethylene and chloroform to carbon dioxide and water. Alternatively, anaerobic reductive dechlorination may produce vinyl chloride and chloromethane, which may degrade in conventional aerobic processes if volatilization losses are minimized. Another scenario involves the mineralization of chlorinated aromatic compounds such as hexachlorobenzene and PCBs in sequential anaerobic–aerobic processes. Reductive dechlorination may occur in anaerobic stages, producing less chlorinated homologs, which may be degraded under conventional aerobic conditions. Indeed, the studies of anaerobic–aerobic systems performed thus far have focused on the degradation of chlorinated compounds, and aerobic reductive dechlorination followed by aerobic degradation of the less chlorinated products has been realized. Table 5 presents a synopsis of some sequential anaerobic–aerobic systems that have been studied.

Tetrachloroethylene, chloroform, and hexachlorobenzene have degraded in a two-stage biofilm reactor consisting of an anaerobic column followed by a conventional aerobic column (*145*). Reductive dechlorination occurred in the anaerobic column, and trichlorinated and dichlorinated products were formed. In the aerobic column, the less chlorinated intermediates were substantially transformed into carbon dioxide and nonvolatile products. The two-stage process resulted in 61, 49, and 23% mineralization of chloroform, tetrachloroethylene, and hexachlorobenzene, respectively. Dechlorination was most extensive when acetate served as the primary substrate, but it occurred to a lesser extent when glucose and methanol served as primary substrates.

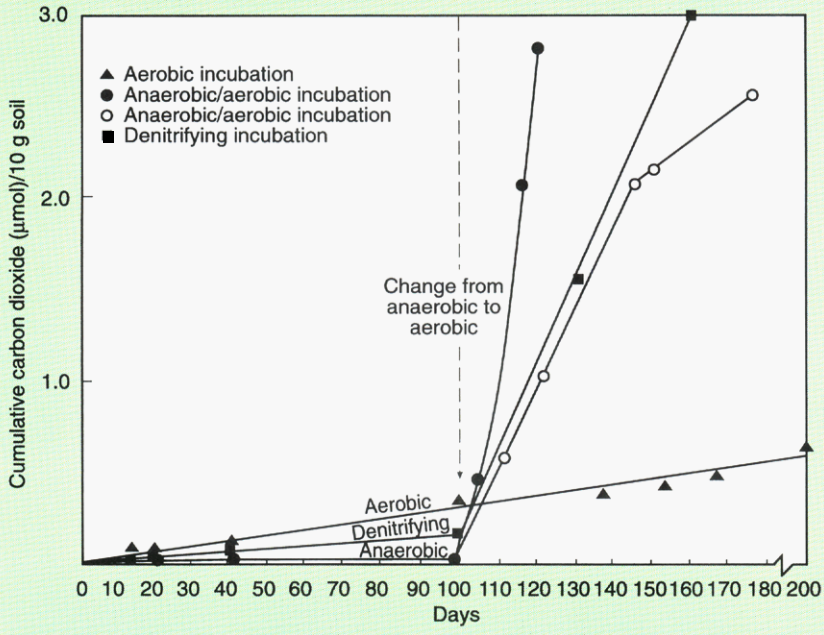
Other compounds that require both reductive and oxidative steps for mineralization have been successfully detoxified under sequential anaerobic–aerobic conditions. Radioactively labeled 1,1-bis (*p*-methoxyphenyl)-2,2,2-trichloroethane (methoxychlor) was degraded by bacteria that were initially incubated for 3 months under anaerobic conditions and subsequently incubated under aerobic conditions (*142*). Cultures exposed to the sequence of environments produced 10- to 70-fold increases in labeled carbon dioxide as compared with cultures maintained under aerobic conditions only. Figure 5 presents the results. At all concentra-

TABLE 5
Sequential anaerobic–aerobic systems[a]

