Degradation Profile of Phenol in Sequential Batch Reactor

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Abstract

Compact well settling granules were cultivated for degrading 3.9 kg m$^{-3}$ d$^{-1}$ of phenols. Formed granules were studied qualitatively using SEM and it was found that a large number of bacterial types including bacterial rod, cocci, diatoms are dispersed in the extracellular polymeric substance (EPS). Degradation studies show that initially the removal rate of phenol was fast but later on it becomes slow. Low concentration of 50 mg l$^{-1}$, 100 mg l$^{-1}$, 200 mg l$^{-1}$ phenol comes down below detection limit in about 170 min of the SBR cycle but high concentration of 400 mg l$^{-1}$, 650 mg l$^{-1}$ took around 240 min for complete removal. This study demonstrates the utilization of aerobic granulation for treating high concentration of phenol and other xenobiotics.

Keywords: Granules, Degradation, Sequential batch reactor, Aerobic sludge.

Introduction

Various industries such as oil refineries, petrochemical plants, coke conversion, pharmaceuticals and resin industries produces many toxic substances as their effluent, phenol being one of it. Phenol is also most frequently found pollutant in rivers and land fill runoff water (Prasad and Ellis, 1978). Phenol concentration of up to 10 000 mg l$^{-1}$ has been reported in much industrial waste water (Fedorak and Hrudey, 1988).

Phenol is toxic at relatively low concentrations and is listed as priority pollutants by the United States Environment Protection Agency (Ghisalba, 1983). The toxicity of phenol often results in the reduction of wastewater biotreatment even at relatively low concentration (Hinteregger et al., 1992).

A wide range of adverse effects have been reported following well documented human exposure to phenol by dermal, oral or intravenous routes. Phenols are also toxic to some aquatic species at low concentration as low as 1 mg l$^{-1}$ (Brown...
et al., 1967) and far lower concentration caused taste and odor problem in drinking water (Rittmann and McCarty, 2001). Hence the removing phenol is significant.

For the removal of aromatic compounds from the groundwater various physical, chemical and biological methods are used (Strier et al., 1980; Atlas et al., 1981; Kobayashi et al., 1982; National Academy of Sciences 1983; Gills et al., 1986). Biological methods have advantage over physical and chemical methods. In chemical and physical methods the intermediate formed are even more toxic than the original compounds (Strier et al., 1980 and Gills et al., 1986). In the case of phenol the use of non biological methods like solvent extraction, mineralization etc. suffers from high cost and formation of hazardous by-products (Loh et al., 2000). Because of lower cost and possibility of complete mineralization and complete oxidation to carbon dioxide and water, biological methods are most commonly used. Phenolic wastewater is treated in activated sludge process. This system is sensitive to high phenol loading rates and to fluctuations in phenol loadings (Kibret et al., 2000 and Watanabe et al., 1999). These difficulties arise because of substrate inhibition, whereby growth (and consequently phenol degradation) is inhibited because of phenol toxicity.

The inhibition difficulties associated with high strength phenolic wastewater can be overcome by strategies such as bioaugmentation (Watanabe et al., 2002) and the other one is cell immobilization (Keweloh et al., 1989). Aerobic granulation technology (a cell immobilization technique) has been developed for treating a wide variety of wastewaters (Peng et al., 1999; Moy et al., 2002; Lin et al., 2003; Yang et al., 2003). This technology is also been extensively investigated (Morgenroth et al., 1997; Beun et al., 1999; Tay et al., 2001; Liu and Tay 2004). Most studies have either focused on granule cultivation through various substrates or treating efficiency of various granule processes (Arrojo et al., 2004; Kim et al., 2004; McSwain et al., 2004; Wang et al., 2004; Hu et al., 2005).

Aerobic granulation represents a cell immobilization technology that has attracted recent research attention (Tay et al., 2001, 2002). Without the inert support for biofilm attachment aerobic granules that are microbial aggregate are cultivated in SBR. The compact microbial structure can confer on aerobic granules the good settleability and the high biodegradation capacity for toxic and recalcitrant compounds (Jiang et al., 2002; Tay et al., 2005a; Yi et al., 2006). Flocculated activated sludge fed with phenol as sole carbon source aerobic granules has been successfully cultivated (Jiang et al., 2002). With the kinetic data it is indicated that phenol degrading aerobic granule have potential to treat wastewater with high phenol loading and also possess highly active compact structure and good settleability.

