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Effect of phenol addition on COD and nitrate removal in an anoxic suspension reactor

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ABSTRACT

In this study, a denitrifying culture was enriched in a continuously re-circulated anoxic suspension reactor fed with glucose and nitrate for about 8 months (stage I) under different organic loading rates (OLR). At the end of stage I, the removal efficiency for NO_3^-–N was 80% with 93% COD (5 g/l) removal at an OLR of 2.5 g/l d. The mean COD/N removal ratio during the whole enrichment was 3.3. The response to phenol as a toxic substance on glucose enriched culture for long time period was investigated by introducing phenol as a co-substrate in the reactor feed in stage II. Phenol was increased gradually to 753 mg/l till termination of the reactor operation. After increasing the OLR or the phenol concentration, fluctuations in removal efficiencies were observed which were partly reversible. At the end of the reactor operation, NO_3^-–N removal was 65% with 81% COD removal at a phenol degradation rate of 207 mg/l d phenol. The OLR of the reactor was 4.3 g/l d COD and a hydraulic retention time (HRT) of 1 day. Phenol degradation in batch assays under anoxic conditions and at low phenol concentrations (188 mg/l) proceeded a removal rate of 1.2 g/l which decreased to 0.67 mg/l d at high phenol concentration (847 mg/l).

1. Introduction

Denitrification after nitrification is commonly employed to remove excess nitrogen in the effluent of wastewater treatment plants in order to minimize nitrogen release to the discharging bodies, thus avoiding problems of eutrophication and nitrite accumulation, since nitrite would be more toxic than nitrate. From nitrate, nitrosamines and nitrosamides could be generated, which both are potentially carcinogenic compounds (Ono et al., 2000). In an anoxic environment there are several possible fates of NO_3, including assimilatory nitrate reduction (immobilization), dissimilatory nitrate reduction to ammonia and denitrification (Tiedje, 1988; Greenan et al., 2006). The terminal end product of denitrification is N_2 gas. The treatment strategy of a system could be disturbed if significant amounts of NO_3 were converted to NH_4 or organic N or inorganic forms such as NO_2, NO, N_2O. Among indicators of inhibition in a wastewater treatment system, the most sensitive and the most accessible is the presence of denitrification intermediates, mainly nitrite or not reduced nitrate in the reactor. The characteristics of organic carbon required for heterotrophic nitrate reduction has a major effect on the denitrification process. An easily available and biodegradable carbon source is a major factor for achieving the optimum denitrification conditions. Similarly, a process failure can occur due to unavailability, toxicity and non-biodegradability of a heterotrophic carbon compound. In a study on nitrification and denitrification of a real wastewater of a metal-processing unit, a system comprised of an aerobic continuously stirred tank reactor followed by an anaerobic packed column was able to achieve high ammonia removal efficiencies of 89%, but nitrate removal was only 15%. This was due to the presence of various unknown and some known substances (e.g. surfactants), in the sewage which inhibited denitrification. It was suggested to use methanol or lactate as a carbon source for optimal denitrification (Kasia et al., 2005). A few studies (Maranon et al., 2008; Gabaldon et al., 2007; Schuch et al., 2000; Buchheister et al., 2000) are available investigating the denitrification of toxic industrial wastewater.

In most studies, a mixed culture was enriched in the presence of toxic compounds (e.g. Sarafaraz et al., 2004; Eiroa et al., 2005; Zhu et al., 2006). The response of denitrifiers, which were proliferated for long periods on non-toxic and easily degradable compounds, to toxic compounds in the influents is not yet well studied. The present paper reports the toxicity effect of phenol towards an anoxic mixed microbial flora in a reactor that removes nitrate with glucose as a carbon source to reveal possibly an inhibition. Phenol is a man-made as well as a naturally occurring aromatic compound and an important intermediate in the biodegradation of natural and industrial aromatic compounds (Schie and Young, 1998). Phenol at high concentrations is a known bactericidal compound and is toxic or inhibitory for non-adapted cultures at already low

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concentrations. The toxicity effects of aromatic organic compounds towards a microbial consortium are expressed by the inhibition of one or several biological steps of the denitrification process. In this study anoxic sludge from a municipal wastewater treatment was used because mixed cultures are more likely to be representative for the populations in reactors than pure cultures. Therefore, working with consortia has the advantage that the results have more relevance for the design and operation of reactors (Fiuza et al., 2002). In literature very few studies are available concerning the microbial exposure to the toxic compound over long operation periods. It is aimed to study the inhibition effect of increasing phenol concentrations on the denitrifiers over a long time period (>1.4 years) as severity of inhibition is related, among other things, to toxicant concentration and exposure time (Yang and Speece, 1985). The effect of phenol was investigated by gradually increasing its concentration in the influent. In this study, nitrate removal in relation to COD removal was chosen to characterize the toxicity of phenol in the influent. Also toxicity bioassays were conducted in continuously stirred batch experiments to determine the phenol toxicity towards sludge taken from the anoxic reactor.

