Aerobic Degradation of Complex Organic Compounds and Cyanides in Coke Oven Wastewater in Presence of Glucose

Naresh Kumar Sharma, Ligy Philip and B. S. Murty

Abstract This study aims at determining the degradation aspect of pollutants from coke oven effluents such as phenols, aromatic hydrocarbons, and cyanide by aerobic mixed culture. Enriched mixed culture was developed from the sludge collected from aeration tank of a sewage treatment plant by serial enrichment technique. The acclimatized culture was able to degrade phenol, cresol, xylenol, quinoline, indole, and cyanide individually, for their concentrations usually found in coke oven wastewater. Xylenol and indole with concentrations above 250 mg/L were highly recalcitrant for biodegradation. A co-substrate such as glucose (1000 mg/L) had an adverse effect on the biodegradation of all the above pollutants; the degradation time was extended with the increase in the concentration of pollutant, although glucose was completely oxidized independent of the pollutant concentration. Biodegradation during mixed pollutant (100 mg/L of each organic compound) conditions were tested in presence of glucose (1000 mg/L) and glucose (1000 mg/L) with cyanide (2.5 mg/L). Aerobic microbes showed increased substrate affinity in the following order phenol > cresol > quinoline > indole > xylenol. The COD at the end of the experiment was found to be less than 0.5 mg/L showing no accumulation of intermediates. In the presence of glucose (1000 mg/L) and cyanide (2.5 mg/L), the lag phase for microbial growth was increased by several days and cyanide was found to be oxidized before the organic pollutants. Xylenol was highly recalcitrant during this experiment and was not degraded even after 20 days. The experimental results highlight the effect of high concentration of co-substrate (1000 mg/L) and the combined toxic influence of cyanide and organics on the microbes treating coke oven wastewater. These results and the ongoing work are aimed at developing

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A. K. Sarma et al. (eds.), *Urban Ecology, Water Quality and Climate Change*, Water Science and Technology Library 84,
https://doi.org/10.1007/978-3-319-74494-0_22

high-rate bioreactors for efficient treatment of phenolics, aromatic hydrocarbons, and cyanide-containing wastes emanating from industrial activities.

Keywords Activated sludge · Glucose · Cyanide · Phenolics · Heterocyclic aromatics

1 Introduction

A mixture of phenolics, heterocyclic aromatics along with cyanide moieties represents one of the toxic combinations of wastewater known to create havoc not only to microbes used for their treatment but also to the ecosystem and to the human race at large. The most common sources of such a combination of these fatal chemicals at lethal doses are from the coal processing and coke manufacturing industries, which satiate our increasing demands for the steels used inconsiderately in this age. Effluents from industries such as coke oven, coal gasification, and coal processing contain myriad of organic and inorganic pollutants (Lai et al. 2008).

The composition of coke oven wastewater is complex and varies from one plant to another, depending upon the quality of raw coal, carbonation temperature, and method used for byproduct recovery (Pal and Kumar 2014). The compounds could be grouped into phenolics, polyaromatic hydrocarbons, nitrogenous heterocyclic compounds, oxygen or sulfur-containing compounds, inorganic compounds, and oil and grease along with suspended solids. Phenol, cresol, xylenol, alkyl phenols are the major contributors in the phenolics category as are quinoline, pyridine, indole in the nitrogen heterocyclic compounds and ammonia, thiocyanate, cyanide, and sulfide in the inorganic group. There are many published reports on the eco-toxicity of these chemical compounds and on the biodegradation of these compounds individually, under different redox conditions (Fetzner 1998). Phenolic compounds can migrate in different aqueous environments and contaminate groundwater (Li and Zheng 2004). Most of the PAHs and NHCs have been reported to be mutagenic and even carcinogenic. Cyanides are toxic to aquatic species and mammals even at low concentrations. Oil and tar form oil slicks over water bodies and influences the distribution of O_2 and CO_2 in water affecting the fishes and aquatic organisms.

Coke oven wastewater (CWW) contains a wide range of PAHs and nitrogen, oxygen and sulfur containing heterocyclic compounds (Zhang et al. 1998; Jianlong et al. 2002). Some of these compounds are known to have long-term environmental impacts and are reported to be mutative and carcinogenic (Qi et al. 2007). Most of the COD that is present after the biological treatment of coke oven wastewater have found to contain these refractory compounds (Lai et al. 2008) because of their nonbiodegradable nature.

