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Anaerobic biodegradation of high strength 2-chlorophenol-containing synthetic wastewater in a fixed bed reactor

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ABSTRACT

In this study the continuous treatment of 2-chlorophenol (2-CP) containing synthetic wastewater at increasing concentrations up to 2600 mg L^{-1} in an anaerobic fixed bed reactor was achieved. As a source of microorganisms municipal sewage sludge was acclimatised to maximally 50 mg L^{-1} 2-CP by 3 successive feedings within 1.5 months. Then, an anaerobic fixed bed reactor was inoculated with this sludge and was operated for 318 d, during which the 2-CP influent concentration was stepwise increased from 50 to 2600 mg L^{-1} within 265 d. At a hydraulic retention time (HRT) of 2.2 d the 2-CP loading rate was 2 g L^{-1} d^{-1} and the average 2-CP removal rate was 0.87 g L^{-1} d^{-1}, accounting for 73% removal. This is the highest 2-CP removal rate ever reported. The negative effect of a 2-CP loading rate of 1.36 g L^{-1} d^{-1} on 2-CP removal was reversible within 2 wk when lower loading conditions (e.g. 0.76 g 2-CP L^{-1} d^{-1}) were re-established. The median chloride ion release per unit 2-CP degraded was 0.24, which was reasonably close to the theoretically expected value of 0.28. In a batch assay, carried out with relatively clear reactor effluent, the highest removal rate of 2-CP was 175 mg L^{-1} d^{-1}. At the time of reactor termination on day 318, the 2-CP removal rate by the biofilm in the reactor was 0.61 g L^{-1} d^{-1}, corresponding to a HRT of 3.4 d and a 2-CP loading rate of 0.76 g L^{-1} d^{-1}. At these very stable conditions removal of COD was 84% and of 2-CP 81%.

1. Introduction

Chlorinated phenols constitute an important class of pollutants because of their wide use as wood preservatives, pesticides, biocides, flame retardants and for pulp and paper production. Due to their high toxicity, recalcitrance, bioaccumulation, strong odour emission, persistence in the environment and suspected carcinogenity and mutagenity, chlorophenols pose serious ecological problems as environmental pollutants (Armenante et al., 1999; Quan et al., 2003). The recalcitrance of chlorophenols results from the carbon–halogen bond, which is cleaved with great difficulty and from the stability of their aromatic structure, resulting in accumulation in nature (Farell and Quilty, 2002). Various chlorophenols, including 2-chlorophenol (2-CP), are termed as priority pollutants (Keith and Telliard, 1979). However many types of microorganisms, such as *Acinetobacter sp., Desulfomonile tiedjei, Desulfitobacterium chlororespirans or Alcaligenes sp.* (Holliger et al., 1999; Kim and Hao, 1999; Gallego et al., 2001) are known to utilize chlorophenols as their sole carbon and energy source. For large scale industrial wastewater treatment, mixed consortia rather than pure cultures are preferred, as they are easier to handle and contain diverse microbial species capable of withstanding various unfavourable operation fluctuations. Treatment of industrial wastewater with biological methods has attracted more attention than mechanical and chemical methods, e.g. by adsorption or wet oxidation. Biological treatment of industrial wastewater that contains high concentrations of chlorophenols is difficult. Conventional treatment systems both, aerobic or anaerobic, often fail to achieve a satisfactory efficiency in treating high concentrations of chlorophenol-containing wastewater due to its toxicity or inhibitory effect on microorganisms, unless these were adapted to or selected for degradation of such contaminants. Generally, for the treatment of highly toxic industrial wastewater anaerobic processes are used as a pre-treatment to remove a high percentage of most of the contaminants, some of which are quite recalcitrant during aerobic treatment (which may be used for final treatment as a polishing step). Anaerobic treatment studies have been done in the past with only low to medium strength 2-CP containing effluents (Table 1), whereas studies with highly concentrated 2-CP containing effluents are rare.

In mixed anaerobic cultures, more than one pathway may be followed for the biotransformation of aromatic compounds. Becker et al. (1999) proposed a pathway in which 2-CP was para-carboxylated to generate 3-chloro-4-hydroxybenzoate, which was subsequently dehydroxylated to yield 3-chlorobenzoate, that accumulated in the system. However, anaerobic biodegradation

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Table 1
Performance of various systems for 2-CP degradation

Reactor	2-CP Concentration ($mg\ L^{-1}$)	2-CP Loading rate 2-CP ($mg\ L^{-1}\ d^{-1}$)	2-CP Removal rate ($mg\ L^{-1}\ d^{-1}$)	Remarks	Ref.
Suspended batch	–		180	Enrichment culture	Dietrich and Winter (1990)
AFBR[a]	2000	70–600	375	Phenol accumulation observed	Dietrich and Winter (1990)
SMBR[b]	25	40	37	Hydrogenotrophic conditions	Chang et al. (2003)
Batch	12.8	–	–	Denitrifying conditions	Bae et al. (2002)
Batch	10–25	–	About 0.3 (maximum)	Experiment carried out in presence and absence of sucrose	Ye and Shen (2004)
UASB[c]	30	45–120	117	$3\ g\ L^{-1}$ Sodium acetate used as co-substrate	Majumder and Gupta (2007)
Suspended batch	50–192	–	115–175	Experiment carried out with acclimatised inoculum	Present study
AFBR	250–2600	50–1365	873 (maximum)	The stable removal rate achievable was $730\ mg\ L^{-1}\ d^{-1}$	Present Study

[a] AFBR – Anaerobic fixed bed reactor.
[b] SMBR – Silicone membrane bioreactor.
[c] UASB – Upflow anaerobic sludge blanket.

of chlorophenols, as reported in the majority of studies (Dietrich and Winter, 1990; Latkar and Chakrabarti, 1994; Smith and Woods, 1994; Wang et al., 1998; Majumder and Gupta, 2007), typically proceeded by reductive dechlorination, whereby the chlorine was replaced by hydrogen, thus releasing chloride into solution. Reductive dechlorination was then followed by cleavage of the aromatic ring, leading to complete mineralization of 2-CP. Biogas and chloride were end products.

