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Research article

Investigating the inhibitory effect of cyanide, phenol and 4-nitrophenol on the activated sludge process employed for the treatment of petroleum wastewater

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ABSTRACT

In this work, the inhibitory effect of cyanide, phenol and 4-nitrophenol on the activated sludge process was investigated. The inhibition of the aerobic oxidation of organic matter, nitrification and denitrification were examined in batch reactors by measuring the specific oxygen uptake rate (sOUR), the specific ammonium uptake rate (sAUR) and the specific nitrogen uptake rate (sNUR) respectively. The tested cyanide, phenol and 4-nitrophenol concentrations were 0.2–1.7 mg/L, 4.8–73.1 mg/L and 8.2–73.0 mg/L respectively. Cyanide was highly toxic as it significantly (>50%) inhibited the activity of autotrophic biomass, heterotrophic biomass under aerobic conditions and denitrifiers even at relatively low concentrations (1.0–1.7 $mgCN^-/L$). The determination of the half maximum inhibitory concentration (IC_{50}) confirmed this, since for cyanide IC_{50} values were very low for the examined bioprocesses (<1.5 mg/L). On the other hand, the IC_{50} values for phenol and 4-nitrophenol were much higher (>25 mg/L) for the tested bioprocesses since appreciable concentrations were required to accomplish significant inhibition. The autotrophic bacteria were more sensitive to phenol than the aerobic heterotrophs. The denitrifiers were found to be very resistant to phenol.

1. Introduction

Petroleum refinery industries result in the production of significant quantities of wastewater from various processes which include desalting, vacuum distillation, hydrocracking, catalytic cracking, catalytic reforming, alkylation (Al Zarooni and Elshorbagy, 2006; Coelho et al., 2006). Petroleum and petrochemical wastewater can contain phenols, hydrocarbons, benzene, toluene, ethylbenzene, xylene, polycyclic aromatic hydrocarbons (PAHs), spent caustic soda, cyanides, heavy metals, sulphides, sodium chloride and other substances (Pérez et al., 2010; Piubeli et al., 2012; Tobiszewski et al., 2012). Several of these substances can inhibit the biochemical reactions that take place during the biological treatment of wastewater resulting in poor treated effluent quality (Di Fabio et al., 2013). Phenol is a toxic substrate and can significantly inhibit biological activity in wastewater treatment plants (WWTPs). However, it is also a carbon source and can be degraded by bacteria (Bumbac et al., 2015). Lu et al. (2013) studied the impact of phenol on nitrite accumulation and sludge performance in the nitritation/denitritation processes. At high phenol concentrations (60–90 mg/L), the nitrite accumulation was attributed to changes in the microbial population of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) which were induced by phenol inhibition. Yi et al. (2006) investigated the biodegradation of *p*-nitrophenol in a sequencing batch reactor (SBR) composed of granules. The increase of *p*-nitrophenol feed concentration resulted in an increase in the biodegradation rate for *p*-nitrophenol concentrations up to 40.1 mg/L. The further increase of the feed *p*-nitrophenol concentrations resulted in a decrease of the biodegradation rate due to inhibition. Stasinakis et al. (2008) examined the inhibitory effect of triclosan and nonylphenol on heterotrophic and autotrophic biomass activity and concluded that autotrophic

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biomass was much more sensitive to these compounds than heterotrophic biomass. Amor et al. (2005) demonstrated that it is possible to obtain effective aerobic biological treatment for wastewater containing high phenol (35–2800 mg/L) and ammonium content. Both phenol and ammonium removal were found to be very high (i.e. >99.9% and 99.8% respectively). Strotmann et al. (1994) studied the inhibitory effects of phenolic compounds by monitoring the growth of activated sludge. The results showed that phenol was the weakest inhibitor of biomass growth in comparison to other phenolic compounds such as 2,4-dinitrophenol and several chlorophenols. Inhibition of biomass activity was observed at high phenol concentrations; specifically, the order which was obtained was the following: phenol < 2,4-dinitrophenol < 3-chlorophenol < 2,3-dichlorophenol < 2,4-dichlorophenol, 2,5-dichlorophenol < 3,4-dichlorophenol < 3,5-dichlorophenol.