The treatment techniques reported in the literature are numerous, including the use of conventional aerobic lagoons, constructed wetlands, photo-bioreactors

(Tamer et al. 2006), bioreactors (aerobic, anoxic, and anaerobic) with different configurations (Zhao et al. 2009; Lai et al. 2008; Maranon et al. 2008; Chakraborti and Veeramani 2005), physicochemical methods (Ghose 2002), membrane bioreactors (Zhao et al. 2009), and the newly enriched bioreactors like anaerobic ammonium oxidation processes. Nevertheless, an economical and effective way of treating coke oven wastewater (CWW) is still a challenge; mainly because the pollutants in CWW are highly varied and it is generally not feasible to remove all the pollutants under similar conditions to the standard discharge levels.

There has been a number of reports on full-scale biological treatment of CWW, but only a few of them follow environmental discharge regulation at a high level of sophistication and price. The bioremediation of contaminant and the rate at which it is achieved depends on the conditions of treatment (pH, temperature, substrate loading, biomass concentration, nutrients, inhibitors concentration), microbial diversity, nature, and chemical structure of the compound being degraded (Haritash and Kaushik 2009). Thus, to devise a biological treatment system, several factors are responsible which must be addressed and explored.

The challenge is to develop a treatment system which could overcome the toxic effects of these chemical mixtures and still maintain the stability in operation and discharge levels. A synthetic wastewater containing the major organic compounds representative of each group corresponds well with the actual wastewater. Henceforth, phenol, cresol, xylenol, quinoline, indole, and cyanide are the target compounds which are selected to represent the actual wastewater from coke oven and coal processing industries. The biodegradation of these compounds with a co-substrate and in presence of an inhibitor contributes to an in-depth study of biodegradation of these pollutants in order to optimize the treatment processes. This knowledge helps in the development of a stable and economically viable treatment plant.

2 Materials and Methods

2.1 Aerobic-Activated Sludge

The activated sludge used in the experiments was obtained from a domestic sewage treatment plant, Chennai and was maintained in the laboratory by a medium containing dextrose as the carbon source. Then the composition of the medium was gradually replaced with phenol, cresol, xylenol, quinoline, indole, and cyanide. Eventually, the activated sludge was cultured in a medium containing these compounds as carbon source over a period of 8 months in a 2 Lx g laboratory unit. The composition of the ultimate medium containing carbon source (in mg/L) was as follows: phenol (500), cresol (100), xylenol (100), quinoline (100), indole (100), cyanide (2.5) along with mineral media having the composition (in mg/L):

NH_4SO_4(230), $CaCl_2$(8), $FeCl_3$(1), $MnSO_4.H_2O$(100), $MgSO_4.7H_2O$(100), K_2HPO_4(500), KH_2PO_4(250), and pH 7.5 ± 0.5 (Saravanan et al. 2008) under agitation condition at room temperature. This medium was supplied to the culture at three days intervals by replacement of 1 L. This acclimated sludge formed light brown flocs and had good settleability.

2.2 Biomass Estimation

To estimate the cell concentration, a known volume of cell suspension was filtered through 0.45 μm filter paper followed by weighing the dried cell mass retained on the filter paper. Protein standard curve was plotted using bovine serum albumin. Protein contents of the acclimated cultures, with known bacterial concentrations (dry weight in mg/L), were determined using Lowry's method (Lowry et al. 1951) and the correlation between protein content of the cells and dry cell weight was established. The modified Lowry's method used was as follows. 2 mL of sample was taken and centrifuged at 8000×g for 8 min. The pellets were then resuspended in 2 mL of phosphate buffer (pH 7) and sonicated at 100 Hz at 15 s intervals (15 s on and 15 s off) for 3 min. The solution was centrifuged again at 8000×g for 8 min. 2 mL of alkaline copper reagent was added to 0.5 mL of supernatant or diluted supernatant of a suitable concentration, incubated for 10 min, followed by addition of 0.2 mL of Folin phenol reagent, and incubated again for 30 min. Reagent blank, containing 0.5 mL of distilled water instead of bacterial suspension, was treated in a similar way. The optical density was measured at 600 nm using a UV–Vis spectrophotometer (Techcom, UK) against the reagent blank. Samples with known bacterial concentrations were used for preparing the calibration curve.

