# Two-Phase Partitioning Bioreactor for the Treatment of Crude Oil-Contaminated Aqueous Solution

**Zainab Ziad Ismail**
Assistant professor
College of Engineering-University of Baghdad
Email: zismail@gmail.com

**Ibtihaj Abdlwahab Abdulrazzak**
Senior Engineer
Ministry of Environment
Email: ibt_mam@yahoo.com

## ABSTRACT

A new application of a combined solvent extraction and two-phase biodegradation processes using two-liquid phase partitioning bioreactor (TLPPB) technique was proposed and developed to enhance the cleanup of high concentration of crude oil from aqueous phase using acclimated mixed culture in an anaerobic environment. Silicone oil was used as the organic extractive phase for being a water-immiscible, biocompatible and non-biodegradable. Acclimation, cell growth of mixed cultures, and biodegradation of crude oil in aqueous samples were experimentally studied at 30±2ºC. Anaerobic biodegradation of crude oil was examined at four different initial concentrations of crude oil including 500, 1000, 2000, and 5000 mg/L. Complete removal of crude oil was achieved biphasic bioreactor after 3 weeks compared to 73-82% in the monophasic bioreactor for the same time period.

**Keywords:** bioreactor, crude oil, biodegradation, biphasic reactor, anaerobic biotreatment

## المفاعل البيولوجي ثنائي الطور لمعالجة المياه الملوثة بالنفط الخام

**زينب زياد اسماعيل**
**استاذ مساعد**
**كلية الهندسة-جامعة بغداد**

**ابتهاج عبد الوهاب عبد الرزاق**
**مهندس أقدم**
**وزارة البيئة**

### الخلاصة

تم في هذه الدراسة ولأول مرة معالجة المياه الملوثة بالنفط الخام باستخدام تقنية جديدة تتضمن عمليتين مشتركة في آن واحد وهي الاستخلاص باستخدام مذيب عضوي والتحلل الاحيائي بوجود المعالجة البيولوجية باستخدام المفاعل البيولوجي ثنائي الطور (TLPPB). يتكون هذا المفاعل (TLPPB) من طورين سائلين احدهما مائي والاخر عضوي (Silicon oil) لمعالجة التراكيز العالية من النفط الخام. ان اختيار استخدام زيت السليكون كمذيب عضوي في (TLPPB) تم على اساس كونه غير قابل للامتزاج بالمياه، لا يؤثر على فعالية ونشاط الاحياء المجهرية , وغير قابل للتحلل. تم اجراء عملية تأقلم وتنمية للخلايا البكتيرية ليتم بواسطتها التحلل الاحيائي للنفط الخام في درجة حرارة 30 ± 2º درجة مئوية. تمت مقارنة التحلل اللاهوائي للنفط الخام في مفاعل ثنائي الطور مع مفاعل البيولوجي احادي الطور لتراكيز مختلفة من النفط الخام تتضمن 500، 1000، 2000، و 5000 ملغم / لتر ، وقد تم تحقيق ازالة تامة للنفط الخام في وسط الماء - ثنائي الطور في خلال 3 أسابيع, مقارنة مع نسبة ازالة 73-82% في المفاعل أحادي الطور.

