A comprehensive evaluation of re-circulated bio-filter as a pretreatment process for petroleum refinery wastewater

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ABSTRACT

Conventional biological treatment process is not very efficient for the treatment of petroleum refinery wastewater (PRW) that contains high-concentration of organic contaminants. Prior to biological treatment, an additional pretreatment process for PRW is required for the effluent to meet the discharge standards. While re-circulated bio-filter (RBF) has been applied as a pretreatment process in several PRW treatment plants, its effects have not been comprehensively evaluated. In this study, the parameters of operation, the changes in pollution indexes and contaminant composition in an engineered RBF have been investigated. We found that mainly highly active de-carbonization bacteria were present in the RBF, while no nitrification bacteria were found in the RBF. This indicated the absence of nitrification in this process. The biodegradable organic contaminants were susceptible to degradation by RBF, which decreased the Biological Oxygen Demand (BOD5) by 83.64% and the Chemical Oxygen Demand (CODCr) by 54.63%. Consequently, the alkalinity and pH value of RBF effluent significantly increased, which was unfavorable for the control of operating parameters in subsequent biological treatment. Along with the decrease of CODCr, the RBF effluent exhibited a reduction in biodegradability. 834 kinds of recalcitrant polar organic contaminants remained in the effluent; most of the contaminant molecules having complex structures of aromatic, polycyclic and heterocyclic rings. The results of this study showed that RBF could efficiently treat PRW for biodegradable organic contaminants removal; however, it is difficult to treat bio-refractory organic contaminants, which was unfavorable for the subsequent biological treatment process operation. An improved process might provide overall guarantees for the PRW treatment.

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INTRODUCTION

Large quantities of wastewater are generated from petroleum refining industries (Freeman, 1995; Yavuz et al., 2010). The typical refractory compounds in wastewater mainly include polynuclear aromatic hydrocarbons (PAHs), long-chain hydrocarbons and nitrogenous heterocyclic compounds, which have been reported to be highly toxic and persistent in the environment (Shariati et al., 2011; Stepnowski et al., 2002). Therefore, Petroleum Refinery Wastewater (PRW) is often toxic and a major environmental pollution source, requiring an adequate treatment before being discharged into water bodies.

Now, wastewater treatment plants are facing new challenges to meet the stricter discharge criterion. The conventional treatment process of PRW involves a series of physic-chemical operations followed by combined biological treatment. However,
it is difficult to effectively treat the wastewater by the process in order to meet the strict discharge standards (Ji et al., 2009; Marañón et al., 2008; Zhao et al., 2010). Therefore, an improved process need to be strongly explored by refinery wastewater treatment plants throughout the world. Recently, re-circulated bio-filter (RBF), as an additional pretreatment process for the biological treatment of PRW, has been considered. RBF has the potential to remove organic contaminants by physical processes (e.g., filtration and sedimentation), chemical adsorption, and biological processes.

RBF has received considerable attention in the treatment of domestic wastewater (Elliott, 2001; Hu and Gagnon, 2006; Roy and Dube, 1994). Leverenz et al. (2000) employed RBF for treatment of septic tank effluent, and achieved 97% and 100% removal of Biological Oxygen Demand (BOD$_5$) and total suspended solids (TSS), respectively. In addition, Lindbo (2004) also employed RBF for treatment of domestic wastewater, reported an average BOD$_5$, Chemical Oxygen Demand (COD$_{Cr}$) and TSS removals of 96%, 80% and 93%, respectively. Although RBF has been employed as a pretreatment process in several PRW treatment plants, its effects have not been comprehensively evaluated.

The aim of the present study was to assess the pollutant removal efficiency of engineered RBF for pretreatment of PRW. To get a better insight into the change in the organic components in the wastewater before and after treatment, Gas chromatography mass spectrometry (GC–MS) and Fourier transform ion cyclotron resonance mass spectrometry (FT–ICR MS) were used. The polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) technique and sequencing analysis were used to identify the dominant microorganisms in a running RBF.

### 1. Materials and methods

#### 1.1. Wastewater

PRW was obtained from the inlet and outlet of RBF unit during PRW treatment process at Huizhou Refinery (Guangdong, China). All the chemical agents were purchased from Reagents Company, China.

#### 1.2. RBF operation parameters

The operation parameters of RBF investigated in the study, included hydraulic retention time (HRT), recirculation ratio, and dissolved oxygen (DO), pH, alkalinity, organic load and sludge retention time (SRT). The parameters are shown in Table 1. Most of the practice parameters were consistent with design parameters. The influence of unmatched parameters will be discussed in another section.

