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COUPLED AEROBIC AND ANAEROBIC TREATMENT OF TOXIC WASTEWATER

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ABSTRACT

Interest in anaerobic biotechnology for industrial wastewater treatment has greatly increased during the past decade. However anaerobic technology has inherent limitations (*e.g.* treatment polishing and chloro-aromatics mineralization). Therefore in many cases where anaerobic treatment is employed, aerobic process is also included for secondary or tertiary treatment. More recently attention has been placed on using anaerobic/aerobic sequence for degradation of toxic compounds, mainly chloro-organics. In the above system, the anaerobic and aerobic bacteria operate in separate units which are complementary to each other. Less chlorinated homologs produced in the anaerobic stage by reductive dehalogenation are then mineralized more easily in conventional aerated units. However knowledge of a single bacterial culture unit combining simultaneously anaerobic-aerobic degrading abilities is still limited. The first study demonstrates the excellent tolerance of the anaerobic granular biofilms to extensive presence of oxygen in the reactor. Large feeding of liquid with dissolved O_2 concentrations as high as 18 ppm into the anaerobic granule bed did not impair the methanogenic activities. Evidently, the peripheral facultative anaerobes consumed the O_2 before it could attain and damage the strict anaerobes of the granule core. In the next study aerobic and anaerobic catabolisms have been combined within a single system for biotreatment of pentachlorophenol (PCP)-containing effluents. PCP degradation has been tested both under anaerobic conditions and in presence of O_2. Actual results show a specific degradation of 10 mg PCP g^{-1} volatile suspended solid (VSS) d^{-1}, in the aerobic/anaerobic system, against 7, in the anaerobic one. Preliminary results indicate that aerobic and anaerobic catabolisms can be coupled in a way which could be easily transferable to a larger scale.

KEYWORDS: anaerobic, aerobic, coupling, anaerobic granule, oxygen transfer, pentachlorophenol, reductive dechlorination, UASB.

INTRODUCTION

Interest in anaerobic biotechnology for industrial wastewater treatment has greatly increased during the past decade, because the anaerobic process is the most elegant method to reduce carbon pollution. With minimum power requirement, anaerobic technology converts 90% of the pollutants into a valuable form (Verstraete *et al.*,

1990). In contrast, aerobic processes transform 50% of the carbon pollution into surplus sludge which still requires other processes to deal with. However, anaerobic technology has inherent limitations. Methanogens, which are at the last stage of the anaerobic process chain, have a limited substrate affinity. As a result, anaerobic systems are inefficient in treatment polishing. In comparison, aerobic treatment permits the removal of trace organics with, in practice, a capacity of purification down to values lower than the required standards (< 30 mg BOD L^{-1}) (Vochten *et al.*, 1988). Furthermore aerobic processes are recognized to have the capability to mineralize a broader range of recalcitrant compounds than anaerobic processes. For example, in pulp and paper wastewater treatment, common compounds refractory to anaerobic treatment include some chlorinated phenolics, tannins, resin acids, terpenes. Despite these drawbacks, anaerobic technology has performed a notable penetration into a difficult sector such as the pulp and paper industry. The volume of pulp and paper wastewater anaerobically treated by the upflow anaerobic sludge bed (UASB) technology has improved by a factor of seven between 1987 and 1991 (Lettinga *et al.*, 1991). The last published survey mentioned that 37 full-scale anaerobic plants were in operation or in construction in 1989 (Lee *et al.*, 1989). However, in all cases where anaerobic treatment is employed, aerobic process is also included for secondary or tertiary treatment. The objective of sequential anaerobic-aerobic system for pulp and paper treatment is to maximize the abatement of the chemical oxygen demand (COD) and to release an effluent that is not acutely toxic. Anaerobic/aerobic sequences are also employed for other applications, such as nutrient (phosphorus, nitrogen) removal.