**الكلمات الدالة:** المفاعل الاحيائي، النفط الخام، التحلل الاحيائي، مفاعل ثنائي الطور، المعالجة الاحيائية اللاهوائية

## 1. INTRODUCTION

Two-phase partitioning bioreactors (TPPB) concept is based on the use of a water immiscible and biocompatible organic solvent that is allowed to float on the surface of a cell-containing aqueous phase. The solvent is used to dissolve large concentrations of pollutants (as substrates) which are readily achievable because most organic contaminants are very hydrophobic, and then partition into the aqueous phase at low levels, **Hernandez et al., 2011**. However, although very high amounts of toxic organic substrates can be added to a bioreactor, the cells experience only very low (sub-inhibitory) concentrations. Moreover, as the cells consume some of the substrate, disequilibrium is created, which causes more of the hydrophobic contaminants to be partitioned into the aqueous phase as the system tries to maintain thermodynamic equilibrium. Thus, not only do appropriate amounts of contaminants get delivered to the cells but also substrate delivery is ongoing until the organic phase becomes completely depleted and the rate is determined by the metabolic activity of the microorganisms, **Yeom,** and **Daugulis, 2001**.The system is well suited for biodegradation of hazardous pollutants, **Janusz** and **Malinowski, 2001**. The two-phase partitioning bioreactor concept appears to have a great potential in enhancing the productivity of many bioprocesses. The proper selection of an organic solvent is the key to successful application of this approach in industrial practice. The integration of fermentation and a primary product separation step has a positive impact on the productivity of many fermentation processes. The controlled substrate delivery from the organic to the aqueous phase opens a new area of application of this strategy to biodegradation of pollutants, **Janusz** and **Malinowski, 2001.** Several studies have been reported concerning the application of TPPB technique for the degradation of toxic organics including phenanthrene and pyrene in the presence of silicon oil as the second phase. Complete phenanthrene biodegradation was achieved within 3 days. Its concentration in the monophasic reactors dropped by 93% within 4 days, but the removal rate remains at that rate till the end of the experiment. Pyrene removal occurred to a limited extent only in the presence of phenanthrene, **Guieysse et al., 2001. Janikowski et al., 2002** used a two-phase partitioning bioreactor (TPPB) utilizing the bacterium *Sphingomonas aromaticivorans* B0695 to degrade 4 low molecular weight polycyclic aromatic hydrocarbons (PAHs). The TPPB achieved complete biodegradation of naphthalene, phenanthrene, acenaphthene and anthracene at a volumetric consumption rate of 90mg/L.h in approximately 30 h. **Tomei et al., 2007**, investigated the performance of two phase liquid-solid systems applied to the removal of 4-nitrophenol, Hytrel 8206 copolymer was utilized in batch kinetic test, this polymer was also employed as the partitioning phase in a lab scale sequencing batch reactor. The completely removal reached after 26 h. **Zilouei et al., 2008**, developed an organic-aqueous two-liquid-phase partitioning system to degrade high concentrations of pentachlorophenol. At the initial biomass concentrations of 7, 25, and 58 mg/l, the volumetric removal rates of PCP obtained were 25.7±0.5, 32.1±0.1, and 39.3 ± 2.9 mg /l.h, respectively. **Zhao et al., 2009**, determined the potential of a two-phase partitioning bioreactor (TPPB) for enhancing the treatment of phenol at high initial concentrations, The maximum volumetric consumption rate of phenol decreased in the order: immobilized microorganisms with organic modified montmorillonite OMMT-PSF capsules in a TPPB (342.4 mg/l.h) > immobilized microorganisms without OMMT-PSF capsules (300 mg/l.h) > free microorganisms with OMMT-PSF capsules in a TPPB (208.4 mg/l.h) > free microorganisms without OMMT-PSF capsules (125.8 mg/l.h). Tomei et al. (2011) 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, Hytrel 8206 was selected from a range of polymers that were tested for their partition coefficient for the target molecules, with the more hydrophobic compound (2,4-dimethylphenol) having a higher

partition coefficient value of 201 compared to 4-nitrophenol partition coefficient of 143. **Ramos et al., 2012**, proposed a two phase partitioning bioreactor to carry out the degradation of the poorly soluble compound anthracene by laccase from *Trametes versicolor*. The organic phase consisted of silicone oil saturated with anthracene. The surfactant, Triton X-100 was added to the aqueous phase at concentration above the critical micelle concentration to enhance anthracene solubility. **Munoz et al., 2013**, investigated an innovative operation mode in a two liquid phase bioreactor (TLPB) for the treatment of volatile organic compounds (VOC), a removal efficiency of 80% was recorded for 26 days. None of the previously mentioned studies concerned of the crude oil biodegradation in a Two-Phase Partitioning Bioreactor (TPPB). Exploring and investigating new environmentally friendly approaches for oil removal from aquatic environment became mandatory due to the wide occurrence of accidental oil spills on local, regional, and global scales. Based on this fact, this study was devoted to evaluate the performance and efficiency of TPPB for crude oil biodegradation.