The main object of this work is to study the aerobic degradation profile of phenol through an aerobic granulation technology by a sequential batch reactor (SBR).

Materials and Methods

Reactor set up and operation

A column type sequential aerobic sludge blanket reactor (5 cm diameter and 150 cm high) made of transparent perfex glass with total volume of 1.5 l (working
volume 1.4 l) was used. The reactor was housed in a thermostat at about 30±2°C. The reactor was fed with phenol as a sole carbon source and was left open for the growth of natural mixed population. At the bottom of column fine bubble aerator was fitted for supplying air at superficial air velocity of 2.67 cm s⁻¹. Reactor was operated sequentially in 4 hrs cycle (2 min of influent filling, 205-230 min of aeration, 2-30 min of settling and 5 min of effluent withdrawal). To avoid excess loss of sludge, the reactor was initially operated at high settling time of 30 min. Later the settling time was reduced to 10 min and finally to 2 min. At the initial stage, feeding time was kept high which was decreased gradually as the operation proceeds. The reactor set-up included two sampling port, arranged along the height of column type reactor, one at a distance of 70 cm and, other at 30 cm from the bottom of the reactor. The effluent was withdrawn through 70 cm port at a volumetric exchange ratio of 50% giving a hydraulic retention time (HRT) of 8 hrs. The HRT of 8 hrs was kept constant during the analysis. Port at 30 cm height was used for the collecting samples for MLSS/MLVSS determinstion and periodical sludge wasting. The operation proceeded with abiotic losses of phenol which were negligible under identical operation condition.

**Seed sludge and wastewater**

The source of aerobic digested sludge with mixed liquor suspended solid (MLSS) and mixed liquor volatile suspended solid (MLVSS) content of 3.5 gm l⁻¹ and 0.43 gm l⁻¹ respectively was taken from secondary clarifier of Star Paper Mills Saharanpur, India. For the period of first 15 days, it was conditioned in an aeration tank where the content was fed with phenol as an only source of carbon along with nutrient (Jiang et al., 2002) and micronutrient (Moy et al., 2002). The acclimatization towards phenol was done in a high precision water bath at a temperature of 30±2°C. After the period of acclimatization, the aeration tank was fed in sequential batch reactor and started with 50 mg l⁻¹ phenol in mineral salt medium which was gradually increased to 650 mg l⁻¹ in a stepwise process.

**Wastewater feed**

A stock solution of phenol was prepared having concentration of 10 gm l⁻¹ (10000 mg l⁻¹). The desired concentrations of phenol for experiments were obtained by proportionate dilution with double distilled water and fed into SBR along with nutrients solution) with following composition (Moy et al., 2002) Table 1. Stock solution of micronutrients was prepared at 1000 times concentration (Table 1). For optimal growth 1 ml of this solution was added to the feed solution. SBR was operated at a constant temperature of 30±2°C and the pH was maintained at around 7.5-7.7 throughout the study.

Microbial granulation depends upon factors such as pH and temperature. A slight alkaline pH (around 7.5) is also necessary for proper aerobic granulation and granulation cease to occur at pH> 8.5 as reported by (Hailei et al., 2006). Yang et al., (2008) reported that a slight low pH favors fungi dominating granules where as a slight alkaline pH favors bacterial granules. Temperature change also affects the reactor performance up to a certain degree. Song et al., (2009) reported that 30°C is optimum temperature for matured granule cultivation.
Table 1 Nutrients composition as given by Moy et al., 2002.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Amount required (gm l⁻¹)</th>
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</thead>
<tbody>
<tr>
<td><strong>Macro Nutrients</strong></td>
<td></td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1.35</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>1.65</td>
</tr>
<tr>
<td>MgSO₄.7H₂O</td>
<td>0.13</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Micro Nutrients</strong></td>
<td></td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>0.05</td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>0.05</td>
</tr>
<tr>
<td>CuCl₂</td>
<td>0.03</td>
</tr>
<tr>
<td>MnSO₄.H₂O</td>
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</tr>
<tr>
<td>Mo₇O₂₄.4H₂O</td>
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<tr>
<td>AlCl₃</td>
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</tr>
<tr>
<td>CoCl₂.6H₂O</td>
<td>0.05</td>
</tr>
<tr>
<td>NiCl</td>
<td>0.05</td>
</tr>
</tbody>
</table>

**Analytical Methods**

Measurement of pH, suspended solids, mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), chemical oxygen demand (COD) were conducted in accordance with the Standard Methods (APHA, 1998). The phenol concentrations were determined spectrophotometrically using the absorbance values at 500 nm λ<sub>max</sub> with a UV/VIS spectrophotometer (GENESYS 20, ThermoSpectronic) according to APHA 1995.