Systems description	Primary substrate	Pollutants transformed anaerobically	Anaerobic products	Pollutants transformed aerobically	Reference
Economical BOD reduction and toxic organics removal					
Anaerobic fluidized bed followed by aerobic trickling filter	Organics in spent bleach liquor from kraft pulping	2,4,6-Trichlorophenol 2,4,5-Trichlorophenol 3,5-Dichloro-2,6-dimethoxyphenol 4,5-Dichloroguaiacol 2,3,4,6-Tetrachloro-phenol 3,4,5-Trichloroguaiacol Trichloroguaiacol Trichlorocatechol 3,4,5-Trichloro-2,6-dimethoxyphenol Pentachlorophenol Tetrachlorophenol	Chloride	ND	137
Anaerobic fluidized bed followed by aerobic activated sludge	Sucrose	4,6-Dinitro-*o*-cresol	ND	ND	138
Anaerobic batch reactor followed by aerobic batch reactor	Organics in a textile wastewater	Azo dye compounds	ND	ND	139
Biodegradation of volatile toxic organics					
Anaerobic suspended growth reactor followed by aerobic suspended growth reactor	Glucose Acetate Benzoate Phenol	2-Chloropropene 1,1-Dichloroethylene *trans*-1,2-Dichloro-ethylene 1,1-Dichloroethane Chloroform 1,1,1-Trichloroethane 1,1-Dichloropropene Carbon tetrachloride Trichloroethylene Tetrachloroethylene 1,2,3-Trichloropropane Hexachloroethane	Vinyl chloride Dichloromethane *cis*-1,2-Dichloro-ethylene	2-Chloropropene 1,1-Dichloroethane *trans*-1,2-Dichloro-ethylene 1,1-Dichloroethane 1,1-Dichloropropene Trichloroethylene Dichloromethane *cis*-1,2-Dichloro-ethylene Vinyl chloride	140
Anaerobic upflow packed bed (sandy loam) followed by aerobic upflow packed bed (coarse sand)	Organics in a hazardous waste leachate (mostly volatile acids)	Benzene Toluene 1,2-Dichloroethane Ethylbenzene Dichloromethane Bis-(2-chloroethyl) ether Phenol Majority of total organic carbon (TOC) removed anaerobically	ND	Benzene Toluene Bis(2-chloroethyl) ether Most remaining total organic carbon (TOC) removed aerobically	141
Insecticide and PCB biodegradation					
Moist sandy loam soil in sealed vessels sequentially exposed to anaerobic and aerobic conditions	No other organic substrates added	1,1-Bis(p-methoxy-phenyl)-2,2,2-trichloro-ethane (methoxychlor)	Unidentified intermediate	Unidentified intermediate	142
Bacteria immobilized in calcium–alginate beads, anaerobic inner bead region, aerobic outer bead region	No other organic substrates added	1,1,1-Trichloro-2,3-bis(4-chlorophenyl) ethane (DDT)	1,1-Dichloro-2,2-bis (4-chloro-phenyl) ethane (DDD) 1,1-Dichloro-2,2-bis (4-chloro-ethylene) (DDE)	Diphenylmethane	143
River sediment in sealed vessels sequentially exposed to anaerobic and aerobic conditions	Methanol	Hexachlorobiphenyls Pentachlorobiphenyls Tetrachlorobiphenyls Trichlorobiphenyls	Dichlorobiphenyls Monochlorobiphenyls	Dichlorobiphenyls Monochloro-biphenyls	144
Miscellaneous					
Anaerobic upflow packed bed (glass beads) followed by aerobic–methanotrophic upflow packed bed (glass beads)	Glucose Methanol Acetate	Chloroform Trichloroethylene Hexachlorobenzene	1,2,3-Trichlorobenzene 1,2-Dichlorobenzene *cis*-1,2-Dichloroethylene Dichloromethane Unidentified volatile and nonvolatile intermediates	Volatile and non-volatile products in anaerobic effluent	145
Anaerobic downflow packed bed (silica beads) followed by aerobic suspended growth reactor	Autoclaved mixed liquor from a municipal treatment plant	2,4,6-Trichloro-phenol	4-Chlorophenol Phenol	4-Chlorophenol Phenol	146

[a]ND, not determined.