ANAEROBIC/AEROBIC SEQUENCE FOR DEGRADATION OF XENOBIOTICS

More recently, attention has been placed on using anaerobic/aerobic sequence processes for degradation of toxic compounds, mainly chloro-organics. Sequential environments are sometimes the best alternative to the detoxification of those organic compounds. Aerobic and anaerobic environments each have limitations in their biodegrading abilities that often complement each other when their are combined. Most highly chlorinated chemicals, which are appreciably degraded under anaerobic conditions, are refractory to conventional aerobic conditions. Some authors have found that common aerobic microorganisms could not employ highly chlorinated aromatic substrates, such as hexachlorobenzene, 1,2,4,5-tetrachlorobenzene, 1,2,4-trichlorobenzene (Zitomer and Speece, 1993), or hetero-substituted aromatics, such as 4-chloro-2-nitrophenol (CNP) (Beunink and Rehm, 1990). Substituents with electron-withdrawing properties (-NO_2, halogens) deactivate oxidative ring-cleavage reactions. The anaerobic step, by reducing such substituents, allows the oxygenase enzyme system to be operative and to subsequently proceed to the oxidative ring-cleavage. While some authors have reported that chloroform, 1,1,1-trichloroethane and trichloroethylene are readily biodegraded aerobically, others have found that the later ones as well as tetrachloroethylene (PCE) and hexachlorobutadiene are not appreciably degraded under conventional aerobic conditions (Janssen *et al.*, 1991). In contrast, investigations done over the past few years have indicated that biologically mediated dehalogenation of highly chlorinated compounds occurs under reduced, anaerobic conditions. The first steps of reductive dehalogenation are the successive removal of the halogens, leading immediately to the formation of less halogenated products that are less toxic and more amenable to further aerobic mineralization (Horowitz *et al.*, 1983; Mohn and Tiedje, 1992). The more halogenated a compound is, the faster the dehalogenation reaction (Vogel *et al.*,

1987). Polychlorobiphenyls (PCB), such as pentachlorobiphenyl, that are resistant to aerobic transformation, will undergo reductive dechlorination into di- and mono-chlorobyphenyls which are aerobically biodegradable (Brown *et al.*, 1987). Similarly, hexachlorobenzene dechlorinates to tri- , di- and mono-chlorobenzene, in anaerobic sewage sludge or anaerobic sediments (Bosma *et al.*, 1988). PCE can be sequentially dechlorinated to vinyl chloride or even ethylene, while CCl_4 can be reductively dechlorinated by acetogens into tri- and di-chloromethane (Galli and McCarty, 1989). The situation is different in the case of pentachlorophenol (PCP). PCP has been shown to be completely mineralized in both aerobic and anaerobic systems providing they have high biomass retention time and a long acclimation period. Aerobic fluidized beds were able to completely mineralize a mixture of chlorophenols (CP) [PCP, 2,3,4,6-tetrachlorophenol (TeCP) and 2,4,6-trichlorophenol (TCP)] at a loading of 217 mg CP L^{-1} d^{-1} (amongst which 18 mg PCP L^{-1} d^{-1}) (Puhakka and Järvinen, 1992). Relatively high rates of mineralization have also been attained (up to 97 mg PCP L^{-1} d^{-1}) with a UASB reactor using volatile fatty acids (VFA) and methanol as co-substrate at an organic loading rate (OLR) of 16 g COD L^{-1} d^{-1} for a hydraulic residence time (HRT) of 0.6 d (Wu *et al.*, 1993). However, in other cases of anaerobic treatment within UASB and anaerobic filter reactors, complete mineralization of PCP did not occur; PCP was transformed in less chlorinated phenols, such as TeCP, 3,4,5-TCP, 2,4,6-TCP, 2,4,5-TCP, 3,4-dichlorophenol and 3- or 4-chlorophenol, with methanol, acetate, phenol and/or glucose as co-substrate (Woods *et al.*, 1989; Hendriksen and Ahring, 1992). Yet, these homologs could be brought to an aerobic system and completely mineralized without limitation as aerobic abilities of degradation of these homologs would be largely above their production rate by dechlorination in the upstream anaerobic system (Shieh *et al.*, 1990; Armenante *et al.*, 1992; Puhakka and Järvinen, 1992).