2. Methods

2.1. Anoxic suspension reactor and operation conditions

A continuously re-circulated suspension reactor was operated for 8 months to investigate nitrate removal with glucose as the only carbon source and, later on, in the presence of phenol as a possibly toxic co-substrate for about ~1.5 years under anoxic conditions. The reactor consisted of a glass cylinder (5.5 cm internal diameter and 45 cm height) fitted with a recirculation system, influent/effluent reservoir, influent/effluent pipes and of a gas outlet, which was connected with a gas meter (Ritter, Bochum). This reactor was an up-flow system. A thick rubber stopper was the seal at the top. The effluent and recirculation pipes were inserted into this stopper. The influent pipe was connected to the recirculation pipe at the bottom. The total working volume of the reactor was 1740 ml. The reactor inoculum was sludge which was taken from the denitrification chambers of the municipal wastewater treatment plant, Neureut. The reactor was operated in two stages i.e. in the absence and in the presence of phenol as a possible inhibitory substance to evaluate the process behaviour under both conditions. In the first stage of reactor operation (Table 1), the influent consisted of synthetic wastewater, containing only glucose as a source of easily degradable organic matter along with essential nutrients described elsewhere (Bajaj et al., 2009). The final COD was 5 g/l. Potassium nitrate was added into this wastewater to maintain a COD/NO_3^-–N ratio in influent from 3.4 to 4. In the second stage of reactor operation phenol was added to the feed solution at steady state conditions on day 246. The treatment of phenol containing synthetic wastewater was evaluated for approximately 1.5 years. Various organic loading rates were applied to the reactor, both, in the absence and in the presence of phenol in the feed. The reactor was monitored for influent and effluent COD, phenol, NO_3^-–N, NO_2^-–N and effluent gas composition.

Table 1
Reactor operating conditions during stage I (glucose as the sole carbon source).

Phase (d)	HRT (d)	OLR (g COD/l d)	NO_3^-–N (g/l)	C/N ratio
I (1–16)	10	0.5	1.56	3.2
II (17–29)	8	0.6	1.60	3.0
III (30–50)	5	1.0	1.60	3.1
IV (51–90)	3	1.7	1.50	3.4
V (90–244)	2.2	2.4	1.60	3.1

2.2. Batch assays

Anoxic batch assays were carried out in serum bottles that were sealed with rubber stoppers and aluminium caps. The exact volume of each bottle was determined by weighing empty and filled bottles with the respective stoppers fitted in. All bottles had volumes ranging from 116 to 119 ml. The inoculum was the sludge described above. The total working volume was kept at 50 ml in each assay. The required amount of substrates in each assay was added as a concentrated solution, as indicated. Anoxic conditions in the assays were maintained by three times evacuating the air from the assay bottles and replacing it with nitrogen at 2 bar. Then the bottles were maintained at atmospheric pressure by releasing the overpressure with a syringe. The required amount of a concentrated anaerobic solution of KNO_3 was added into all assays with a syringe. The assays were incubated at 25 + 2 °C on a shaker. All assays were performed in duplicate.

2.3. Analytical

Samples of influent and mixed liquor from the reactor and from the batch assays were collected and centrifuged in an Eppendorf Microfuge at 12,500 rpm for 5 min (Eppendorf, Hamburg, Germany). The supernatant was used for further analyses. The dissolved chemical oxygen demand (COD) was determined by the method of Wolf and Nordmann (1977). The concentration of phenol and fatty acids (for batch assays) were determined by gas chromatography (GC) with flame ionization detection. The GC operating conditions are described else (Bajaj et al., 2009). The concentration of NO_3^-–N was determined using the 2,6-dimethyphenol (DMP) method of German Standard Methods (DEV, 1998). The DMP reagent (1 ml) was added to centrifuged sample (1 ml) acidified with 8 ml of a 1:1 mixture of H_2SO_4 and *ortho*-H_3PO_4 acid. A colour development after 15–30 min incubation due to the NO_3^- and DMP interaction was measured with a spectrophotometer at 324 nm. Since NO_2^- causes interference in the analysis, it was removed by adding some crystals of amidosulfonic acid before acidification of the sample. The presence of nitrite in the samples was determined either with Merckoquant® NO_2^- test strips (Merck, Darmstadt) or spectrophotometrically according to German standard methods DEV (1998). Nitrite forms an intensive pink colour in acidified solution with sulfanilamide and N-(1-napthyl)-ethylenediamine, which could be measured at 540 nm after 90 min incubation of the sample with 1 ml of acidified solution to make 50 ml total volume. Total and volatile suspended solids were determined according to American standard methods (APHA, 1992). All chemicals were of analytical grade and were purchased from Merck/VWR (Darmstadt), Fluka (Taufkirchen) or Carl Roth (Karlsruhe), Germany.

3. Results and discussion

3.1. Enrichment of an anoxic denitrifying culture in the presence of glucose

Enrichment of an anoxic denitrifying sludge was divided into five phases in stage I that represented different loading conditions (Table 1). Under anoxic conditions NO_3^-–N is sequentially reduced to nitrogen and all intermediates of nitrate reduction may be released under certain non-optimal conditions. During the first 245 days of reactor operation, glucose was the sole carbon source and electron donor in the synthetic wastewater feed to improve proliferation of denitrifies and to observe their responses to changes in OLR. The average dissolved COD concentration during the first 200 days was 5018 mg/l. The COD removal ranged from