Optical density (O.D.₆₀₀) was calculated using UV/VIS spectrophotometer at 600 nm as specified by Ziagova et al., 2007. O.D.₆₀₀ gives density of the microbes present in the SBR. All the experiments were performed in duplicate.

Morphology and surface structure of granules were observed qualitatively with a scanning electron microscope (STEREO SCAN 360). Granules were prepared for SEM image by washing with a phosphate buffer and fixing with 2% glutaraldehyde overnight at 4°C. Fixed granules were washed with 0.10 M sodium cacodylate buffer, dehydrated by successive passages through 25, 50, 75, 80, 90, 95 and 100% ethanol and dried with a CO₂ Critical Point Dryer.

**Result and Discussion**

Phenols are most common compounds, distributed in waste discharge of pulp and paper industries. The microorganisms are already acclimatized for phenol and its derivatives through natural selection. In our experiment conditioning period was reduced due to already acclimatized sludge. This is the reason for choosing the aerobic sludge from Star Paper Mills Saharanpur, India. However, for getting more specific microbes, the sludge was initially acclimatized for about 15 days to allow biomass to adopt phenol. The SBR cycle was performed 45 days.
Microbial density in SBR system (O.D.\textsubscript{600})

Optical density (O.D.\textsubscript{600}) gives the density of microbes present in the SBR. Figure 2 shows the O.D.\textsubscript{600} of the acclimatized sludge is around 0.63 which increases after inoculating into SBR. This acclimatized sludge shows two sharp decreases on day 13 and day 23 due to reduction settling time from 30 to 10 min and from 10 to 2 min. From first day to day 12, when the settling time was 30 min, O.D.\textsubscript{600} increases from 0.63 to 0.82, but on day 13 when the settling time was reduced to 10 min the O.D.\textsubscript{600} of SBR decreases to 0.69 and then increases gradually up to day 22 with same settling time. There is again a decrease in the in the value of O.D.\textsubscript{600} to 0.98 on day 23 due to reduction in settling time to 2 min and then the value of O.D.\textsubscript{600} increases for rest of the cycle till day 38 and then stabilized at 1.65.

Shape and Size of Microbial Granule

In SBR stable granule were obtained. Stable granules were compact and strong but not much spherical. However, high degradation efficiency of granules is due to their compact structure (Khan et al., 2009) and not effected by their shape. It has also been proved earlier that granules have a high resistant to toxic xenobiotic due to their compact structure (Glancer et al., 1994; Jiang et al., 2002, Tay et al., 2005b, Bergsma-vlami et al., 2005) The diameter of formed granules is about 1-2 mm. Figure 3 shows close packed structures with evenly distributed channels which facilitates movement of waste water inside the granules.
Effluent and Influent concentration

Figure 4 shows concentration graph of effluent and influent with respect to number of days. According to the graph, initially the concentration of effluent is high 27.56 mg l\(^{-1}\) due to the absence of compact granules although the concentration of influent added was quite low 52 mg l\(^{-1}\). The concentration of effluent did not decreases remarkably in the first few days of the SBR cycle but decreases gradually for the rest of the cycle inspite of being increase in the influent concentration from 52 to 656 mg l\(^{-1}\).

Effluent and Influent COD (mg l\(^{-1}\))

Figure 5 shows COD graph of effluent and influent Vs days. This graph shows that initially, the COD of effluent was high 215 mg l\(^{-1}\) even though the COD of influent was 516 mg l\(^{-1}\), because the lack of stability in aerobic granules. After few days of operation, compact granules were formed and the COD of influent was very much high but the effluent COD was low. At the end of cycle, influent and effluent COD were 7418 and 263.31 mg l\(^{-1}\) respectively.

Phenol Removal Profile

The degradation profile shows that initially the degradation of phenol was high later on degradation rate decreases till the end of cycle (Figure 6). This is due to the fact that initially microorganism has large amount phenol available, which is food for them, so they degrade it faster in the beginning. During the first 60 min of 240 min cycle (with 50% volumetric exchange giving 8 hrs HRT) sufficient amount of phenol was removed but in next 180 min its concentration reduces to as low as 2 mg l\(^{-1}\). Figure 4 also shows that concentration of 50, 100 mg l\(^{-1}\) of phenol decreases to below detection limit in just 150 min but high concentration of phenol (200, 400, 650 mg l\(^{-1}\)) took around 220-230 min for complete removal. Just after addition of influent, a sharp
A decrease in phenol concentration was observed (as shown in Figure 6 first 20 min), this is due to dilution.

