OPTIMIZATION CONDITION OF BIO-PROCESS FOR PHENOL DEGRADATION IN OIL REFINERY WASTEWATER

Erni Martani¹, Sri Astuti Rahayu², Bardi Marachman³, and Noegroho Hadi Hs.²

ABSTRACT


Imperfect bio-process of oil refinery wastewater will cause environmental pollution due to the high concentration of phenol and ammonia. Therefore, this study was done to investigate possible alternatives in improving the bio-processing unit.

The oil refinery wastewater sample was taken from inlet of wastewater treatment of an oil industry in West Java. Several treatments in bio-process were conducted by adjusting aeration flow rate, N/P ratio (by urea addition), bacterial inoculation, and their variation. An optimal condition for bio-process was based especially on the phenol degradation. Ammonia content and the COD level during incubation period were analysed as supporting data.

Chemical analyses of the wastewater sample showed that BOD, COD, phenol, and ammonia concentration were higher than allowed concentration in oil refinery wastewater. Microbial examination indicated that Pseudomonas and Bacillus were dominating the wastewater. Therefore, they were chosen as bacterial inoculants. The experiments showed that phenol degradation was enhanced by aeration, and it was greatest in flow rate of 20 L/min. Complete phenol degradation was observed in this flow rate within 48 hours. This optimal flow rate was used in combination treatment with nitrogen addition and bacterial inoculation. Based on phenol degradation, single aeration treatment at 20 L/min was the best compared to other treatments. However, complete phenol degradation rate was not always followed by COD and ammonia reduction. Studies are still required to get a better quality of wastewater based on several environmental quality parameters.

Key words: oil refinery wastewater, bio-process, optimization, phenol degradation.

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INTISARI

Erni Martani, Sri Astuti Rahayu, Bardi Murachman, and Neograho Hadin Has "Optimal kondisi bioproses untuk degradasi fenol dalam limbah cair industri minyak bumi. Biotek. 21(9): 553-565.

Bioproses yang tidak sempurna dalam penanganan limbah industri pengolalan minyak bumi dapat menyebabkan pencemaran lingkungan akibat tingginya kandungan pencemar, seperti fenol, amoniak dan BOD-COD. Penelitian ini dilakukan untuk mengamati gejala dan bioproses untuk memperbaiki kualitas limbah industri pengolalan minyak bumi.


Kata kunci: limbah cair, kilang minyak, bioproses, optimal, degradasi fenol.

INTRODUCTION

Oil refinery wastewater contains organic and inorganic substances potential for causing pollution, such as: phenol and ammonia. Threshold odor for phenol concentration in water is 1.0 mg/l, but 5 mg of phenol/l caused changing in color and odor of water (Fawell & Hunt, 1988). At 1-10 mg/l, phenol was toxic to fish (Anonymous, 1975). Phenol consumption in rats and mice caused carcinogenicity and reproductive toxicity (Fawell & Hunt, 1988). Phenol concentration in oil refinery wastewater is depend on the process of each industry. The more complex of process, the higher phenol concentration in the wastewater (Uditharbo & Neograho-Hadin, 1987). Many studies were done concerning degradation of phenol or phenolic compounds. Most information stated that degradation of aromatic compounds, including phenols, was more rapid in aerobic condition (Alexander, 1994; Atlas & Bartha, 1993). Moreover, Stah & Gibson (1988) showed that substituted phenols were degraded aerobically by Pseudomonas putida F1 and Pseudomonas sp. strain J80. However, recent studies showed that bacteria which function under anaerobic condition are frequently high versatile. They destroyed variety of compounds, such as phenolic compounds, benzene, aromatic hydrocarbons, chloroalkanes and alkenes (Alexander, 1994). Although hexa-chlorocyclohexane was converted in both with or without O2, anaerobic conversion was more rapid (Alexander, 1994).

Phenolic compounds were degraded by bacteria of Pseudomonas sp. (Alexander, 1994; Bandhopadhyay et al., 1988). This bacteria also degraded petroleum hydrocarbons (Martani & Ijutono, 1984) and chlorophenolic compounds (Spain & Gibson, 1988). Other phenol degrading bacteria were: Achromobacter, Acinetobacter, Arthrobacter, Bacillus, Corynebacterium, Flavobacterium, and Mycobacterium (Alexander, 1994). Most of those bacteria were aerobic or facultative anaerob. Naturally, degradation of pollutants in wastewater or other polluted environments is always done by mixed microbial communities. The types of interaction among those communities which resulted in the pollutant degradation is still unclear. Co-metabolism and synergism are often play an important role in pollutant degradation (Atlas & Bartha, 1993; Martani & Seto, 1991).

