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Effect of cyanide on phenolics and aromatic hydrocarbons biodegradation under anaerobic and anoxic conditions

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HIGHLIGHTS

- Coke oven wastewater contains phenolics, aromatic hydrocarbons along with cyanide.
- Earlier studies focused on biodegradation of mixed organic compounds in aerobic conditions.
- Study focused on degradation of complex organic pollutants with cyanide in anoxic/anaerobic conditions.
- Anoxic condition was more effective for pollutants removal than anaerobic condition.
- A mathematical model is proposed to model the degradation of mixed pollutant system.

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ABSTRACT

The objective of this study was to investigate the biodegradation of a mixture of pollutants commonly found in coke oven wastewater such as phenol, cresol, xylenol, quinoline, indole and cyanide under anaerobic and anoxic conditions. It was found that xylenol was highly recalcitrant under anaerobic and anoxic conditions. In presence of free cyanide (>2.5 mg/L), the organic compounds (100 mg/L) were degraded before the biodegradation of free cyanide under both the conditions. However, the biodegradation of pollutants prolonged under anaerobic conditions compared to anoxic conditions. All the organics, especially xylenol and indole, inhibited the degradation of cyanide. Experimental results highlighted the detrimental effects of the combined toxic influence of cyanide and organics on the anaerobic and anoxic microbes treating coking wastewater. The proposed mathematical model was able to simulate the biodegradation of organic compounds along with cyanide satisfactorily.

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1. Introduction

Coal processing wastewater contains toxic organic pollutants, which include phenolics, nitrogen containing aromatic hydrocarbons, free and metal complexed cyanides, thiocyanate and ammonia, among other pollutants [1]. Usually such toxic wastewater is treated by employing various combinations of physico-chemical and biological units [1]. Different configurations of anaerobic, anoxic and aerobic reactors are utilized to minimize treatment cost and achieve standard discharge levels. Nevertheless, it is reported that, many of the full scale coal processing and coke oven wastewater treatment plants do not achieve standard discharge levels as the biological systems get destabilized with respect to time [1,2]. This performance failure is mostly attributed to incomplete understanding of the interactive effects of various classes of pollutants on one another and on the microbes involved in their treatment, under different environmental conditions.

There have been vast literatures on the eco-toxicity and biodegradation of pollutants found in coke oven wastewater (CWW) in order to determine the different metabolic pathways involved in the use of these compounds as microbial carbon and energy source. However, there has been very limited study on the biodegradation of xylenol under both aerobic and anaerobic conditions [3]. It is also reported that xylenol is 34 times more toxic than phenols under aerobic conditions [3–5]. The refractory compounds in CWW get accumulated in the aerobic activated sludge, leading to potential pollution problems during sludge disposal [6]. Anaerobic biodegradation involves partial breaking down of polycyclic rings and depending on the microbes and culture conditions (methanogenic, nitrate or sulfate rich), these fission ringed products are then funneled to Krebs cycle through different pathways [7]. Capability of anaerobic cultures to effectively mineralize individual phenolic compounds has been demonstrated by others [8]. Xylenol is reported to be more refractory and inhibitory than phenols and

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cresols [9] even under anaerobic conditions. There are several reports elucidating the potential of aerobic and anaerobic microbes in utilizing both free and metal cyanides as sole carbon and nitrogen sources [10]. Microbes such as *Escherichia coli* strain BCN6 and *Pseudomonas fluroscence* NCIMB 11764, were able to degrade cyanide aerobically whereas *Klebsiella oxytoca* was degrading cyanide under anaerobic conditions [11]. Studies using double pollutant degradation is limited to similar classes of pollutants such as phenol–cresol [12], quinoline–indole [13], pyridine–quinoline [14], pyridine–indole [15]. However, the effect of cyanide on anaerobic phenol – degrading microbes have been investigated earlier [16–18] and it was concluded that methanogens were highly susceptible to cyanide concentration than phenol-degraders. There have been no reports on the influence of cyanide in the removal of other pollutants present in CWW such as cresols, xylenols, quinoline and indole. Under aerobic conditions, it have been shown that the combination of xylenol (200 mg/L) and cyanide (20 mg/L) completely inhibited microbial growth [19]. Therefore, in order to delineate the causes of failure of CWW treatment plants, it is important to understand the effect of cyanide on the removal of other pollutants present in CWW under different redox conditions.

There have been a few investigations on the biodegradation of phenolics and nitrogen containing aromatic hydrocarbons under nitrate rich conditions which demonstrated the advantages of non-methanogenic anaerobic conditions [6,20]. It is reported that phenols, cresols (100–1500 mg/L), quinoline, pyridine and indole (100–300 mg/L) could be individually mineralized better under anoxic conditions than under methanogenic conditions [21]. Licht et al. [22] studied the transformation of quinoline and indole by *Desulphobacterium* and found that quinoline and indole were hydroxylated by similar set of inducible enzyme system during degradation under anoxic conditions. Previous studies indicated that nitrate reducing condition is effective in eliminating many of the NHCs including quinoline [6]. There are only limited investigations on biodegradation of xylenol under anoxic conditions [3,4]. Moreover, there have been relatively fewer studies under anoxic conditions investigating the influence of cyanide upon removal of other pollutants such as xylenols, quinoline and indole which are prominently found in CWW.

