Enhanced Crude Oil Biodegradation in a Two-liquid Phase Partitioning Bioreactor

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The biodegradation of crude oil at relatively high concentrations in an aquatic environment is constrained by the inherent toxicity of crude oil. In this study, a new application of an aqueous-organic two-liquid phase partitioning bioreactor (TLPPB) was developed to degrade high concentrations of crude oil up to 5000 mg L\(^{-1}\). Silicon oil was selected as the sequestering organic phase to control the delivery of crude oil in aqueous phase by absorbing, and subsequently releasing the crude oil to the microorganisms. The efficiency of the silicon oil-based TLPPB has been compared to the conventional monophasic bioreactor. Regardless of the crude oil concentrations and the type of aqueous phase, complete removal of crude oil up to 100% was achieved in the two-liquid phase partitioning bioreactor (TLPPB) compared to 75–80% removal efficiency of crude oil in the monophasic conventional bioreactor. In addition, the performance of TLPPB for crude oil removal was evaluated in terms of the salinity effect using distilled, river, and marine water samples. High salinity of aqueous phase proved to slightly inhibit the microorganisms-bioactivity and hinder the rate of crude oil degradation in the TLPPB. This study indicates the potential of TLPPB for enhancing the transport and biodegradation of crude oil in aqueous solutions.

Key words: crude oil, biodegradation, biphasic bioreactor, salinity, silicon oil

Introduction

One of the most dramatic environmental problems is the crude oil spill into natural aquatic systems and the subsequent environmental risks represented by endangering parts of the aquatic and ecosystem due to oil seepage from damaged pipelines into water supplies. Accidental leakages during hydrocarbon fuels transportation and other activities are inevitable, making these hydrocarbons the most common global environmental pollutants. The spill of oil into water is raising concerns of human health as well as ecological damage. Biological treatment is considered a cost-effective environmentally friendly technology that is often used to treat oil spills in soils, groundwater, and surface water. Due to the obvious benefit of the actual removal of oil from the environment and the fact that it can be cost effective compared to other physical and chemical treatment technologies, biodegradation has been, and currently remains, the subject of considerable research. In the biotreatment of any organic compound, the most significant challenge is substrate delivery. That is, the addition of substrate at high concentrations will inhibit or even kill the organisms, whereas substrate addition at too low concentrations will cause the cells to starve and result in a sub-optimal process performance, so that precise and controlled delivery of these materials is exceedingly important. The two-phase partitioning bioreactor concept (TPPB) appears to have great potential in enhancing the productivity of many bioprocesses. The proper selection of an organic solvent is the key to the successful application of this approach in industrial practices. The controlled substrate delivery from the organic to the aqueous phase opens a new area of application of this strategy to biodegradation of pollutants. In such a two-phase aqueous-organic system, the substrate is solubilized in the immiscible organic phase and allowed to transfer into the organic phase. The microorganisms degrade or transform the substrate at the aqueous/organic interface and/or in the aqueous phase. Thus, the substrate concentration in the biotic phase can be maintained below the inhibitory level. The partition process itself is controlled to some extent by the metabolic activity of microorganisms. The system is well suited for biodegradation of hazardous pollutants. The idea of a two-
phase partitioning bioreactor was first proposed by Collins and Daugulis\textsuperscript{8} for the biodegradation of xenobiotic pollutants that originally exists in the organic solvent. Tomei\textit{et al.}\textsuperscript{7} compared the performance of a TPPB, relative to single phase operation, in which a small volume (5 \%, v/v) of the beads polymer Hytrel 8206 was used to treat aqueous mixtures of 2,4-dimethylphenol and 4-nitrophenol. Zuniga\textit{et al.}\textsuperscript{9} studied the biodegradation of methane, and the accumulation of poly-hydroxybutyrate using a methanotrophic consortium and an isolated strain in a TPPB. Ramos\textit{et al.}\textsuperscript{11} proposed a two phase partitioning bioreactor to carry out the degradation of the poorly soluble compound anthracene by laccase from \textit{Trametes versicolor}. The organic phase consisted of silicone oil saturated with anthracene. Juang\textit{et al.}\textsuperscript{12} used a two-phase partitioning bioreactor equipped with an agitator to remove phenol from an organic phase that was dispersed in an aqueous mineral salt medium containing \textit{Pseudomonas putida} BCRC 14365. Kerosene was selected as the organic solvent. Munoz\textit{et al.}\textsuperscript{13} investigated an innovative operation mode in a two-liquid phase partitioning bioreactor (TLPPB) for the treatment of volatile organic compounds (VOC). The TLPPB was implemented in a 2.5 L stirred tank reactor using 10 \% (v/v) of silicone oil as the organic phase and hexane as model VOC. A removal efficiency of 80 \% was recorded for 26 days. However, none of the previously reported studies involved the crude oil biodegradation in a two-liquid phase partitioning bioreactor (TLPPB). Ismail and Abdulrazzak\textsuperscript{14} studied the anaerobic biodegradation of crude oil in mineral salt media (MSM) using small bench scale-TLPPB. The results revealed that complete removal of crude oil was achieved in biphasic bioreactor after 3 weeks compared to 73–82 \% in a monophasic bioreactor for the same period.