This study aimed to evaluate the performance of a bench-scale two-phase partitioning bioreactor (TPPB) for the degradation of crude oil in aqueous phase compared to the performance of conventional monophasic bioreactor.

## 2. MATERIALS AND METHODS

### 2.1 Microorganisms, nutrient, and MSM

A mixed culture was freshly collected from the aeration tanks in Al-Rustamia Sewage Treatment Plant, Baghdad. The stock culture was stored at 4$^{o}$C. The nutrient media (NM) contained 15000 mg/L meat papain peptone, and 15000 mg/L Tryptone. The composition of mineral salt media (MSM) in (mg/L) were, $(NH_4)_2SO_4$ (100), $KH_2PO_4$ (350), $K_2HPO_4$ (775), $MgSO_4$ .$7H_2O$ (100), $CaCl_2$ (40), $FeSO_4.7H_2O$ (1), $MnSO_4.H_2O$ (1), $NaMoO_4$ (0.21), and Sodium Chloride (5000). The MSM was prepared at pH 6.8, which was accurately controlled by daily measurement. Sulfuric acid (0.1M) and potassium hydroxide (0.1M) were used for pH adjustment. The prepared MSM was sterilized in an autoclave at 121$^{o}$C for 30 min, **Ted** and **Christine, 2010.**

### 2.2 Crude oil samples

Crude oil samples were obtained from midland refineries company /Al-Dora Refinery. The properties of the crude oil are given in **Table 1**.

### 2.3 Organic solvent

The organic phase used in this study was silicone oil (polydimethylsiloxane) of purity 100%, purchased from Gainland Chemical Company (GCC).

### 2.4 Analysis

The chemical oxygen demand (COD) concentrations in aqueous samples were measured using the COD analyzer Type: Lovibond, RD 125. Oil content analysis was carried out using the oil content analyzer Type: HORIBA OCMA-350, based on infrared analysis. It includes a single-beam, fixed wavelength, non-dispersive infrared filter-based spectrophotometer. Infrared radiation from a tungsten lamp is transmitted through a cylindrical, quartz cuvette containing a sample extract. The radiation which passed through the extract enters a detector containing a filter that isolates analytical wavelengths.

### 2.5 Biomass measurements

Engineers have traditionally used volatile suspended solids (VSS) as an indication of biomass concentration. The VSS parameter has the advantage of fitting directly into mass balance equations, **(Degenaar et al., 2000).** Volatile suspended solids were determined according to the procedure reported in the Standard Methods **(APHA, 1998).** Additional approach known as (Standard Plate Count) was used in this study to quantitatively measure the bacteria growth **(Ted & Christine, 2010)**. The major part of the procedure deals with a series of successive dilutions of the original culture in sterilized bottles using sterilized water. Thereafter, the diluted culture is poured into Petri dishes along with the nutrient agar, and then number of colonies is counted after incubation. The production of biogas was considered in this study as an additional approach to examine the growth and activity of microorganisms.

### 2.6 Enrichment of microorganisms

The freshly collected activated sludge samples were centrifuged at 3000 rpm for 10 min to separate the biomass from liquid phase. Thereafter, the culture suspension was reactivated and enriched at 30±2$^{o}$C in 250 ml mineral salt media (MSM), into which 20 mg/L of crude oil was added to 10 mL of mixed culture suspension in a 500 mL - Erlenmeyer flask to be adapted for 24h. Prior to starting the adaptation process, the flasks were flushed with nitrogen to achieve anaerobic environment, and then agitated by an orbital shaker at 100 rpm to enrich the culture seeds.