In most of the above cases, anaerobic transformation is thus incomplete, and less chlorinated aliphatics or aromatics are the endproducts of the anaerobic processes. In contrast, aerobic microorganisms are efficient degraders of less chlorinated organic compounds up to complete mineralization. Anaerobic digestion is thus indicated as a primary treatment step to convey less chlorinated or dechlorinated compounds-containing effluents to an aerobic polishing unit.

ANAEROBIC/AEROBIC COUPLING

In the above treatment schemes, the anaerobic and aerobic bacteria operate in separate units that complement each other. Despite its great potential, the anaerobic/aerobic sequence might not be the optimal arrangement. In some cases, anaerobic partial degradation results in products which are just as or more toxic than the primary molecule. These products can accumulate in the anaerobic stage where they could inhibit anaerobic microorganisms themselves prior to being released into the subsequent aerobic unit. This might decrease the effectiveness of the overall system from a certain feeding level. One economical scheme may be a single bacterial culture unit exposed to a cyclic anaerobic-aerobic sequence. Knowledge on these operations is still limited. With this regard, a bioprocess integrating both aerobic and anaerobic populations would create an effective synergism between the oxidative and reductive catabolisms. Several phenomena are already known. On one side, a significant number of strict aerobes have been detected in anaerobic mixed cultures (Toerien and Hattingh, 1969). On another side, under oxygen-limiting conditions, obligatory anaerobic bacteria will survive in activated sludges (Wu *et al.*, 1987) or in

aerated suspended cultures (Gerritse *et al.*, 1990). Aerobic and anaerobic microorganisms can grow in the same habitat provided that the input of O_2 is lower than the potential rate of consumption, which causes O_2-limited environments. This is typical of biofilm systems. Limitation in the molecular diffusion of O_2 results in abrupt O_2 concentration downward gradients, leaving a large portion of the biofilm volume free of O_2 (Table 1). In all cases shown, over 63 % of the total biomass is anaerobic.

TABLE 1. LIMITATION IN O_2 MASS TRANSFER IN VARIOUS TYPES OF BIOFILMS

System	Trickling filter slime [a]	Mycelial pellet [b]	*Enterobacter cloacae* in alginate bead [c]
Dissolved O_2 in bulk liquid (ppm)	8.5	7.8	7.4
Biofilm thickness or radius (mm)	0.4-1	3	1.5
Depth of O_2 penetration (μm)	150	135	150
Relative volume free of O_2 (%)	63-85	87	73

[a] Chen and Bungay, 1981; [b] Huang and Bungay, 1973; [c] Beunink *et al.*, 1989.

This disadvantage of biofilms was exploited to develop a co-culture of a strict aerobe (*Alcaligenes*) with a facultative anaerobe (*Enterobacter cloacae*), both immobilized within Ca-alginate beads of *ca.* 3 mm of diameter (Beunink and Rehm, 1990). Both microorganisms which were initially distributed homogenously within the bead matrix, were rapidly shared out differentially amongst the inner and the outer space of the bead, due to the selective pressure exerted by the oxygen in the outer space, and its drastic limitation in the inner space. The authors showed that only this synergistic arrangement was able to completely degrade the 4-chloro-2-nitrophenol (CNP). Otherwise CNP is totally refractory to pure aerobic cultures of *Alcaligenes* alone, while in the presence of a pure anaerobic culture of *Enterobacter* alone, CNP transformation was limited to the 4-chloro-2-aminophenol. The nitro-group first had to be anaerobically reduced before dioxygenases of *Alcaligenes* were able to cleave the aromatic ring and proceed to its mineralization. Even though the application of this laboratory system at a large scale is questionable (use of pure strains; oxygen tolerance of the anaerobic species which is facultative; use of alginate beads as immobilization carriers), it represents an excellent model which demonstrates the interest of coupling reductive and oxidative catabolisms. The next part of the present paper will present preliminary results exploring the possibility of coupling aerobic and anaerobic treatments in a way that could be transferred to a larger scale.