#### 1.3. Wastewater quality analysis

The 5-day BOD$_5$ was measured according to the Chinese standards HJ 505-2009. The COD was determined by the potassium dichromate oxidation method (GB/T 11914-1989). Alkalinity was determined by a national standard method (GB/T 11911-1989). The 1 mL wastewater samples were added with 20 μL 30% (v/v) NH$_4$OH to accelerate the deprotonation of acidic compounds in ion ESI. The mass spectrum was overlapped by 64 scan spectra in order to reduce S/N and increase resolution. Moreover, 1 mL wastewater samples were added with 20 μL 30% (v/v) NH$_4$OH to accelerate the deprotonation of acidic compounds in ion ESI. The data was obtained on the software of Xmass version 6.0 (Bruker Daltonics, USA) and analyzed by the Kendrick method.

#### 1.4. GC–MS analysis

GC–MS was used to analyze the organic compounds in PRW on a Thermo-Finnigan SSQ710 GC/MS with HP-5MS elastic silica capillary columns (60 m × 0.25 mm × 0.25 mm). The pretreated samples (1 mL) were analyzed by GC/MS. Pure helium at 37 kPa was used as the carrier gas at a flow velocity of 1 mL/min. The analytical conditions were as follows: initial temperature of 50°C, with isothermal operation for 1 min; heating to 120°C at a constant rate of 20°C/min; and heating to a final temperature of 310°C at a constant rate of 4°C/min, with a 30 min isothermal. Mass spectrometer conditions were: ionization mode, EI; electron energy, 70 eV; filament current, 100 μA; multiplier voltage, 1200 V; full scan.

#### 1.5. ESI FT–ICR MS analysis

Electrospray Ionization-Fourier Transform-Ion Cyclotron Resonance-Mass Spectrometry (ESI FT-ICR MS) was used to quantitatively analyze PRW (Hughey et al., 2007), which was operated in the negative-ion mode on an Apex-ultra FT-ICRMS (Bruker Daltonics, USA) with a 9.4 T actively shielded magnet. Ions were generated from a micro-electrospray source equipped with a 50 μm i.d. fused silica-capillary. The operation conditions were as follows: flow rate of PRW pretreated samples 180 μL/hr, mass to charge ratio (m/z) scanning 115–1000 and signal to noise (S/N) > 4. Each mass spectrum was overlapped by 64 scan spectra in order to reduce S/N and increase resolution. Moreover, 1 mL wastewater samples were added with 20 μL 30% (v/v) NH$_4$OH to accelerate the deprotonation of acidic compounds in ion ESI.

#### 1.6. DNA extraction

Genomic DNA was extracted from 0.25 g activated sludge as described in the manufacturer’s manual of the genomic extraction kit (MOBIO, Carlsbad, CA, US) and was detected by 0.8% (w/v) agarose gel electrophoresis.

#### 1.7. PCR-DGGE analysis

The extracted DNA was amplified by PCR in a peqSTAR 96 universal thermo-cycler (PEQLAB Biotechnology, Germany).

### Table 1 – Operation parameters of RBF.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Design value</th>
<th>Operation value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydraulic retention time (hr)</td>
<td>–</td>
<td>14</td>
</tr>
<tr>
<td>Recirculation ratio (%)</td>
<td>20–30</td>
<td>20</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/L)</td>
<td>2–5</td>
<td>3.4</td>
</tr>
<tr>
<td>pH</td>
<td>–</td>
<td>8.2</td>
</tr>
<tr>
<td>Alkalinity (mg/L as CaCO$_3$)</td>
<td>–</td>
<td>799.35</td>
</tr>
<tr>
<td>Organic load (kg COD/(m$^3$·day))</td>
<td>–</td>
<td>1.667</td>
</tr>
<tr>
<td>Sludge retention time (day)</td>
<td>15</td>
<td>12</td>
</tr>
</tbody>
</table>

RBF: re-circulated bio-filter.

T16488-1996) and the pH was measured by a pH meter (pH-3D, Leici Corporation, China).
One primer pair of variable region V3 of the 16S rRNA gene, 357F (5′-CCTACGGGAGGCAGCAG-3′) and 518R (5′-ATTACCGCGGCTGCTGG-3′) was used. The PCR amplification was performed as follows: 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 55°C for 30 sec and 72°C for 30 min and final extension at 72°C for 10 min. For DGGE analysis, DNA fragments were separated on 7% polyacrylamide gels with a 50%–100% linear gradient of denaturant. The gel was conducted at 60°C, 120 V for 12 hr on Dcode Universal Mutation Detection System (Bio-Rad, US). After electrophoresis, the gel was stained using EB-staining method followed by visualizing on Gel Doc XR system (Bio-Rad, US). Specific gel bands were excised and dissolved in 100 μL sterile water at 4°C overnight. 1 μL solution as DNA template was reamplified using the primers 357F/518R. The DNA fragments were cloned by pMD19-T plasmid vector system (TaKaRa, Japan) and transferred into competent Escherichia coli DH5a (TaKaRa, Japan). The positive colonies of each sample were sequenced by a commercial service (BGI, China).