### 2.7 Sequential cultivation process

The growing cells were collected after centrifugation of the enriched suspension at 3000 rpm for 10 min. The collected activated cells were re-suspended and re-inoculated into fresh culture media in 500-ml Erlenmeyer flasks with the addition of 80 mg/L crude oil. After inoculation, the flasks were flushed with $N_2$, capped and placed in an orbital shaker controlled at 100 rpm and 30±2$^{o}$C. This cultivation cycle were sequentially repeated for 20 cycles with increased initial concentrations of crude oil up to 5000 mg/L. For each cultivation cycle, the initial and final concentration of cell, were determined.

### 2.8 Two-Phase Partitioning Bioreactors

Eight bench scale two phase portioning bioreactors (TPPB) were prepared and setup with four different initial concentrations of crude oil including 500, 1000, 2000, and 5000 mg/L using alternatively mineral salt media (MSM) and distilled water (DW). Silicon oil was used as the organic liquid phase. In order to examine the efficiency of TPPB towards the conventional bioreactor, additional eight bioreactors were identically set up without using the silicon oil as the mono-phasic bio reactors. Also, a set of four reactors were set up to examine the effect of abiotic processes including sorption, volatilization, and chemical degradation. Accordingly, a total of 16 bench reactors were prepared as given in **Table 2** which summarizes the experimental set up and data related to the amounts and types of the reactors contents, respectively. All experiments were conducted in duplicate in 250-mL Erlenmeyer flasks. The flasks were closed with butyl septa, tightly sealed with parafilm and incubated on a rotary shaker at 120 rpm and 30 ± 2ºC. All flasks were flushed with nitrogen to maintain anaerobic environment.

## 3. RESULTS AND DISCUSSION

### 3.1 Enrichment and acclimation of cultures

As mentioned in section 2.5, results of the acclimation process are presented by 3 different approaches as follows:

1- Characterization of the dominant cultures

Results of this method indicated the presence of several types of microorganisms, in particular *E.Coli, Pseudomonas seroginosa*, and *Bacillus subtilis* which represent the dominant and the most widely available types of organisms in activated sludge composition. However, **Table 3** illustrates the dominant types of species and their numbers before and after the acclimation process.

2- Visual characterization of biomass granules

Results of SEM technique are given in **Fig. 1a &b** before and at the end of the acclimation period, respectively. The bacterial colonization and dense growth at the end of acclimation process are well characterized as shown in **Fig. 1b** indicating the dense growth of microorganisms.

3- Measurements of produced biogas

As a result of the anaerobic biodegradation process, the biogas mainly represented by $CH_4$ was released as the end product of this anaerobic bioprocess. Accordingly, the released amounts of $CH_4$ could be another potential approach for indicating the startup progress and the end of anaerobic biodegradation process of crude oil during the acclimation period. **Fig. 2** presents the amounts of released biogas during the acclimation process using 20, 80, 280, 400, and 500 mg/L crude oil. **Fig. 3** illustrates the bioreactors before and after acclimation period.

### 3.2 Significance of TPPB application

The sets of experiments were alternatively conducted with distilled water (DW) and mineral salt media (MSM) to examine the effectiveness of each system for the anaerobic biodegradation of crude oil, also to investigate the potential of biphasic bioreactors compared to monophasic bioreactors. Results revealed that higher removal efficiency was achieved up to 100% in the DW-biphasic bioreactor after less than 3 weeks. However, lower efficiencies of crude oil removal were observed in the MSM-monophasic, MSM-biphasic, and DW-monophasic bioreactors indicating the effectiveness of the DW-biphasic bioreactor system compared to the other reactors. Table 4, illustrates the removal efficiencies (ranging from 73% to 100%) of the four bio-systems at different initial concentrations of crude oil. **Figs. 4 to 7** illustrate the profiles of crude oil removals with time in the bioreactors. However, results revealed complete removal of crude oil in the DW-biphasic bioreactors at different initial concentration of crude oil**.** The effect of silicone oil existence as the immiscible organic phase in the bioreactor was well observed in controlling the dispersion and transfer of crude oil into the aqueous phase to feed the biomass. Silicone oil exhibited as a sponge adsorbed the high initial concentration of crude oil, and then gradually desorbs batch doses of oil as a substrate for the starving microorganisms to prevent the substrate shock loading which may cause consortiums toxicity and death.