2.3 Individual Pollutant Analysis

2.3.1 High-Performance Liquid Chromatography

Phenol, o-cresol, m-xylenol, quinoline, and indole concentrations were quantified by high-performance liquid chromatography (HPLC) (Dionex, Ultimate 3000). The aqueous samples of the suspended culture were centrifuged at 8000×g for 10 min and filtered through a 0.45 μm filter paper. Then the cell-free supernatants were determined for residual chemical concentrations in the solution. HPLC was performed on a reverse phase C-18 column with acetonitrile/water (50/50, v/v) mobile phase at a flow rate of 1.0 mL/min, and detected using UV at 275 nm.

2.3.2 UV-Spectrophotometry

Free cyanide ion concentrations were analysed using standard methods (APHA 1985) using (Chloramine-T) pyridine-barbituric acid reagent and the light pink color developed was measured colorimetrically at 578 nm using UV-spectrophotometry (Techcom, UK).

2.4 Chemical Oxygen Demand

COD of liquid samples was estimated from the centrifuged (8000×g for 10 min) and filtered samples and the supernatant was analysed by the closed reflex method as suggested in standard methods (APHA 1985). Closed reflux digestion was conducted in HACH COD digester (Model No 45600, USA) and the remaining $K_2Cr_2O_7$ was titrated with FAS to calculate the amount of organics.

2.5 Aerobic Biodegradation Studies

All biodegradation experiments using the acclimated mixed culture were performed in 500 mL Erlenmeyer flask containing 100 mL of MSM containing glucose and pollutants at different concentrations. The initial pH of the reaction mixture was 7.5 ± 0.5. The initial dissolved oxygen concentration was between 6 and 6.5 mg/L in the entire batch degradation studies and it never dropped below 3 mg/L during the biodegradation studies. Upon incubation of the flasks at 30 °C under agitation condition (150×g), samples were withdrawn at regular time intervals, centrifuged (10,000×g for 5 min), filtered, and analysed for the residual pollutant, COD, and protein concentration. For each concentration, duplicate experiments were performed under the same conditions and average values are reported. Each experiment was carried out for a period until the residual concentration of the pollutants and the amount of biomass in the flask had reached asymptotic values with time. Abiotic controls were also monitored during the study.

3 Results and Discussion

3.1 Effect of Co-substrate on Individual Pollutant Biodegradation

An attempt was made to study the degradation of different pollutants, with varying concentrations, along with 1000 mg/L of glucose. Such a high COD of glucose was

chosen to represent the COD of cww. However, 1000 mg/L of glucose had an adverse effect on the rate of biodegradation of all pollutants. The degradation time was extended with the increase in the concentration of the pollutant, although glucose was completely oxidized independent of pollutant concentration; as corroborated by the biomass growth and COD at the end of the experiment. Similarly, Lob and Tar (2000) observed decreased degradation rate of phenol when glucose concentration exceeded 1 g/L and attributed it to the catabolite repression by glucose, which has been observed by other researchers also (Papanastasiou 1982; Satsangee and Ghosh 1990). Phenol (200 mg/L) which degraded within 10 h took a maximum degradation time of more than 14 h in presence of 1000 mg/L of glucose as shown in Fig. 1.

Optimum concentration of glucose during which degradation rate of the pollutant observed to be higher was around 350 mg/L reported by Lob and Tar (2000) and concentrations above 780 mg/L increased the pollutant removal time. The COD at the end of the batch was less than 0.1 mg/L which indicated the complete oxidation of glucose and metabolic intermediates.