The main objective of this study was to evaluate for the first time the biodegradation of crude oil in actual river and marine water samples using TLPPB inoculated with mixed culture at anaerobic conditions. The effects of aqueous phase salinity, substrate initial concentration, and agitation rate were evaluated.

\section*{Materials and methods}

\subsection*{Aqueous and non-aqueous solutions}

Two types of actual aqueous solutions were examined in this study which were river water (RW) samples freshly collected from the Tigris River (Iraq), and marine water (MW) samples collected from the Arabian Gulf. For comparison purpose, a set of experiments was conducted with distilled water (DW) to demonstrate the effect of total dissolved solids (TDS) concentration on the biotreatment process. The properties of the water samples are given in Table\ 1. The aqueous samples were synthetically contaminated with crude oil obtained from the Midland Refineries Company-Al-Dora Refinery (Iraq). The properties of the crude oil samples are given in Table\ 2. Silicone oil (polymethylsiloxane) was selected in this study as the organic phase solvent, supplied by Gainland Chemical Company (GCC), UK.

\begin{table}[h]
  \centering
  \caption{Properties of water samples}
  \begin{tabular}{lccc}
    \hline
    Constituent & Average concentration, mg L\textsuperscript{-1} & \text{distilled water (DW)} & \text{river water (RW)} & \text{marine water (MW)} \\
    \hline
    TSS & – & 23 & 117 \\
    TDS & – & 567 & 46000 \\
    BOD\textsubscript{5} & – & 1.3 & 12 \\
    COD & – & Nil & 21 \\
    Oil content & – & Nil & Nil \\
    NH\textsubscript{4}\textsuperscript{+} & – & 0.36 & 1.25 \\
    NO\textsubscript{3}\textsuperscript{–} & – & 4.7 & 6.9 \\
    PO\textsubscript{4}\textsuperscript{3–} & – & 0.38 & 0.46 \\
    Cl\textsuperscript{–} & – & 138 & 24400 \\
    SO\textsubscript{4}\textsuperscript{2–} & – & 243 & 4212 \\
    pH & 7.0 & 8.0 & 7.7 \\
    \hline
  \end{tabular}
  \label{table:water-properties}
\end{table}

\begin{table}[h]
  \centering
  \caption{Properties of the Al-Basrah crude oil}
  \begin{tabular}{lcc}
    \hline
    Constituent & Value & Unit \\
    \hline
    Density & 0.8745 & mg mL\textsuperscript{-1} \\
    Viscosity & 0.055 & N \cdot s m\textsuperscript{-2} \\
    API gravity & 30.3 & – \\
    Pour point & –30 & °C \\
    Water content & nil & %wt \\
    Salt content & 10 & Pb\textsuperscript{*} \\
    Sulphur & 3.1 & %wt \\
    CCR & 5.9 & %wt \\
    Wax & 1.2 & %wt \\
    Asphaltene & 2.6 & %wt \\
    ASTM distillation cracking point & 339 & °C \\
    \hline
  \end{tabular}
  \label{table:crude-oil-properties}
\end{table}\textsuperscript{*pound per thousand barrels}
Microorganisms and growth media

The mixed culture was freshly collected from the bottom of an aeration tank at a local sewage treatment plant (Baghdad, Iraq). The stock cultures were stored at 4 °C. The Mineral Salt Media (MSM) used for maintenance and culturing of the microorganisms was prepared at pH 6.8. The composition of MSM in (mg L⁻¹) was, (NH₄)₂SO₄ (100), KH₂PO₄ (350), K₂HPO₄ (775), MgSO₄ · 7H₂O (100), CaCl₂ (40), FeSO₄ · 7H₂O (1.0), MnSO₄ · H₂O (1.0), NaMoO₄ (0.21), NaCl (5000), meat papain peptone (15000), and Tryptone (15000). Prior to use, the MSM were sterilized in an autoclave at 121 °C for 30 minutes. The pH was fully controlled by daily monitoring and the final pH was 7.