FIGURE 5
Biodegradation of methoxychlor under sequential environmental conditions



Source: Reference 142.

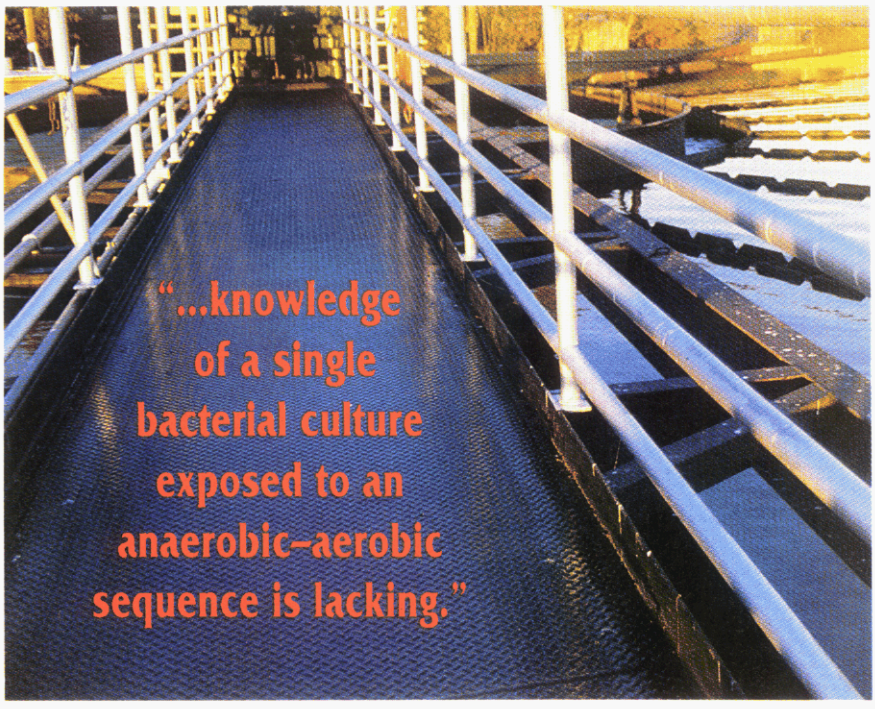
tions of radio-labeled methoxychlor studied, the anaerobic–aerobic system produced significantly more radio-labeled carbon dioxide than the purely aerobic system did. In conclusion, the authors state: "One of the effects of sequential changes in metabolic regimes is that persistent chemicals in the environment could be more readily degraded than would be expected under a single environmental condition."

The successful mineralization of methoxychlor has encouraged others to investigate sequential anaerobic–aerobic processes for the degradation of related compounds such as 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane (DDT). It has been found that DDT would reductively dehalogenate, and diphenylmethane would be oxidized in a co-culture containing anaerobic and aerobic bacteria (*143*). An anaerobe, *Enterobacter cloaca,* maintained at an oxidation–reduction potential (E_H) below –200 mV, reduced DDT to 4,4′-dichlorodiphenylmethane (DDM). The aerobe, *Alcaligenes* sp., mineralized diphenylmethane. Anaerobic and aerobic environments were maintained concomitantly when bacteria were entrapped in calcium–alginate beads. The outer bead regions were aerobic due to dissolved oxygen in the surrounding bulk liquid. However, inner bead regions were anaerobic because oxygen diffused into the beads relatively slowly and was consumed by bacteria in the outer bead region. It was hoped that reductive dechlorination would yield diphenylmethane, but the DDM actually produced was difficult to aerobically degrade. Therefore, the system did not mineralize DDT, but future efforts may demonstrate the mineralization of DDT in sequential anaerobic–aerobic systems.