2. Results and discussions

2.1. Wastewater characteristics

The PRW quality was evaluated by determining the COD<sub>Cr</sub>, BOD<sub>s</sub>, alkalinity, pH and other physical and chemical characteristics. The results are shown in Table 2. The COD<sub>Cr</sub> was 2554 mg/L, indicating a characteristic of high-concentration organic components. The BOD<sub>s</sub>/COD<sub>Cr</sub> (B/C) was 0.469, meaning the substrates in the wastewater were biodegradable. The ratio of C:N:P was 100:5.466:0.183, with the value for P below the conventional treatment standard of 100:5:1. On the basis of the wastewater quality analysis, it could be inferred that the PRW that contains high-concentration organic contaminants cannot be efficiently treated by conventional biological process.

2.2. Treatment efficiency in RBF system

The investigation was carried out for 25 days and the COD<sub>Cr</sub>, BOD<sub>s</sub>, BOD<sub>s</sub>/COD<sub>Cr</sub> (B/C), pH and alkalinity variation of PRW were used to assess the operation performance of RBF. The results are shown in Fig. 1. The final COD<sub>Cr</sub> of the effluent decreased to around 1159 mg/L (mean value) after the RBF treatment, showing that the COD<sub>Cr</sub> of the wastewater was removed about 54.63%. After the RBF treatment, the BOD<sub>s</sub> of the wastewater declined to 196 mg/L, showing that the BOD<sub>s</sub> of the wastewater was removed about 83.64%. The data reveal that, even though BOD<sub>s</sub> can be removed efficiently from the wastewater, the COD<sub>Cr</sub> value still remains high. This may be due to the presence of refractory compounds such as PAHs. Considering the change of COD<sub>Cr</sub> and BOD<sub>s</sub>, it also could be inferred that RBF has a strong ability to remove biodegradable organic contaminants. The pH increased to almost 8.2 from 7.6 after the RBF treatment. Consequently, the alkalinity increased to almost 800 mg/L (calculated as CaCO₃). The alkalinity and pH values of RBF effluent significantly increased, besides, the effluent of RBF exhibited a decreased.

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**Table 2 – Characteristics of PRW.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>COD&lt;sub&gt;Cr&lt;/sub&gt; (mg/L)</th>
<th>BOD&lt;sub&gt;s&lt;/sub&gt; (mg/L)</th>
<th>pH</th>
<th>ALK (mg/L as CaCO₃)</th>
<th>TN (mg/L)</th>
<th>TP (mg/L)</th>
<th>Oil (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>2554</td>
<td>1198</td>
<td>7.6</td>
<td>672.67</td>
<td>139.6</td>
<td>4.67</td>
<td>1.85</td>
</tr>
</tbody>
</table>

COD<sub>Cr</sub>: Chemical Oxygen Demand; BOD<sub>s</sub>: Biological Oxygen Demand.

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**Fig. 1 – COD<sub>Cr</sub>, BOD<sub>s</sub>, B/C, pH and alkalinity changes in the RBF. COD<sub>Cr</sub>: Chemical Oxygen Demand; BOD<sub>s</sub>: Biological Oxygen Demand; B/C: BOD<sub>s</sub>/COD<sub>Cr</sub>; RBF: re-circulated bio-filter.**
biodegradability (BOD₅/CODₐ from 0.531 to 0.169), resulted by the reduction in BOD₅, which all were unfavorable to the control of operating parameters in subsequent biological treatment.

2.3. GC–MS analysis

The organic components in RBF influent and effluent were analyzed by GC–MS and the results are shown in Fig. 2. The detected organic compounds can be categorized as eight major categories including organic acids, esters, alcohol, heterocyclic compounds, alkanes, aromatic, hydrocarbons, aldehydes and ketones and phenols (Table 3). Obviously, organic acids were the most dominant category accounting for 50.94% of the total number of compounds in the influent. After RBF treatment, phenols were absent in the effluent, suggesting that they were effectively degraded by the microorganisms in RBF (Tong et al., 2013). The category of esters (C₉–C₂₀), accounting for 39.72% of the total number of compounds, were found to be most dominant fraction. Small molecules of organic acids, aldehydes, ketones, phenols and other biodegradable organic contaminants were found to be effectively degraded by microorganisms (Tong et al., 2013), which was consistent with the increase in alkalinity and pH value, and decrease in B/C. Besides, the relative abundance of heterocyclic compounds and aromatic hydrocarbons relative increased during the treatment, possibly resulted by the reduction of other organic compounds. The results showed that the compositions of the effluent might be more complex and bio-refractory (Wang et al., 2000).