Analytical procedure

Analysis of oil content was carried out using the oil content analyzer (Model: HORIBA OCMA-350) based on infrared analysis. It included a single-beam, fixed wavelength, non-dispersive infrared filter-based spectrophotometer. Infrared radiation from a tungsten lamp was transmitted through a cylindrical, quartz cuvette containing a sample extract. The radiation that passed through the extract entered a detector containing a filter that isolated analytical wavelengths in 3400 to 3500 nanometer range. Chemical oxygen demand (COD) concentrations in aqueous samples were measured using the COD analyzer (Model: Lovibond, RD 125). Volatile suspended solids (VSS) concentrations were measured according to Standard Methods¹⁵. Measurements of produced biogas were carried out using gas chromatography (Model: PACKARD 438A). Biomass growth was observed using scanning electron microscopy (SEM) (model: VEGA /easy probe). An additional approach known as standard plate count was used in this study to quantitatively measure the bacteria growth.

Microorganisms’ cultivation

Freshly collected activated sludge samples were centrifuged at 3000 rpm for 10 minutes to separate the active biomass from liquid phase. The separated suspension of cultures were activated and enriched at 30±2 °C in 250 mL nutrient media (MSM), into which 20 mg L⁻¹ of crude oil was added to 10 mL of mixed culture suspension in a 500 mL-Erlenmeyer flask to be adapted for 24 h. Prior to starting the adaptation process, the flasks were flushed with nitrogen to achieve an anaerobic environment, and then agitated by an orbital shaker at 100 rpm to enrich the culture seeds. The growing mixed cells were collected after centrifugation of the enriched suspension at 3000 rpm for 10 minutes. The collected activated cells were re-suspend-
three types of aqueous solutions could be attributed to the effect of salinity because high TDS concentration is a stressful agent for most of organisms which may hinder their activity and the subsequent rate of the oil biodegradation process.

These findings are in a good agreement with the previously reported investigations. Minai-Tehrani et al. observed 41% crude oil degradation in soil samples with no NaCl added, while only 12% was obtained in samples from the same soil subject to 50 g L⁻¹ NaCl after 120 days. The negative impact of increasing salinity on hydrocarbons biodegradation is also observed in environments where halotolerant and/or slightly halophilic microorganisms tend to be dominant, as the case of mangroves. Even in typical hyper-saline environments, a negative impact on hydrocarbon biodegradation is induced by increasing salinity. Mille et al. reported that the amount of oil degraded decreased with increasing salt concentrations.

**Effect of agitation rate**

A set of experiment were carried out using river water as an aqueous phase to study the effect of agitation rate on oil removal in TLPPB. Fig. 3 illustrates the effect of agitation rate on the crude oil removal. It is well observed that at 250 rpm agitation rate, the crude oil removal rate was faster than at 120 rpm as more time was required to achieve equilibrium. These results could be attributed to the fact that higher mass transfer can be obtained by increasing the mixing rate. These findings are in a good agreement with the previously reported results by Juang et al. for the removal of phenol in (TPPB).
However, the main objective of mixing is to maximize the process rate. It would be reasonable to believe, nonetheless, that increasing the agitating rate could increase the total surface contact and consequently the speed of reaction. A minimum requirement for the process is that the concentration is well distributed throughout the mixing volume.

Comparison of crude oil removal between monophasic and biphasic bioreactors

To quantify the action of the organic phase on the degradation rate, a set of experiments were conducted without using silicon oil as the organic extractive phase. Results given in Table 3 indicate that the microbial activity for degradation of crude oil was greatly enhanced in the biphasic system. In case of monophasic bioreactor, microorganisms were in direct contact with high concentration of crude oil up to 5000 mg L\(^{-1}\) and the microbial growth seemed to be limited or hindered. Comparative biodegradation of crude oil in the biphasic and monophasic system emphasize the role of the organic extractive phase. The silicon oil seems to act as a sponge that absorbs the crude oil droplets from the aqueous phase, and then by continuous shaking and agitation the organic phase will gradually release the substrate to the microorganism in the aqueous media.