Xylenol, the most toxic organic compound did not degrade to a concentration higher than 200 mg/L even in the presence of glucose (Fig. 2), although complete

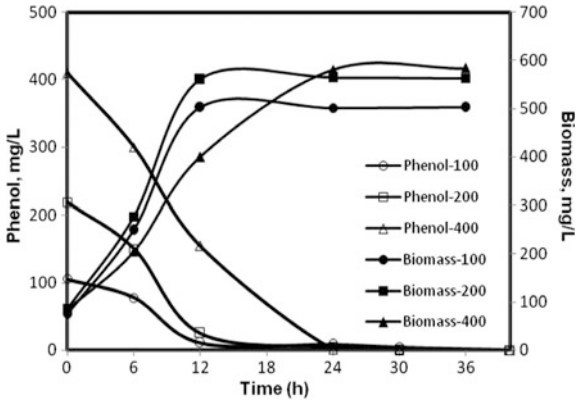

Fig. 1 Phenol biodegradation and biomass growth in presence of glucose (1000 mg/L)

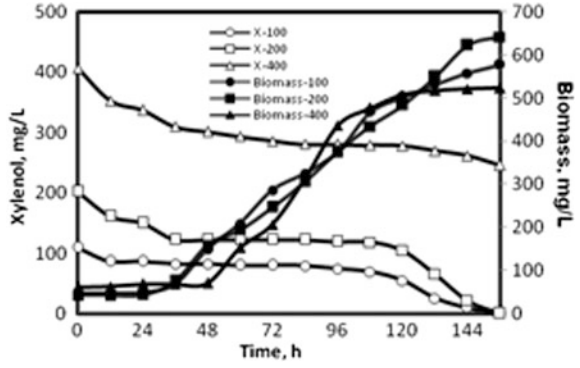

Fig. 2 Xylenol biodegradation and biomass growth in presence of glucose (1000 mg/L)

oxidation of glucose and biomass growth was observed as shown in Fig. 2. Xylenol has already been reported to be 32-fold more toxic than phenol by Acuna-Arguelles et al. (2003). Other organic pollutants such as cresol (Fig. 3), quinoline (Fig. 4), and indole (Fig. 5) showed increased removal time period in presence of glucose as co-substrate. The abiotic losses of the organic compounds were always found to be less than 1%.

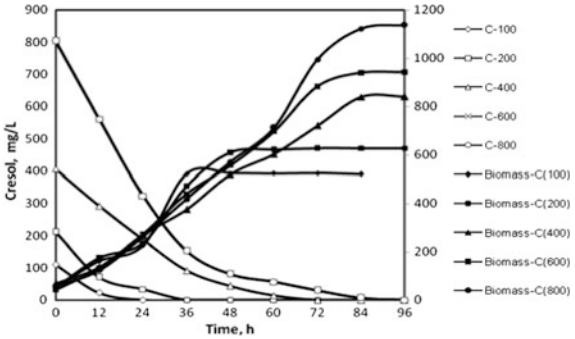

Fig. 3 Cresol biodegradation and biomass growth in presence of glucose (1000 mg/L)

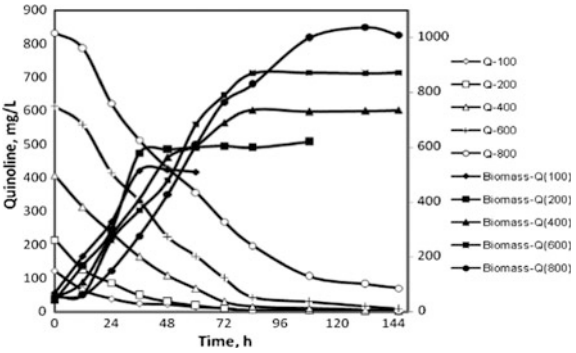

Fig. 4 Quinoline biodegradation and biomass growth in presence of glucose (1000 mg/L)

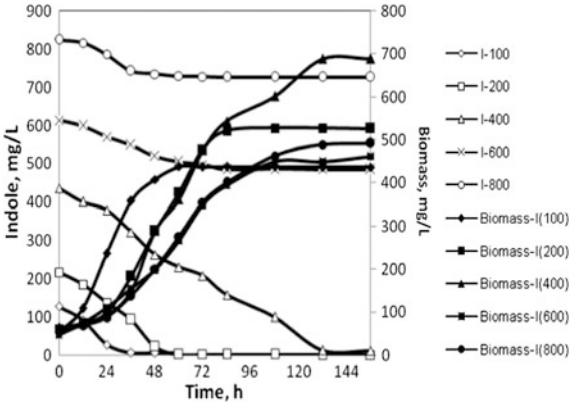

Fig. 5 Indole biodegradation and biomass growth in presence of glucose (1000 mg/L)