In spite of the degrader, phenol degradation in oil refinery wastewater was also affected by available nutrients, such as C sources other than phenol itself, also N and P compounds. The optimal C (as BOD)/N/P ratio in wastewater treatments was 100:5:1 (Anonymous, 1975). Due to the high concentration of available C source, Alexander (1994) assumed
that in oil refinery wastewater treatment, petroleum degradation was depend on optimal N/P ratio, that was 5:1. To meet optimal condition, sometimes urea was amended into wastewater treatment as additional N source (Martani et al., 1999).

This study was done to investigate optimal condition to improve quality of oil refinery wastewater, especially on phenol concentration. The effect of aeration and other treatments, including nutrient amendment, bacterial inoculation, and their variations were conducted in respect to the phenol degradation in oil refinery wastewater sample.

MATERIALS AND METHODS.

1. Oil refinery wastewater sample was obtained from an oil refinery industry located in West Java. The important physical, chemical and biological characters of the wastewater were analyzed using standard methods for each component.

2. Isolation and identification of bacteria. Surface plating methods on Nutrient Agar were done for bacterial isolation. The pure culture of isolates were kept on Nutrient Agar slants and examined each morphological and biochemical characteristic.

3. Aeration treatments. A series of variation in aeration (0, 5, 10, 15 and 20 l/min) was conducted into the wastewater sample. Optimal flow rate for aeration was based on residual phenol concentration during incubation time.

4. Nutrient amendments. When optimal flow rate for phenol degradation was obtained (3), the wastewater was amended with nutrients, so that some specific N/P ratios were found. In this research three N/P ratios, 10:1, 10:2 and 10:3, were used. As in experiment 3, optimal nutrient amendments was based on the phenol degradation in the wastewater.

5. Bacterial inoculation. Finally, the wastewater was inoculated with pure culture or mixed cultures of selected bacterial isolates. It was incubated at optimal flow rate (3) and/or nutrient amendment (4). Each treatment was measured its effect on phenol degradation in the wastewater sample.

Decreasing COD, ammonia concentration and bacterial growth were measured as supporting data.

RESULTS AND DISCUSSIONS

Chemical analysis of wastewater sample.

<table>
<thead>
<tr>
<th>No</th>
<th>Parameter</th>
<th>Oil refinery wastewater (inlet)</th>
<th>Kep. No 42/MENTAL/H/10/1996</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pH</td>
<td>9.8</td>
<td>6.0 - 9.0</td>
</tr>
<tr>
<td>2</td>
<td>IOD$_5$</td>
<td>459</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>COD</td>
<td>947</td>
<td>200</td>
</tr>
<tr>
<td>4</td>
<td>Phenol</td>
<td>325</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>Oil &amp; fat</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>6</td>
<td>Ammonia</td>
<td>42.25</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>Dissolved O$_2$</td>
<td>0.7</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>Total bacteria (cell/ml)</td>
<td>1.8 x 10$^8$</td>
<td>not written</td>
</tr>
</tbody>
</table>

Note: *Not analyzed; *Not written.

Table 1 showed characters of wastewater sample and quality standard of important parameters of oil refinery wastewater. A proper treatment of oil refinery wastewater is required, especially in the case of phenol concentration, BOD, COD, and ammonia concentration. Biochemical characters (fermentation of carbohydrates, O-F test, Catalation, O$_2$ requirement, etc.), these isolates were identified as the genus of Alcaligenes, Bacillus, Chromobacterium, Neisseria, Pseudomonas, and Streptococcus (Table 2).
**Table 2. Bacteria isolated from the wastewater sample**

<table>
<thead>
<tr>
<th>No.</th>
<th>Genera</th>
<th>Total strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Pseudomonas</em></td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td><em>Bacillus</em></td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td><em>Alcaligenes</em></td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td><em>Chromobacterium</em></td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td><em>Streptococcus</em></td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td><em>Neisseria</em></td>
<td>2</td>
</tr>
<tr>
<td>Total isolated</td>
<td>48</td>
<td></td>
</tr>
</tbody>
</table>