ANAEROBIC GRANULES IN OXIC ENVIRONMENT

The present study investigated whether anaerobic granules suspended in UASB-like reactors, can be operated in oxic environments. The major difference with the former model is that anaerobic granules contain large amount of strict anaerobes such as acetogens and methanogens, which are highly sensitive to traces of oxygen (Patel *et al.*, 1984). Carbohydrate-fed anaerobic granules, which can be several millimetres in diameter, include a wide variety of bacterial trophic groups (acidogenic fermenters, sulphate-reducers, proton-reducing acetogens, hydrogenophilic and acetoclastic methanogens). In the recent past, we have amongst others, observed a structured organization of the granule consortium, the core of which is almost exclusively colonized by *Methanothrix*-like methanogens, while the peripheral layer contains a large variety of morphotypes which include fermentative species (MacLeod *et al.*, 1990; Guiot *et al.*, 1991). Consequently gradients of trophic activities are expected (Arcand *et al.*, 1994). To separately assess the specific activities of granule concentric portions, calibrated abrasions were sequentially processed on sucrose-fed granules to progressively strip their outermost biomass particles off. Large amount of granules were submitted anaerobically to a controlled fluidization at a superficial liquid velocity of 30 m h^{-1} in the presence of a mineral abrasive (sand particles sized between 60 and 180 µm, 20 g L^{-1} sludge bed). Detached particles were separated from the residual granules and sand by gravity. Mass balances and activities were assessed on all abraded particles and on the residual granule cores at the end of the process. Results on acidogenic activities are presented in Table 2.

TABLE 2. PARTITION OF THE GLUCOSE SPECIFIC ACTIVITY AS A FUNCTION OF THE GRANULE DEPTH

Layer Location	Relative mass (%)	Glucose specific activity (std. dev.) (mmol g^{-1} VSS d^{-1})
External	34	12.3 (0.96)
Median	23	4.8 (0.25)
Central	43	1.3 (0.21)

Average equivalent-diameter of granules: 1.95 mm ± 0.49. Reactor dilution rate: 1.9 d^{-1}. Organic loading rate: 3.7 g COD L^{-1} d^{-1}. Specific substrate removal rate: 0.31 g COD g^{-1} VSS d^{-1}. Substrate : sucrose added with NH_4Cl, $(NH_4)_2SO_4$, NaH_2PO_4, K_2HPO_4, $NaHCO_3$, $KHCO_3$, yeast extract and trace metals.

The first abrasion resulted in the detachment of 34 % of the total biomass (on a dry weight basis), and the second one to 23 % (corresponding respectively to a 14 and 13 % decrease in diameter). The glucose specific activity drastically decreased when penetrating in the granule inner mass, indicating a clear predominance of acidogenic fermenters (partly facultative anaerobes) in the external layer of the granule. Glucose specific activity was ten fold larger in the outermost layer than in the inner core. It was thus conceivable that the peripheral layer could easily shield the strict anaerobic granule core against the detrimental effect of oxygen. This was verified experimentally. Exposure of an anaerobic granule bed to a significant supply of oxygen over a relatively long period of time had been conducted to test the anaerobic granule tolerance to oxygen. An upflow sludge bed and filter (UBF) reactor was operated during three months with variable concentrations of dissolved O_2 in the liquid