However, the relatively lower efficiency of MSM-biphasic bioreactor compared to the DW-biphasic bioreactor could be attributed to the availability of regular substrate (MSM) to the microorganisms making them less longing for unconventional type of substrates (crude oil).

## 4. CONCLUSION

The main conclusions that can be drawn from this research are represented by a successful bacterial acclimation process which was accomplished to tolerate the high concentrations of crude oil as the sole toxic organic pollutant in the aqueous solution samples. Also, results

demonstrated that the removal efficiency of crude oil at 500, 1000, 2000, and 5000 mg/L initial concentrations in DW-biphasic bioreactor was 100% after 3 weeks, compared to 73-86% in the MSM-monophasic, MSM-biphasic, and DW-monophasic bioreactors for the same time period.

**ACKNOWLEDGMENT**
Financial and technical support for this work by the Ministry of Environment is greatly appreciated.

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**NOMENCLATURE**

COD = chemical oxygen demand, mg/L.
DW = distilled water.
MSM = mineral salt media.
NM = nutrient media.
OMMT = organic modified montmorillonite.
TLPPB = two-liquid phase partitioning bioreactor.
TPPB = two-phase partitioning bioreactor.
VOC = volatile organic compounds.
VSS = volatile suspended solids.

**Table 1** Properties of the crude oil.

| Constituent | Value | Unit |
|---|---|---|
| Density | 0.8745 | gm/ml |
| Viscosity | 55.0 | cp |
| API gravity | 30.3 | - |
| Pour point | -30 | $^{o}$C |
| Water content | Nil | %wt |
| Salt content | 10 | Ptb* |
| Sulphur | 3.1 | %wt |
| Wax | 1.2 | %wt |
| Asphaltene | 2.6 | %wt |