More promising results have been obtained from investigations describing sequential anaerobic–aerobic processes for the destruction of PCBs in river sediment. The biotransformations that occurred when a mixture of PCB congeners (Aroclor 1242) in sediment was incubated under anaerobic conditions and then under aerobic conditions are described (*144*). Methanol was added as a primary substrate. During the anaerobic period, reductive dechlorination occurred, and tri-, tetra-, penta-, and hexachlorobiphenyl mass decreased, whereas mono- and dichlorobiphenyl concentrations increased. Under subsequent aerobic conditions, significant degradation of all mono- and dichlorobiphenyl homologs occurred. Only 43% of the 300 mg of PCBs/kg of soil initially added remained after treatment. The authors also describe a conceptual model in which the in situ mineralization of PCBs may be accomplished by injecting methanol or other primary substrates into river sediment, monitoring the extent of dechlorination, and finally injecting hydrogen peroxide and methanol to stimulate aerobic mineralization of less chlorinated homologs.

In some situations, the primary function of aerobic stages in sequential anaerobic–aerobic systems has been efficient BOD reduction. Anaerobic stages have been necessary for the transformation of specific organic chemicals. For example, chlorinated compounds, which often cause spent pulp bleaching liquors to exhibit toxic effects, have been successfully detoxified in an anaerobic fluidized bed reactor followed by an aerobic trickling filter (*137, 147*). The toxicity of highly chlorinated bleaching wastewater from the pulp and paper industry has been reduced by this process. Toxicity reduction was primarily accomplished in the anaerobic stage, whereas the aerobic stage's main function was BOD reduction. In addition, researchers have found that removal of 4,6-dinitro-*o*-cresol can be accomplished using an anaerobic fluidized bed reactor followed by the aerobic activated sludge process (*138*). Sucrose was added as a primary substrate for the anaerobic degradation of 4,6-dinitro-*o*-cresol, and the activated sludge stage removed excess sucrose from the synthetic wastewater. Also, wastewater containing azo dyes has been treated in a sequential anaerobic–aerobic system (*139*). The anaerobic stage was necessary for transformation of the dye compounds, and BOD removal was accomplished in the aerobic stage. It was concluded that anaerobic treatment provided significant decolorization of azo dye and enhanced subsequent aerobic biodegradation.

The ability of sequential environments to biotransform a combination of compounds has also been investigated. A serial anoxic–anaerobic–aerobic packed bed scheme for the treatment of a landfill leachate that contains trichloroethylene, tetrachloroethylene, methylene chloride, and other priority pollutants has been described (*141*). The influent to the anoxic–anaerobic column contained nitrate. Denitrification occurred as evidenced by the evolution of nitrogen gas, and methane was also produced. Only trace amounts of toluene and methylene chloride were found in the anoxic–anaerobic column effluent, whereas benzene, 1,2-dichloroethane, and ethylbenzene were not detected. It is possible that these latter compounds were metabolized during



denitrification or that volatilization losses in the feed reservoir and in the packed beds may have resulted in the disappearance of these priority pollutants. The investigators state that "the concentrations of volatile species in the leachate reflect levels before volatilization losses from the PVC feedbags occurred." The concentration of priority pollutants in effluent gas from the packed beds was not reported. Volatile priority pollutants were not found in the aerobic column effluent. Of the two nonvolatile pollutants studied, phenol was removed by the anaerobic column, bis(2-chloroethyl) ether was primarily removed in the anaerobic column; the remaining portion was partially removed in the aerobic column.

The unique abilities of an aerobic–methanotrophic treatment stage have also been investigated. An anaerobic suspended growth reactor is followed by an aerobic–methanotrophic suspended growth reactor for the treatment of groundwater containing chlorinated propenes, ethenes, ethanes, and methanes (*140*). The concentration of individual chlorinated compounds was 120 ppb, and the effluent from the sequential treatment contained less than 2 ppb of each of the pollutants. Anaerobic treatment was most effective for removal of the highly chlorinated compounds, and compounds not removed anaerobically were degraded in the aerobic–methanotrophic reactor. Also, less chlorinated products of anaerobic reductive dechlorination, such as vinyl chloride and dichloromethane, were degraded in the subsequent aerobic–methanotrophic reactor.