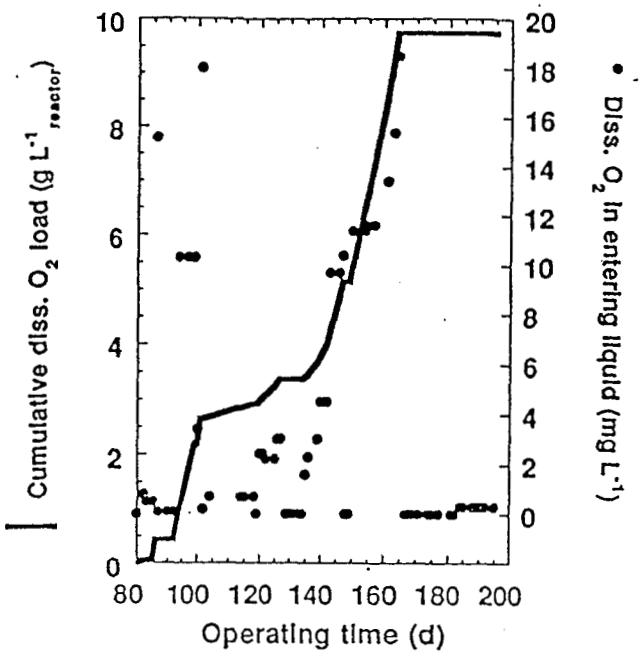

Fig. 1. Change in the dissolved O_2 content of the liquid entering the anaerobic UBF reactor and cumulation of the O_2 supplied and consumed in the reactor.

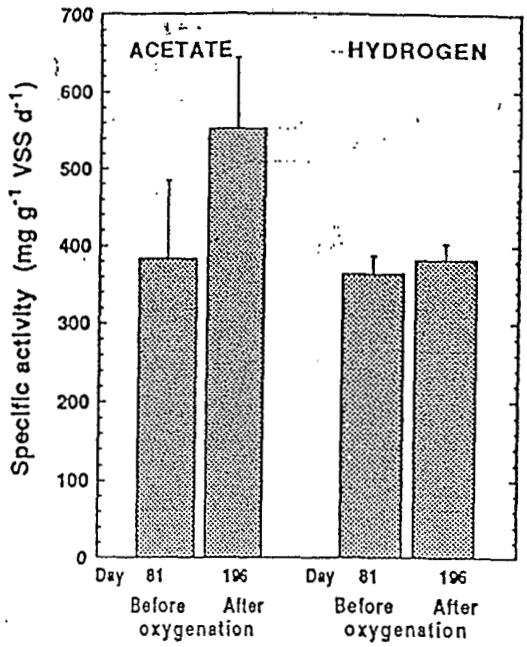

Fig. 2. Change in the methanogenic specific activities before and after the period of oxygenation of the reactor.

entering the reactor: from traces up to 18 ppm. Organic feeding was done with a sucrose synthetic wastewater at an average OLR of 2.44 (±0.42) g COD L^{-1} d^{-1} with a HRT of 1.1 (±0.07) d. Biomass content in the reactor ranged from 7.4 to 12 g VSS L^{-1}. Fig. 1 shows the variation in the O_2 content of the liquid entering the reactor and the resulting cumulative dissolved O_2 supply to the reactor. It has been estimated that the granule bed has received a total of *ca.* 10 g O_2 L^{-1} of reactor over the three months of experimentation. Methane production rate fluctuated between 0.5 and 1 vol (STP) vol^{-1} d^{-1} (0.66 on average). Comparison of methanogenic activities before and after the period of oxygenation of the reactor are shown in Fig. 2. Neither the acetoclastic nor the hydrogenotrophic methanogenic specific activities were affected by the continuous oxygenation of the reactor. In fact, dissolved oxygen was never detected in the liquid above the granule bed. This indicated that the oxygen input was never exceeding the potential rate of consumption. Evidently, the O_2 consumption by the peripheral acidogens (partly facultative anaerobes) limited the penetration of O_2 towards the inner space of the granule on a short distance, if any. Hence strict anaerobes in the granule core were shielded against oxygen. In contrast, acetoclastic specific activity passed from 400 (± 100) to 540 (± 80) mg acetate g^{-1} volatile suspended solid (VSS) d^{-1} after three months of such a regime of oxygenated liquid feeding. It is possible that oxygen stimulated facultative organisms which have a role in supplying growth factors to methanogens (Pirt and Lee, 1983). So the limitation in oxygen mass transfer in anaerobic systems may be used to prospect the possibility of developing anaerobic and aerobic microbial processes within a single system.