Cyanide compounds are produced from various industrial activities such as mining, coke, steel, chemical and petroleum industry. Cyanide can be present in different forms which include (i) free cyanide, (ii) weak and moderately strong metal-cyanide complexes of Ag, Cd, Hg, Cu, Zn, Ni and (iii) strong cyanide-metal complexes of Fe (Botz et al., 2005; Fatone et al., 2009). Free cyanide is the sum of hydrogen cyanide (HCN), and the cyanide ion (CN^-). Free cyanide is much more toxic than cyanide found in complexes. However, free cyanide can be degraded more easily than cyanide complexes (Di Fabio, 2009). Cyanide biodegradation is quite complex and can take place via four general pathways: hydrolytic; oxidative; reductive; and substitution/transfer. The pathway that is followed depends on factors such as the presence or absence of oxygen and carbon source, the pH, the cyanide concentration (Ebbs, 2004). Several reviews describe the pathways of biochemical cyanide removal (Knowles and Bunch, 1986; Raybuck, 1992; Dubey and Holmes, 1995). Han et al. (2014) examined the inhibition caused to nitrification by acrylonitrile, acrylic acid, acetonitrile and cyanide in acrylonitrile wastewater. The authors concluded that the presence of cyanide-degrading bacteria as well as the step-wise increase of influent cyanide concentration can mitigate the inhibitory effect of cyanide. Furthermore, nitrification inhibition could be recovered a few days after the feed of cyanides had ceased. The presence of phenol and cyanide in wastewater which is treated biologically can cause process instability which can lead to the biomass inhibition/washout from the bioreactor. Previous works have shown that even low concentration of free cyanide of the order of 1.0–2.0 mg/L (i.e. in the form of HCN or CN^-) can inhibit nitrification and denitrification in the activated sludge process (Kelly et al., 2004; Kim et al., 2008; Park and Ely, 2008). Kim et al. (2011) studied the inhibition caused to nitrification and denitrification by cyanide and found that, in a batch system, a concentration of 0.07 mg/L of free cyanide slightly reduced the initial rate of nitrification, while a concentration of 0.2 mg/L caused a distinct lag phase on nitrification. Denitrifying bacteria were more tolerant to cyanide than nitrifiers; however, a free cyanide concentration of 5 mg/L resulted in an almost complete inhibition of denitrification. Comparing the toxicity of free cyanide and phenol, it can be concluded that free cyanide is much more toxic to biomass, since much lower concentrations result in inhibition of bioprocesses (Neufeld et al., 1986).

The biological treatment of wastewater in an SBR failed when electroplating wastewater with high CN^- concentration (23.0 ± 2.2 mg CN^-/L) and low biodegradable content (10 ± 3 $mgBOD_5$/L) was fed to the SBR. However, when electroplating wastewater was used to supplement the treatment of Thai-rice noodle wastewater, with a feed cyanide concentration of 2.3 ± 0.2 mg CN^-/L it was very effective in supplementing wastewater as the nitrogen source (Sirianuntapiboon et al., 2008). Kong et al. (1996) examined the inhibition caused by 3,5-dichlorophenol, copper and cyanide on organic carbon oxidation and nitrification. In general, the nitrification process was found to be more sensitive to these substances than organic carbon oxidation with the exception of copper. The results indicated that cyanide is a non-competitive inhibitor for organic carbon oxidation and a mixed inhibitor for nitrification. Tomei et al. (2003) examined the biodegradation process of 4-nitrophenol in an SBR process. The authors concluded that the removal kinetics of 4-nitrophenol was described by the typical substrate inhibition pattern which is predicted by the Haldane equation. Ricco et al. (2004) studied the toxicity of 4-nitrophenol and other organic compounds on municipal activated sludge by monitoring the specific oxygen uptake rate (sOUR). The sOUR inhibition was 17% at 4-nitrophenol concentration of 16 mg/L, and increased to 68% at 160 mg/L; the IC_{50} is found to be approximately 150 mg/L.

The oil and gas industry in Kazakhstan is a major producer of wastewater in both upstream and downstream processes. In oil refinery plants the petroleum wastewater is treated by the activated sludge process to deliver a treated effluent which is of adequate quality for discharge. However, the inhibitory effect of several substances could render the process problematic. For instance, Di Fabio et al. (2013) examined biomass activity in a pilot scale submerged membrane bioreactor (MBR) treating petrochemical wastewater. The authors measured the sOUR for heterotrophic biomass which was on average between 7.8 and 11.0 mgO_2/gVSS h for the different experimental periods. The specific ammonium uptake rate (sAUR) was 0.12–0.34 mgN/gVSS h and the specific nitrogen uptake rate (sNUR) was 1.03–2.70 mgN/gVSS h depending on the operating conditions. The rates are much lower compared to the typical ones met in municipal wastewater treatment plants, due to the inhibitory effect of certain petrochemical compounds.

This work examined the inhibitory effects of cyanide, phenol and 4-nitrophenol on aerobic heterotrophic biomass, nitrifiers and denitrifiers. The inhibitory effect of these compounds was examined in batch reactors in which specific concentrations of these compounds were added. This work offers new insight on the inhibitory effects of three compounds which are frequently met in petroleum and petrochemical effluents and result in a decreased activity of the various bioprocesses.

2. Materials and methods

2.1. Batch reactor inhibition tests

The inhibitory effect of cyanide, phenol and 4-nitrophenol was examined on activated sludge. These chemicals were added in the forms of KCN, phenol and 4-nitrophenol (Merck). The experiments were carried out in batch reactors (1 L Erlenmeyer flasks) in which fixed concentrations of the aforementioned chemicals were added. Activated sludge was obtained from the recycle stream of the activated sludge process of a municipal WWTP in Astana of Kazakhstan.

The inhibition of heterotrophic biomass in aerobic conditions was examined by monitoring the sOUR, while inhibiting the nitrification process. Biomass was placed overnight under aeration. Then, 400 mL of activated sludge was placed in each of the 1 L Erlenmeyer flasks under continuous aeration and agitation. Temperature was maintained at 20 ± 2 °C and the pH was adjusted at 7.8 ± 0.4 by adding $NaHCO_3$. Nitrification was inhibited by adding 10–12 mg/L allyl-thiourea into the activated sludge. After 30 min of continuous aeration and agitation, the biomass was placed in a BOD flask under very mild agitation without aeration and the DO was recorded with time. The slope of DO decrease with time corresponded to the endogenous oxygen uptake rate ($OUR_{endogenous}$).