There are no reports available on the effect of cyanide on the biodegradation of cresol, xylenol, quinoline, and indole, with some exceptions on phenol. Therefore, experiments have been carried out to understand the effect of varying concentrations of cyanide on the biodegradation of phenolics and aromatic hydrocarbons under aerobic conditions. Initial concentrations of phenol, quinoline, cresol, indole, and xylenol were kept at 250 mg/L, while the cyanide concentration was varied from 0 to 20 mg/L. Phenol (250 mg/L) degraded completely within 24 h. Phenol degradation was insignificant as long as cyanide was present in the system, and the degradation of phenol was very fast once the cyanide disappeared from the system. A similar trend was observed in case of cresol and other organics (Sharma et al. 2012).

3.2 *Effect of Co-substrate on Mixed Organic Pollutant Biodegradation*

Effect of glucose (1000 mg/L) in mixed pollutants (100 mg/L of each organic compound) was studied of inorganic inhibitors. The lag phase for degradation of pollutant was increased mainly because of the combined influence of each pollutant on the aerobic consortia. Inhibition due to the presence of multiple pollutants has been reported by several authors (Lai et al. 2008; Zhang et al. 1998). Phenol and cresol were degraded in less than 4d in presence of other organic pollutants and were most preferable substrates, xylenol on the other hand with only one methyl group excess to cresol was the most toxic organic compound even compared to the heterocyclics like quinoline and indole. The substrate affinity for aerobic consortia was found to be phenol > cresol > quinoline > indole > xylenol as shown in Fig. 6. The degradation of xylenol was found to start only after the oxidation of

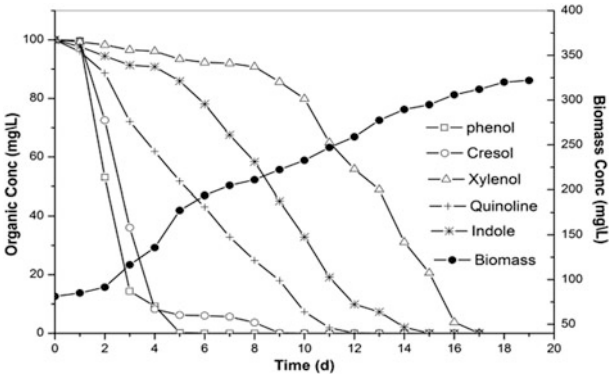

Fig. 6 Mixed organic substrate biodegradation and biomass growth in presence of glucose (1000 mg/L)

other organic compounds. It has been seen from the single substrate studies that xylenol was found to be the most recalcitrant compound due to excess methyl group present in its structure. Also, the degradation time was longest for xylenol than for other single-target compounds. Hence, it was found that the acclimated biomass had a high proclivity toward other organic substrates compared to xylenol. Other studies have shown that the recalcitrant nature of the pollutant increases with increase in the methyl group along the benzene ring of the compound (O'Connor and Young 1996).

Mixed bacterial culture was able to biodegrade the mixed pollutants in absence of cyanide without any accumulation of organic intermediates as evident from the TOC analysis. The initial biomass concentration was maintained at 50–80 mg/L while the concentrations of target organic pollutants were at 100 mg/L each along with 2.5 mg/L of cyanide. Coke oven wastewater contains many inorganic toxic inhibitors such as ammonia, sulfide, thiocyanate, and cyanide. Effect of the presence of cyanide (2.5 mg/L) on mixed organic pollutant degradation was studied. Cyanide had a detrimental effect on the aerobic consortia even at very less concentration (2.5 mg/L). Cyanide was oxidized before other organic compounds, whose concentrations were 40 times more than cyanide concentration. Thus, the time period for organic degradation increased, without any change in the organic substrate affinity.