Biodegradation of pollutants under single redox conditions may not be effective especially for the treatment of complex wastewaters [1]. Current literature on individual and double pollutant biodegradation under different redox conditions is not sufficient to correlate and elucidate the biodegradation mechanisms to devise bioreactors for effective removal of pollutants using sequence of optimized environments [1,2]. Thus it is necessary to study the pollutant removal mechanisms in biological reactors under different redox conditions for mixed pollutants, and in presence of inhibitors like cyanides. In the present work, batch experiments were performed separately under anaerobic and anoxic conditions for single pollutants, organic pollutants in presence of cyanide and mixture of pollutants to simulate the biological treatment of wastewater generated from coal processing industries. Biokinetic parameters for individual pollutants under anaerobic and anoxic conditions were evaluated using Haldane model. An attempt was made to predict the behavior of pollutant degradation in presence of cyanide, using the biokinetic parameters obtained from the individual pollutant systems.

2. Materials and methods

2.1. Seed sludge

2.1.1. Anaerobic sludge

The inoculum for anaerobic acclimatization was obtained from anaerobic digester unit of a municipal wastewater treatment plant located in Nesapakkam, Chennai, India. The composition of the synthetic medium used in the study was (mg/L): $NaHCO_3$ (5000), NH_4Cl_4 (280), $CaCl_2{\cdot}2H_2O$ (10), K_2HPO_4 (250), $MgSO_4$ (100), yeast extract (100) and l mL of micronutrients stock solution which contained (mg/L): $FeCl_2{\cdot}4H_2O$ (2000), H_3BO_3 (50), $ZnCl_2$ (50), $CuCl{\cdot}2H_2O$ (38), $MnCl_2{\cdot}4H_2O$ (500), $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (50), $AlCl_3{\cdot}6H_2O$ (90), $CoCl_2{\cdot}6H_2O$ (2000), $NiCl{\cdot}6H_2O$ (142), EDTA (1000) and HCl 36% (1 mL) [23]. In order to remove oxygen content in the basal medium, 100 mg/L of $Na_2S{\cdot}9H_2O$ was also added. The carbon source included the organic pollutants (mg/L); phenol (250), o-cresol (100), 3,5-xylenol (100), quinoline (100), Indole (100) and free cyanide (20). The pH and temperature during acclimatization of anaerobic biomass was 7.5–8 and 27–31 °C, respectively.

2.1.2. Anoxic sludge

The primary seed for anoxic cultures was developed using defined mixture (1:1) of aerobic activated sludge and anaerobic digester sludge obtained from the return sludge of the activated sludge unit and anaerobic digester of domestic sewage treatment plant, Nesapakkam, Chennai, India. The enrichment of cultures was initiated by several subcultures transferred to fresh anoxic medium containing 2 g/L of KNO_3 and pollutants (mg/L) such as phenol (250), o-cresol (100), 3,5-xylenol (100), quinoline (100), indole (100) and cyanide (20). A mineral salt medium containing the following constituents was used for enrichment (g/L): $Na_2HPO_4{\cdot}2H_2O$ (3.1); KH_2PO_4 (1.6); NH_4Cl (0.5); $MgSO_4{\cdot}7H_2O$ (0.1); $CaCl_2{\cdot}2H_2O$ (0.02) and 1 mL of micronutrients $FeCl_2{\cdot}4H_2O$ (2000), H_3BO_3 (50), $ZnCl_2$ (50), $CuCl{\cdot}2H_2O$ (38), $MnCl_2{\cdot}4H_2O$ (500), $(NH_4)_6Mo_7O_24{\cdot}4H_2O$ (50), $AlCl_3{\cdot}6H_2O$ (90), $CoCl_2{\cdot}6H_2O$ (2000), $NiCl{\cdot}6H_2O$ (142) [20]. The pH of the basal medium was 7.5–8 and temperature was between 27 and 31 °C during the acclimatization of anoxic biomass. Biomass growth under anoxic conditions was detected within 3 weeks, by the presence of light brown biomass flocs.

2.2. Batch degradation experiments

2.2.1. Anaerobic studies

Anaerobic batch biodegradability experiments were conducted in 125 mL serum bottles. The batch experiments were performed using the adapted anaerobic sludge. After flushing the dissolved oxygen from the media using ultra pure nitrogen gas at a flow rate of 4 LPM for 5 min, the serum bottles were sealed with 12 mm thick Teflon coated butyl rubber septa and sealed with aluminum caps. The inoculum was transferred to the serum bottles, which contained sterile (autoclaved) 100 mL of the anaerobic basal medium. The desired amounts of pollutants were added with a syringe to the serum bottles and incubated at 30 °C with agitation (150 rpm). At regular time intervals, the samples were centrifuged (10,000×*g* for 5 min) and analyzed until the residual pollutant, TOC and amount of biomass in the flask had reached asymptotic values with respect to time. For each concentration, duplicate experiments were performed under the same conditions and average values are reported. Cell-free abiotic controls were maintained using same concentrations of pollutants as in the experiments.

2.2.2. Anoxic studies

For anoxic experiments, silicone septum secured with aluminum capped 125 mL serum bottles with 100 mL anoxic basal medium was used. Before the start of the batch experiment, the medium was flushed with O_2 free N_2 gas at a flow rate of 4 LPM for 5 min. The head space was sealed with N_2 gas. The medium was autoclaved and cooled, later pollutants and adapted microbial culture were added using syringe needle, followed by incubation at 30 °C with agitation (150 rpm). At regular time intervals, samples were withdrawn, centrifuged (10,000×*g* for 5 min) and analyzed for residual pollutant, TOC and biomass concentrations. For each

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