AEROBIC AND ANAEROBIC SYNCHRONOUS TREATMENT OF PCP-CONTAINING WASTEWATER

Anaerobic granules adapted for a long time to PCP-containing influents have been tested for PCP degradation under both anaerobic and oxic conditions. Briefly, both anaerobic reactor and aerobic/anaerobic system were operated at 35°C and fed with a mixture of sucrose, butyrate and ethanol at a constant OLR of 1.41 (± 0.23) g COD L^{-1} d^{-1} on average in a first phase (day 1 to 56) and of 2.89 (± 0.13) g COD L^{-1} d^{-1} in a second phase (day 57 to 132). The synthetic feed was prepared with deionized water and supplemented with metal and nutrient salts free of chloride. Also a constant HRT of 2.1 (± 0.1) d on average was maintained during the experimentation period presented here i.e. 4.3 months. The PCP loading was gradually increased to reach at the present stage of the experiment values of 100 mg PCP L^{-1} d^{-1}. The oxygen consumed in the aerobic/anaerobic system was on average *ca.* 350 mg O_2 L^{-1} d^{-1} during the first phase and *ca.* 650 mg O_2 L^{-1} d^{-1} in the second phase, *i.e. ca.* 23 % of the COD-based OLR. Hence a large portion of the carbon pollution had still to be anaerobically converted. PCP removal was assessed from the balance between the measured PCP contents of influent and effluent and compared to the rate of discharge of inorganic chloride released with the effluent. Perfect stoichiometry between PCP removed and chloride released indicated that PCP removal effectively resulted from biologically mediated complete dechlorination. In fact, only traces of less chlorinated intermediates were detected in the effluent, if any. At the present stage of the study, both anaerobic reactor and aerobic/anaerobic system were capable of degrading PCP at rates near to 100 mg PCP L^{-1} d^{-1}. This is well comparable to the highest rate of anaerobic dechlorination observed to date in the literature (Wu *et al.*, 1993). However, when PCP dechlorination rate was reported to the biomass content, aerobic/anaerobic system showed specific rates superior to those of the anaerobic reactor: 10 against 7 mg PCP g^{-1} VSS d^{-1}, respectively, at the end of the experimental segment presented here. This was due to a lower biomass content in the aerobic/anaerobic system (10 g VSS L^{-1}) than in the conventional anaerobic reactor (15 g VSS L^{-1}). However, it is premature to conclude from these results that the aerobic/anaerobic system is more efficient than the anaerobic one with regard to their PCP degradation function, since it is not known yet whether the reactors were transport or kinetics limited. The experiment is still under progress. At least, it can be concluded that aerobic/anaerobic coupling is workable in a way that is transferable to a larger scale. Methanogenic activity was not affected by the O_2 presence since CH_4 production rate of the coupled aerobic/anaerobic reactor, during the second phase, was on average 0.49 (±0.16) vol (STP) vol^{-1} d^{-1} against 0.60 (±0.16) in the conventional anaerobic reactor. The lower CH_4 production rate of the aerobic/anaerobic system indicated that part of the co-substrates were effectively aerobically converted. This is consistent with aerobic growth observed in the aerobic/anaerobic system. Counts of strict aerobic microorganisms on Plate count agar from representative sludge samples of both reactors on operating day 91 revealed that the number of strict aerobic colony forming units (CFU) was significantly higher in the aerobic/anaerobic system than in the anaerobic reactor, $7.2 \cdot 10^9$ CFU g^{-1} VSS against $3.5 \cdot 10^7$, respectively.

CONCLUSIONS

Numerous insights from literature indicate that the combined reductive and oxidative degradation can be regarded as an advantageous alternative to purely oxidative degradation of xenobiotics. Coupled reductive and oxidative degradation

of xenobiotics might be of great industrial potential, if it could be developed at a scale able to deal with large volumes of pollution. The experimental project, partially presented here, augurs well that coupling aerobic/anaerobic populations within a single system is feasible and workable in a way that could have an immediate applicability at the industrial scale.

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