Results shown in Table 2 were supported by previous studies which showed that *Pseudomonas* spp. dominated the microbial population in oil refinery wastewater treatments (Udiharto & Noegroho Hadi, 1987). *Pseudomonas* sp. is ubiquitous bacteria (Atlas & Bartha, 1993), which degrade many kinds of organic pollutants including phenolic compounds (Bandyopadhyay et al., 1998; Spain & Gibson, 1988; Udiharto & Noegroho Hadi, 1987), petroleum hydrocarbons (Martani & Jutono, 1984; Whyte et al., 1997), and also some chlorinated organic compounds (Atlas & Bartha, 1993).

Another genus which usually found in the wastewater was *Bacillus* (Udiharto & Noegroho Hadi, 1987). Therefore, a strain of *Pseudomonas* and *Bacillus* were chosen as bacterial inoculant. Strain selection was based on its growth rate in simulated wastewater (data not shown). The selected strains were *Pseudomonas* sp. PP-9 and *Bacillus* sp. PB-5.

**Effect of aeration on phenol degradation.**

In the experiment using aeration flow rate of 0 - 20 l/min, aeration increased phenol degradation (Fig. 1). Rapid degradation was observed especially when aeration flow rate was enhanced from 0 to 5, 10, and 15 l/min. Although enhancement of phenol degradation was slower in the increasing aeration from 15 to 20 l/min (from 36.5 to 41.1 %), the highest phenol degradation was found at flow rate of 20 l/min.

![Figure 1. Effect of aeration on phenol degradation within 24 hours.](image)

**These results showed that in the wastewater, oxygen was required for phenol degradation. It was supported by Alexander (1994) and Atlas & Bartha (1993), who stated that aromatic degradation was more rapid in aerobic condition. Cleavage of aromatic rings required the presence of oxygenases (Atlas & Bartha, 1993). Based on these data, it was decided that variation of aeration would be focused between 15 to 20 l/min. The conducted flow rates were 15, 18 and 20 l/min, and incubation time was prolonged to 72 hours. The results of phenol degradation were shown in Fig. 2.**

Within 72 hours, phenol residue was still high in the non-aerated sample. Higher aeration enhanced phenol degradation. Within 48 hours, complete degradation was observed in sample aerated at 20 l/min. Complete phenol degradation in samples aerated at 15 and 18 l/min was observed after 72 hours. These results showed that higher aeration accelerated process for complete phenol degradation. In this study, phenol degradation was maximum in aeration of 20 l/min. Although the acceleration of phenol degradation from flow rate of 20 to 18 l/min only 24 h, it enhanced the efficiency of wastewater treatment.

![Figure 2. Effect of aeration on the degradation of phenol in the wastewater sample](image)

In-spite of phenol degradation, bacterial growth were also measured. Significant growth was detected between 24 to 48 hour incubation, especially in aerated sample (Fig. 3). They increased from 10^6 to 10^7 cell/ml. These results were coincided with Fig. 2, in which phenol degradation in aerated samples was detected especially after 24 hours. These data showed that phenol was degraded by indigenous microorganisms in wastewater sample, and the degradation activity was accelerated.
by aeration, Martani & Seto (1991), showed that degradation of organic compounds was started when the population of the bacterial degrader reached to $10^4 - 10^5$ cell/ml depended on the chemical concentration.

Although no phenol degradation was observed in non-aerated sample (Fig. 2), the bacteria still grew to $10^6$ cell/ml in 72 hour incubation (Fig. 3). It was suggested that they grew by utilizing C-sources other than phenol.