![SEM image of mature granule at 15000 magnifications.](image)

Figure 3. SEM image of mature granule at 15000 magnifications.

![Concentration of Effluent and Influent VS time.](image)

Figure 4 Concentration of Effluent and Influent VS time.

**COD Removal Profile**

Similarly the removal rate of COD was high in the beginning but later on it goes on decreasing. Just after addition of influent in the SBR there is sharp decrease in COD which is attributed to dilution but this value of COD further increases to give COD concentration below 50 mg l\(^{-1}\) by microbial action (Figure 7).
COD Removal Efficiency with Phenol Concentration

Figure 8 illustrates that COD removal efficiency increases with phenol concentration and attains maxima (94.54%) at 600 mg l\(^{-1}\). This is because at high concentration of phenol, influent COD was itself very high hence high removal efficiencies were achieved by using the following formula

\[
\text{% COD removal} = \left(\frac{\text{Influent COD} - \text{Effluent COD}}{\text{Influent COD}}\right) \times 100
\]

However, at very high concentration of phenol, removal efficiency decreases owing to microbial inhibition caused by high substrate concentration.

Biomass Concentration (MLSS and MLVSS)

The initial sludge has MLSS concentration of 3.5 gm l\(^{-1}\) and MLVSS of 0.43 gm l\(^{-1}\). After conditioning period, MLSS increases 5.2 gm l\(^{-1}\) and MLVSS to 0.82 gm l\(^{-1}\). Then the sludge was inoculated in to SBR for the cultivation of aerobic granules. Aerobic granules developed along with amorphous flocs were first observed after 10 days of inoculation. However the biomass concentration of MLSS and MLVSS reduces to 3.5 gm l\(^{-1}\) and 0.94 gm l\(^{-1}\) due to reduced settling time from 30 min to 10 min. The biomass with small settling velocity had been washed out of the reactor. Small granules grew rapidly in subsequent week, while more floc like sludge washed out from reactor gradually. The MLSS and MLVSS concentrations increases till 5.6 gm l\(^{-1}\) and 2.1 gm l\(^{-1}\) and again shows a sharp decrease, due to decrease in settling time from 10 to 2 min on day 23. The granules become dominant form of biomass as evident from gradual increase in biomass concentration beyond day 24. MLSS and MLVSS concentration finally shows an upward trend and stabilizes at 7.3 gm l\(^{-1}\) and 4.1 gm l\(^{-1}\) (Figure 9).
Figure 6 Concentration profile of phenol during single cycle.

Figure 7 COD removal profile of phenol during single cycle.
Figure 8 COD removal efficiency VS phenol concentration.

Figure 9 Variation of MLSS and MLVSS concentration of Phenol during its degradation

Conclusion

This study illustrate that compact aerobic granule cultivated in SBR can tolerate fluctuation in COD load of 1000 mg l⁻¹ and higher COD load of 7500 mg l⁻¹. Optical density (O.D. 600) of SBR system was stabilized at 1.65 which shows
maximum retention of biomass. The concentration and COD of influent and effluent shows that after the formation of compact granule phenol degradation was more efficient. The COD removal efficiency was above 94%. Degradation profile illustrates that during first hour of cycle removal rate of phenol and COD were very fast but later on becomes slow. MLSS and MLVSS stabilized at 7.3 g m⁻¹ and 4.1 g m⁻¹. The process appears very useful for treating waste water containing phenols and other toxic xenobiotics. This technology is advantageous as the same biomass can be used over a long period. This technology can withstand high organic load as well as fluctuation in organic load so it can be exploited for treating high strength municipal and industrial waste both.

Acknowledgments

Authors wish to thank Prof. (Mrs.) A. Lal, Chairperson, Department of Chemistry for providing necessary research facilities. F.K. and M.Z.K. thank UGC for providing fellowship.

Authors' contributions: Farah Khan performed experiment, wrote the manuscript and performed calculations; and Mohammad Zain Khan performed a portion of experiment and helped in the calculations and Dr. Suhail Sabir (Associate Professor), contributed in experiment design and final editing of the manuscript;

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