2.4. FT-ICR MS analysis

ESI FT-ICR MS is known for ultra-high resolution power and ultra accurate mass determination of many compounds in complex mixtures and has been widely applied (Lu et al., 2010). In this case, negative-ion ESI FT-ICR MS analysis was applied to the characterization of polar compounds of RBF influent and effluent and the results are presented in Fig. 3. In each spectrum, most peaks were assigned a unique molecular formula. These formulas could help identify the relationships among peaks between different samples (Mesfioui et al., 2012). These formulas finally could be grouped into CHO-containing molecules, CHNO-containing molecules, CHNOS-containing molecules, CHSO-containing molecules, CHS-containing molecules,
and CHNS-containing molecules. As shown as in Fig. 4, the number of peaks in all formulas dropped to 834 from 990 after the RBF treatment, however, the groups of all formulas was hardly altered. Obviously, CHO-formula was both the predominant formula both before and after RBF treatment. The total number of CHO-formula in PRW decreased from 417 to 387 after the RBF treatment, however, the relative abundance of CHO-formula increased. This change indicated that RBF selectively oxidized lower molecular weight compounds to O-compounds (such as acidic compounds) or mineralized.

Important graphic information of the complex FT-ICR mass spectral data are modified by the van Krevelen diagrams (Fig. 5). The H/C ratio separates compounds according to the degree of saturation, whereas O/C ratios separate according to O classes. Data points corresponding to compounds in different alkylation series appear along lines that intersect at H/C = 2 on the y-axis (Fig. 5) (Wu et al., 2004). Furthermore, among compounds in the same class, those with same number of carbons have identical O/C values. For those species, as the number of rings and double bonds increases, the H/C value decreases to generate a series of data points falling on a single vertical line (Fig. 5). Such plots make it easy to recognize changes in chemical structure of organic compounds (Mesfioui et al., 2012).

Obviously, the peaks in O1 class disappeared in RBF effluent, which was consistent with the fact that the peaks number decreased and phenols disappeared. Furthermore, the number of peaks in O2, O3 and O4 classes was all almost constant during the treatment, showing that the recalcitrant polar organic contaminants remained in the wastewater; most of the contaminant molecules having complex structures of aromatic, polycyclic and heterocyclic rings. ESI FT-ICR

![Fig. 4 – Number and relative abundance of polar compounds in RBF influent and effluent in negative-ion mode.](image)

![Fig. 5 – Two-dimensional modified van Krevelen diagram for oxygen-only containing classes identified in RBF influent and effluent.](image)
MS analysis showed that RBF has little effect on removing bio-refractory organic compounds.

2.5. Bacterial community structural and biological activity analysis

The PCR-DGGE technology based on 16S ribosomal DNA (rDNA) gene is the one of most common molecular biological approaches to assess the bacterial community. In this study, this technology was applied to investigate the bacterial community structures in RBF. The results are shown in Fig. 6 and each DGGE band could be considered as individual bacterial species (Luca et al., 2002). As shown in Fig. 6, DGGE banding patterns from the RBF showed differences in number, abundance and position of bands, suggesting that each bacterial may play different roles in degrading organic compounds in the petroleum refinery wastewater.

The 16S rDNA sequences from the strong DGGE bands 1–13 were obtained by sequencing and most of them were identical compared to the bacterial sequences presented in the gene-bank (Table 4). The bacterial species in the RBF belonged to the Verrucomicrobia bacterium (band 1), Acidovorax sp. (band 2), Acidobacteria bacterium (band 3 and band 5), Pseudomonas sp. (band 4), Soehngenia sp. (band 6), Comamonadaceae bacterium (band 7), Bradyrhizobiaceae bacterium (band 8), Bacteroidales bacterium (band 9), Clostridiales bacterium (band 11), Thauera sp. (band 12), and Gluconacetobacter sp. (band 13) species. Uncultured bacterial clone (band 10) was also observed, obviously, the Soehngenia sp. (band 6), Comamonadaceae bacterium (band 7) and Clostridiales bacterium (band 11) were the predominant species (brighter bands). Most of them had been reported to be able to degrade hydrocarbons (Cheng et al., 2013; Hasanuzzaman et al., 2007; Shinoda et al., 2004; Vinas et al., 2005).