The rate of dechlorination of multi-chlorinated aromatic compounds depends upon the degree of chlorination. It increases progressively as the highly chlorinated chlorophenol molecules become more dechlorinated. The rate of mineralization of the resulting monochlorophenols is usually slower than the rate of dechlorination of multiple chlorinated compounds, resulting in an intermediary accumulation of monochlorophenol in the system (Kohring et al., 1989; Zhang and Wiegel, 1990). Thus complete mineralization of the monochlorophenols is very important for the full biodegradation of polychlorinated chlorophenols.

Keeping the above discussion in mind, the aims of the present study were (i) To adapt sewage sludge to 2-CP degradation, which is a common intermediate of biodegradation of highly chlorinated phenols. It very likely accumulates and thus affects wastewater treatment processes. (ii) To utilise the acclimatised sludge as a seed inoculum for an anaerobic fixed bed reactor (AFBR) in order to investigate reactor performance and microbial adaptation at increasing loading rates and concentrations of 2-CP from 250 to $2600\ mg\ L^{-1}$ in the influent, (unlike the usual strategy, where the 2-CP concentration in the influent is very low and the loading is increased by decreasing the hydraulic retention time HRT). This approach would provide useful information for industrial wastewater treatment of effluents containing high and changing chlorophenol concentrations). (iii) To carry out batch assays at different 2-CP-concentrations to determine maximal 2-CP degradation rates.

2. Material and methods

2.1. Preparation of a 2-chlorphenol-degrading seed inoculum

The seed sludge was taken from an anaerobic reactor of the municipal sewage treatment plant, Berghausen, Germany. It was incubated at 37 °C with $50\ mg\ L^{-1}$ of 2-CP and its degradation was monitored. Upon complete removal after about 3 wk, this sludge was spiked again twice with 2-CP to enrich 2-CP-degrading organisms. Then the sludge or the turbid supernatant (containing the bacteria) was used for further experiments.

2.2. Reactor design

A continuous AFBR was started by introducing the supernatant of the previously 2-CP-acclimatised sludge after gravity sedimentation of the solids for 1 h as a seed inoculum. The AFBR consisted of a glass cylinder which was 80% packed with clay beads (Liapor®, Forchheim, Germany) as a support material for biofilm formation. The diameter of the beads was 12 ± 1 mm. The density of the material was $274\ g\ L^{-1}$ and wet and dry porosities were 42% and 48%, respectively. Two plastic sieves were provided at the top and bottom of the reactor to fix the carrier material in place and to avoid initial floating. The liquid working volume of the AFBR was 1.2 L. The reactor was maintained at 37 ± 2 °C through a jacket of warm water regulated with a thermostat. The influent was provided at the bottom using a peristaltic pump (Gilson, model Minipuls 3, Hanau) and the same amount of effluent was displaced at the top of the reactor through a siphon. At the top a gas outlet was connected to a wet gasometer (Ritter, Bochum). The influent feed was a synthetic wastewater (SWW) consisting of 2-CP as the main carbon substrate. The SWW was prepared in a 50 mM phosphate buffer of pH 7.3 that contained the following nutrients or salts ($g\ L^{-1}$): peptone, 0.12; yeast extract, 0.12; NaCl, 0.007; $MgSO_4 \cdot 7H_2O$, 0.002, $CaCl_2 \cdot 2H_2O$, 0.004 and $200\ \mu L\ L^{-1}$ of a trace metal solution (Sarafaraz et al., 2004). The amount of 2-CP in the feed was gradually increased from $0.25\ g\ L^{-1}$ to $2.6\ g\ L^{-1}$ as described in Table 2. Replacement of peptone and yeast extract by $0.5\ g\ L^{-1}$ NH_4NO_3 and $1.3\ ml\ L^{-1}$ vitamin solution (Kafkewitz et al., 1996) is indicated where applicable.

2.3. Batch assay

An assay was carried out to determine the rate of 2-CP degradation under batch conditions using reactor effluent as an inoculum. The reactor effluent was rather clear with an optical density of only 0.066 at 578 nm. In order to accumulate more biomass, the treated wastewater was collected over days 149–157 of reactor operation and stored at 37 °C. Before using the biomass for batch tests, it was allowed to sediment and the turbid lower layer was utilised as an inoculum. By this procedure the biomass was enriched about 3-fold. Batch tests were carried out in serum bottles with 50 mL working volume, incubated at 37 °C on a mechanical shaker. To ensure anaerobic conditions, the bottles were evacuated to remove air and filled with N_2 gas and maintained at atmospheric pressure. Gas samples were taken with a gas-tight syringe to measure the methane content. Liquid samples were taken out with 1 mL syringes, previously flushed with N_2 to analyse 2-CP and phenol concentrations as described below.