Summary and future applications

Pollution control engineers and scientists currently employ sequential environments to enhance the removal of BOD, produce sludge with good settling characteristics, and facilitate biological nutrient removal. In addition, recent research suggests that the degradation of a broader range of toxic organic compounds may be more efficiently accomplished during anaerobic–aerobic biological treatment. Compounds that biodegrade most readily through a combination of reductive and oxidative steps, such as chlorinated benzenes, PCBs, and highly chlorinated aliphatics may be more completely degraded in sequential environments. It has been shown that chlorinated organic chemicals will undergo a biologically catalyzed reductive dehalogenation during anaerobic periods, and the less chlorinated products are much more amenable to aerobic or anoxic treatment.

As effluent toxicity standards become more stringent, sequential treatment processes may be more widely employed for the degradation of specific organic compounds. In addition, more stringent regulation of volatile organic compounds released to the atmosphere during aeration may stimulate the development of anaerobic pretreatment for the partial degradation of chlorinated volatiles such as carbon tetrachloride, tetrachloroethylene, chloroform, and 1,1,1-trichloroethane. For these reasons, future research describing the reductive dechlorination of volatile compounds under sequential anaerobic–aerobic conditions would be beneficial. One economical scheme may be a sequential anaerobic–aerobic single-sludge activated sludge process. It is possible that such a system would effectively reduce BOD and concomitantly degrade many chlorinated volatile compounds. In addition, it is possible that anaerobic stages could be economically installed in existing treatment plants that experience effluent toxicity or air emission problems. The viability of anaerobic bacteria during aerobic periods and aerobic bacteria during anaerobic periods needs to be determined.

Other possible applications of sequential environments pertain to the treatment of hazardous waste, soil, and groundwater contaminated with chlorinated solvents, insecticides, and PCBs. Most of the hazardous waste treated biologically is very high in BOD and may contain chlorinated organics. Anaerobic–aerobic sequences may reduce hazardous waste BOD to low levels and concomitantly degrade these chlorinated compounds. Also, the in situ biodegradation of chlorinated solvents in groundwater may be possible if a carbon and electron source, such as methanol, is continuously added, and an electron acceptor, such as hydrogen peroxide, is added in a pulse mode. This may cause alternating anaerobic and aerobic sequences to exist in polluted aquifers. It has been reported that the sequential anaerobic–aerobic condition stimulates reductive dechlorination and that PCBs may be similarly detoxified in river sediments (*135, 144*).

Much work remains to be done before sequential environments are employed to their fullest potential. It is easy to assume that aerobic bacterial processes will behave in a conventional manner even if proceeded by anaerobic processes. Indeed, this may be the case when anaerobic bacteria and aerobic bacteria are contained in separate unit operations in series. However, knowledge of a single bacterial culture exposed to an anaerobic–aerobic sequence is lacking. Under what conditions will anaerobes survive periodic aeration? It has already been shown that, under oxygen-limiting conditions, obligatory aerobic and anaerobic (methanogenic) bacteria will survive in a mixed culture (*148, 149*). What detoxifying abilities might such a

system have? It has even been suggested that methanogenesis is enhanced by traces of oxygen during the digestion of complex biomass (*150*). In addition, methanogenic viability has been maintained in anaerobic contact processes that incorporate a short aeration period prior to sedimentation. These are certainly concepts that challenge the current dichotomous description of anaerobic and aerobic processes, and they encourage more complete investigations of the benefits of sequential environmental processes.

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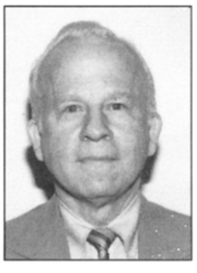

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