Biodegradation of crude oil in lab-scale TLPPB

Experimental work was extended to study the performance of the lab-scale TLPPB system alternatively using river and marine water samples-loaded with crude oil at initial concentration of 5000 mg L\(^{-1}\). The results revealed that complete removal of crude oil up to 100 % in river and marine water samples were achieved after 10 and 20 days, respectively (Fig. 4). In these experiments, no by-products were observed since complete biodegradation of oil was achieved. However, measurements of the released biogas indicated that CH\(_4\) and CO\(_2\) were the end-products of the oil anaerobic biodegradation in TLPPB.

The results of this study indicated complete removals of oil, which is in a good agreement with the previously published investigations. Yeom et al.\(^{22}\) reported that the removal efficiency of benzene was more than 99 % within 24 h in a Lab-scale TPPB. MacLeod & Daugulis\(^{23}\) reported that phenanthrene and pyrene were completely degraded within 4 days in a Lab-scale TPPB.

In order to examine the validity and reliability of the suggested approach, it was necessary to test the aqueous samples actually contaminated with crude oil resulted from an accidental spill. These samples were collected from the Tigris River (Baiji, Iraq). Table 4 presents the quality of the actually contaminated aqueous samples. A set of experiments was conducted using the actual samples of crude oil-contaminated river water alternatively in Lab-scale monophasic and biphasic bioreactor (TLPPB). The results revealed complete removal of crude oil after 6 days in TLPPB at an average...
initial concentration of 3000 mg L\(^{-1}\), while the removal efficiency of crude oil in the monophasic reactor did not exceed 85 % and remained constant (Fig. 5).

**Biomass granulation**

Upon achieving equilibrium in the TLPPB, the biomass granules formation was clearly visible. This granulated biomass had a typical brownish-dark gray color and 1–2 mm size of granules. The Scanning Electron Microscopy (SEM) images of these bio-granules (Fig. 6) revealed a porous and spongy structure with some cracks on the surface of the layers. A similar observation was reported by Amro\(^24\) for biomass growth on oil contaminated-soil samples using the SEM technique.

**Conclusion**

This study confirmed the potential of the biphasic bioreactor (TLPPB) for the anaerobic biodegradation of crude oil for concentrations up to 5000 mg L\(^{-1}\) in aqueous phase using silicon oil as the extractive organic phase solvent. The TLPPB significantly enhanced the biological degradation

### Table 4 – Quality of the river-water samples actually contaminated with crude oil

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Average concentration, mg L(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS</td>
<td>41</td>
</tr>
<tr>
<td>TDS</td>
<td>314</td>
</tr>
<tr>
<td>BOD(_5)</td>
<td>0.90</td>
</tr>
<tr>
<td>COD</td>
<td>3100</td>
</tr>
<tr>
<td>Oil content</td>
<td>3070</td>
</tr>
<tr>
<td>NH(_4^+)</td>
<td>0.21</td>
</tr>
<tr>
<td>NO(_3^-)</td>
<td>3.80</td>
</tr>
<tr>
<td>PO(_4^{3-})</td>
<td>0.16</td>
</tr>
<tr>
<td>Cl(^-)</td>
<td>64.6</td>
</tr>
<tr>
<td>SO(_4^{2-})</td>
<td>97</td>
</tr>
<tr>
<td>pH</td>
<td>7.80</td>
</tr>
</tbody>
</table>

![Fig. 5](image-url) Profiles of substrate removal in actually contaminated river-water (ARW) samples in monophasic and biphasic bioreactors

![Fig. 6](image-url) SEM images for mixed culture (a) before growth and (b) after growth in TLPPB
rate of crude oil and reduced the inhibitory and toxicity effect of crude oil on mixed culture. Under the conditions studied, the crude oil was completely removed and biodegraded in the lab-scale TLPPB within 10 and 20 days at 5000 ppm crude oil in river and marine water samples, respectively. The salinity of the aqueous phase affected and hindered the biomass activity causing longer time necessary for the complete removal of crude oil.

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References