3.3 Effect of Cyanide on Mixed Pollutant Biodegradation in Presence of Glucose

Coke oven wastewater contains many inorganic toxic inhibitors such as ammonia, sulfide, thiocyanate, and cyanide. Effect of presence of cyanide (2.5 mg/L) on mixed organic pollutant degradation in presence of glucose as co-substrate (1000 mg/L) was experimented. Abiotic loss of compound was very minimal. In case of cyanide, which is highly volatile below pH 8.4, the abiotic loss was less than 1%. Cyanide ion hydrolyzes in water to form molecular hydrogen cyanide (HCN) and hydroxyl ions (OH^-), with a corresponding increase in pH. Though HCN has a high vapor pressure, the rate of volatilization depends on the HCN concentration (a function of total cyanide concentration and pH); the surface area and depth of the liquid; temperature and transport phenomena associated with mixing (Huiatt et al. 1983). Moreover, at a pH of 7.1–8.4, it was reported that at low cyanide concentrations (<50 mg/L), the volatilization was less than 1%, while at high concentrations of cyanide (100–150 mg/L) the removal due to volatilization was about 14%, under similar conditions of batch experiments (Haghighi-Podeh and Siyahati-Ardakani 2000). Many researchers who conducted experiments at pH of 7–8.5 and at room temperature (25–30 °C) observed cyanide volatilization loss to be less than 1%, for different initial KCN concentrations (Chen et al. 2008). Cyanide had a detrimental effect on the aerobic consortia, although very less in

concentration (2.5 mg/L). Cyanide was oxidized before other organic compounds whose concentrations were 40 times more than cyanide concentration. Thus, the time period for organic degradation was increased, without any change in the organic substrate affinity. Xylenol has been reported to be 32-fold more toxic than phenol (Acuna-Arguelles et al. 2003). Xylenol was found to be very recalcitrant in presence of cyanide and mostly remained in the medium even after 20d as shown in Fig. 7; this shows the combined toxicity of cyanide and organic substrates on the aerobic consortium.

Semple and Cain (1997) observed that xylenol was significantly oxidized only after the consumption of phenols by a pure culture of *O. dancia*. After 12 h, the synthetic wastewater which was colorless initially turned into dark pinkish brown color, representative of actual coke oven wastewater. This color development was mainly due to the oxidation of aromatics like quinoline and indole, as observed by other researchers (Bai et al. 2009, 2010). The mixture of the pollutants without microbial consortia remained visibly colorless for several days and the abiotic loss of each of the compounds was always less than 10%.

In absence of glucose, Phenol and cresol were degraded in less than 96 h in presence of other organic pollutants and were most preferred substrates. Xylenol, on the other hand, with only one methyl group excess to cresol was the most toxic organic compound even compared to the heterocyclic compounds like quinoline and indole. The biomass concentration was found to increase up to 330 mg/L.

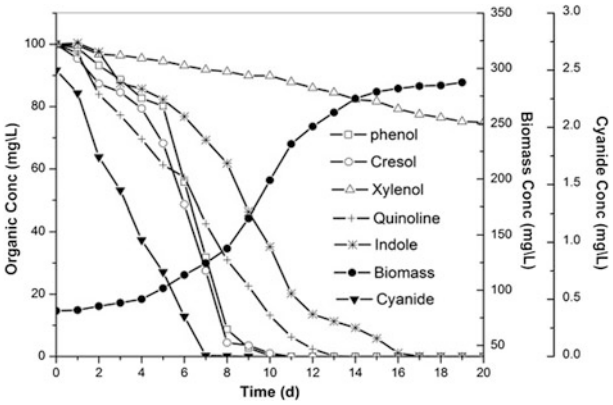

Fig. 7 Mixed substrate biodegradation and biomass growth in presence of glucose (1000 mg/L) and cyanide (2.5 mg/L)

4 Conclusion

Our experiments indicated that in presence of an external carbon source (1000 mg/L) the degradation time for all the pollutant tested was increased, most probably due to additional organic loading and catabolic repression. Xylenol which has been showed to be 32-fold more toxic than phenols was also observed to be the most toxic pollutant to inhibit microbial growth above 200 mg/L, similarly indole beyond 400 mg/L proved inhibitory to aerobic microbes. In the mixed organic study, substrate affinity of aerobic microbes was found to be phenol > cresol > quinoline > indole > xyelnol. Nevertheless, the presence of co-substrate increased the biomass concentration. In presence of cyanide, xylenol was not removed. Thus, the combination of xylenol and cyanide was proved to be fatal to the aerobic microbes by completely arresting the microbial growth and substrate degradation.

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