Available nutrients affected the rate of degradation (Alexander, 1994; Anonymous, 1975; Martani & Seto, 1991). In this study, urea was added into the sample so that the N/P ratio in the samples reached around 10:1; 10:2; and 10:3. Results showed that additional urea nutrition affected phenol degradation in both aerated and non-aerated wastewater samples (Fig. 4). The most rapid phenol degradation was detected in non-aerated sample with N/P ratio of 10:1, in which 97.2% of phenol was degraded within 72 hours. In the cases of N/P ratio 10:2 and 10:3, phenol residues were still above 65%. No significance increase of phenol degradation in N/P ratio 10:3 compared with the control. In aerated samples (Fig. 4 B), the phenol degradation was highest in sample with no additional nutrition, followed by urea-added samples of 10:1, 10:2 and then 10:3. These results indicated that natural chemical contents in wastewater was high enough to support phenol degradation by aerobic indigenous microorganisms. The differences among aerated and non-aerated samples might be due to species differences of active microorganisms in both samples. Alexander (1994) stated that many anaerobic bacteria are frequently highly versatile, and they destroy a variety of organic compounds, including phenolic compounds. These anaerobic bacteria required additional nutrients.

*Pseudomonas* sp. PP-9, Bacillus sp. PB-5, and their mixture were inoculated into non-sterilized samples with initial population $10^4 - 10^5$ cell/ml. There was no significant bacterial growth within 72 hours (data not shown), which might be due to the balance between available C-sources in the wastewater and the bacterial population level. Martani & Seto (1991) showed that when *Pseudomonas* sp. E6 was inoculated into a groundwater sample at the levels lower than $10^5$ cell/ml, it grew significantly and degraded 0.1 ppm of 2,4-DCP. However, when it was inoculated at $10^5$ cell/ml, although they degraded 2,4-DCP, no significant growth was detected.

Figure 5 shows that phenol was degraded completely within 48 hours in non-inoculated sample. Inoculation of *Pseudomonas* sp. PP-9 or Bacillus sp. PB-5 or mixed culture of them could not increase phenol degradation. Complete degradation was detected at 72 hours in mixed culture inoculated sample. Competition between indigenous and the inoculated bacteria to gain available nutrients in wastewater may have responsibility to the long phenol complete degradation (Martani & Seto, 1991). Theoretically, bio-remediation of microbial inoculation was done when there was scarcity of microbial degrader in.
a natural environment (Alexander, 1994). In that case, inoculation will increase pollutant degradation (Martini & Seto, 1991). In this study, indigenous bacteria were relatively high enough (10^6 cell/ml, Table 1) and they did degrade phenol in aerated samples (Fig. 3). Therefore, bacterial inoculation did not affect phenol degradation (Fig 5).

Figure 5. Effect of bacterial inoculation on phenol degradation in aerated wastewater sample at 20 l/min.

Phenol = inoculated with Pseudomonas sp.; Bacill = inoculated with Bacillus sp.; Mixed = inoculated with mixed cultures; Control = not inoculated

Figure 6 shows the changes of COD and ammonia during incubation time. Both of them decreased in all samples. The highest decreasing of COD was detected in mixed cultures inoculated-aerated sample, in which COD level was 87 mg/l (Fig. 6 A). Within 72 hours, COD level was highest in control sample. These data showed that ration, nutrient addition and bacterial inoculation enhanced reduction of COD. However, except of mixed culture-inoculated sample, the COD levels were still higher than its maximal level based on Quality Standard (Table 1). In spite of carbon assimilation, organic degradation may lead through co-metabolism or preference, which require the presence of degradable compounds (Atlas & Bartha, 1993; Martini & Seto, 1991). In this wastewater sample, many C sources other than phenol were present (Table 1).

Similar evidences were found in ammonia contents (Fig. 6 B). No significance differences between treated and non-treated samples. Ammonia residues were still around 23 - 25 mg/l. These levels were more than twice of maximal standard values. Failure of microbial inoculation to degrade ammonia might be due to the failure of indigenous nor inoculated bacteria to transform ammonia.

CONCLUSIONS

1. Phenol was degraded completely in oil refinery wastewater when it was treated with aeration of 20 l/min, with or without nutrition addition, or mixed culture inoculation. The most rapid degradation was observed in sample treated with aeration only.

Ammonia in the wastewater was derived from N-organic decomposition, and then it was biologically transformed to nitrates through nitrification (Argaman, 1991). It was suggested that in this wastewater sample, the indigenous nor inoculated bacteria could not transform ammonia to nitrates. In this study nitrification and denitrification activities of bacterial isolates was not detected. However, some species of Pseudomonas have denitrification activity (Buchanan & Gibbons, 1993). These activity may has responsibility to enhance ammonia concentration.
2. Complete degradation of phenol did not always followed by the decreasing of other pollutants, such as COD and ammonia.

REFERENCES