The PCR-DGGE results showed that most of identified bacteria had been reported to be able to degrade hydrocarbons, however, most of their abundance were low (darker bands), which could be inferred from the fact that high active de-carbonization bacteria were mainly inhibited. Furthermore, nitrification bacteria were not found, which indicated the absence of nitrification function. It could be due to the reduction of SRT, which inhibited the growth of nitrification bacteria. Further study needs to be conducted to reveal the detailed functions and roles of each bacterial species in RBF.

3. Conclusions

RBF was comprehensively evaluated as a pretreatment process for PRW. It was found that RBF was effective at treating biodegradable organic contaminants. It achieved a mean $\text{BOD}_5$ degradation efficiency of 83.64%, and a mean $\text{COD}_{cr}$ degradation efficiency of 54.63%. However, the RBF achieved a decreased biodegradability ($\text{BOD}_5/\text{COD}_{cr}$ from 0.531 to 0.169) and an increased alkalinity and pH value, which were unfavorable for the control of operating parameters in subsequent biological treatment. GC-MS and FT-ICR MS indicated that RBF can effectively remove lighter hydrocarbons, whereas some organic contaminants with high molecular weight and complex molecular structure are found to be difficult to remove. Consequently, most of the contaminant molecules of the RBF effluent had the complex structures of aromatic, polycyclic and heterocyclic rings, which was unfavorable for subsequent biological treatment of PRW for meeting discharge standards. PCR-DGGE revealed that high active de-carbonization bacteria were mainly inhibited in RBF. Furthermore, nitrification bacteria were not found, which indicated the absence of nitrification function. Our results demonstrated that RBF technology had a limited engineered application for the pre-treatment of PRW.

Acknowledgments

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Table 4 – Comparisons of nucleotide sequences and abundance of sequenced DGGE bands.

<table>
<thead>
<tr>
<th>Band</th>
<th>Relatives</th>
<th>Accession number</th>
<th>Similarity</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Verrucomicrobia bacterium</td>
<td>JF410432.1</td>
<td>99%</td>
<td>Verrucomicrobiun</td>
</tr>
<tr>
<td>2</td>
<td>Acidovorax sp. CPO 4 0017</td>
<td>KC302440.1</td>
<td>98%</td>
<td>Gammaproteobacteria</td>
</tr>
<tr>
<td>3</td>
<td>Acidobacteria bacterium SH5</td>
<td>KC715862.1</td>
<td>95%</td>
<td>Acidobacteria</td>
</tr>
<tr>
<td>4</td>
<td>Pseudomonas sp. S2P1061</td>
<td>KC145944.1</td>
<td>100%</td>
<td>Gammaproteobacteria</td>
</tr>
<tr>
<td>5</td>
<td>Acidobacterium D199A</td>
<td>KC845239.1</td>
<td>100%</td>
<td>Acidobacteria</td>
</tr>
<tr>
<td>6</td>
<td>Soehngenia sp. L35B_140</td>
<td>JF946902.1</td>
<td>100%</td>
<td>Sarcina</td>
</tr>
<tr>
<td>7</td>
<td>Comamonadaceae bacterium B1-08</td>
<td>JF754519.1</td>
<td>99%</td>
<td>Betaproteobacteria</td>
</tr>
<tr>
<td>8</td>
<td>Bradyrhizobiales bacterium GJW-30</td>
<td>HF970589.1</td>
<td>94%</td>
<td>Alphaproteobacteria</td>
</tr>
<tr>
<td>9</td>
<td>Bacteroidales bacterium M6</td>
<td>KC762919.1</td>
<td>99%</td>
<td>Bacteroides</td>
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<tr>
<td>10</td>
<td>Uncultured bacterium OX G09</td>
<td>FN429550.1</td>
<td>100%</td>
<td>–</td>
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<tr>
<td>11</td>
<td>Clostridiales bacterium De1161</td>
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<td>12</td>
<td>Thauera sp. BC0187</td>
<td>KC166840.1</td>
<td>95%</td>
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<tr>
<td>13</td>
<td>Gluconacetobacter sp. T61213-21-1a</td>
<td>B778532.1</td>
<td>100%</td>
<td>Rhodospirillales</td>
</tr>
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</table>

PRW: petroleum refinery wastewater; DGGE: denaturing gradient gel electrophoresis.

National Offshore Oil Corporation (CNOOC-KJ125 ZDXM 15 LH007 LH12) project.

REFERENCES