* Pounds per thousand barrel

**Table 2** Types and classification of the bench scale bioreactor.

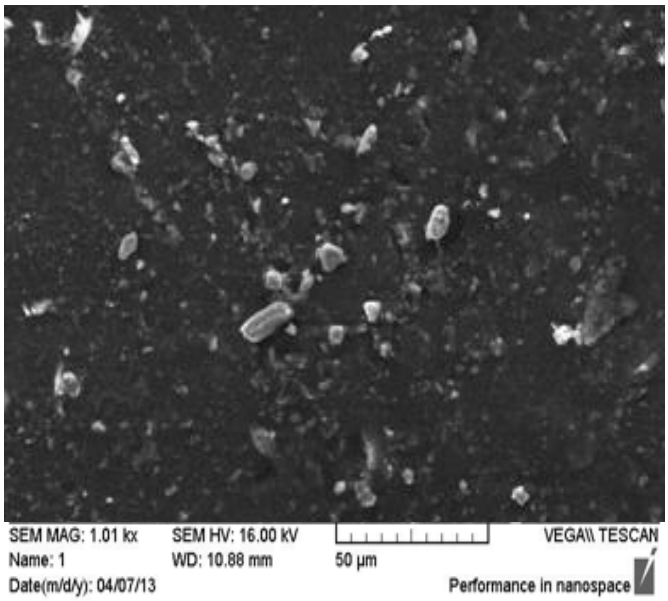
| Reactor | Contents | | | | | Liquid phases |
|---|---|---|---|---|---|---|
| | MSM (mL) | DI (mL) | Crude oil conc. (mg/L) | Culture (mL) | Silicone oil (mL) | |
| **Anaerobic biodegradation processes** | | | | | | |
| BR1 | 450 | - | 500 | 15 | - | Single |
| BR2 | 300 | - | 500 | 10 | 150 | Two |
| BR3 | - | 450 | 500 | 15 | - | Single |
| BR4 | - | 300 | 500 | 10 | 150 | Two |
| BR5 | 450 | - | 1000 | 15 | - | Single |
| BR6 | 300 | - | 1000 | 10 | 150 | Two |
| BR7 | - | 450 | 1000 | 15 | - | Single |
| BR8 | - | 300 | 1000 | 10 | 150 | Two |
| BR9 | 450 | - | 2000 | 15 | - | Single |
| BR10 | 300 | - | 2000 | 10 | 150 | Two |
| BR11 | - | 450 | 2000 | 15 | - | Single |
| BR12 | - | 300 | 2000 | 10 | 150 | Two |
| BR13 | 450 | - | 5000 | 15 | - | Single |
| BR14 | 300 | - | 5000 | 10 | 150 | Two |
| BR15 | - | 450 | 5000 | 15 | - | Single |
| BR16 | - | 300 | 5000 | 10 | 150 | Two |
| **Control reactors for examination of abiotic processes effects** | | | | | | |
| R1 | - | 450 | 1000 | - | - | Single |
| R2 | - | 300 | 1000 | - | 150 | Two |
| R3 | - | 450 | 5000 | - | - | Single |
| R4 | - | 300 | 5000 | - | 150 | Two |

*****MSM, mineral salt media
**DW, distilled water

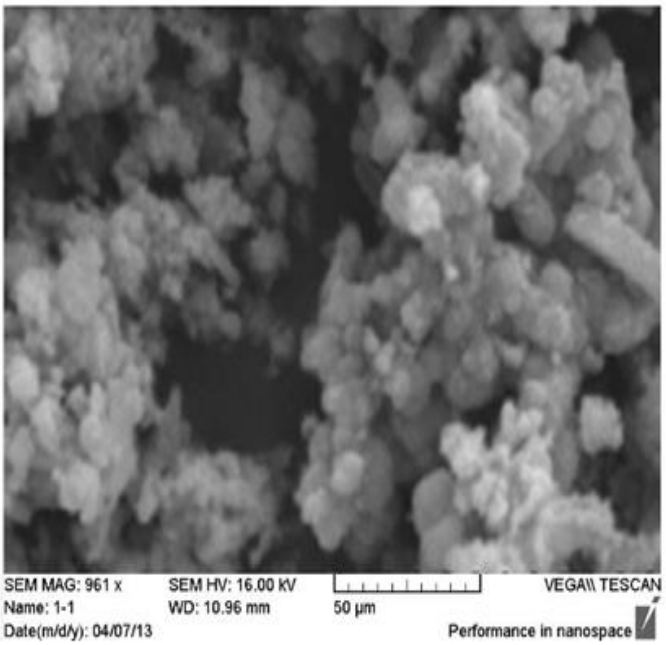
**Table 3** Types of organisms in the mixed culture samples

| Microorganisms | Aeromonas hydrophilla (colonies/mL) | |
|---|---|---|
| | before acclimation | after acclimation |
| Pseudomonas aeroginosa | $2.0x10^3$ | Heavy growth |
| Fecal Coliform | $1.6 x10^3$ | $3.7 x10^5$ |
| E.Coli | $6.5x10^7$ | Heavy growth |
| Bacillus subtilis | $8.5x10^4$ | $6.2 x10^7$ |
| Total Coliform | $1.2 x10^3$ | $3.0 x10^6$ |
| T.P.C. | $9.8 x10^7$ | Heavy growth |

| **Table 4** The removal efficiencies of the tested systems..**Types of reactor** | **Average initial concentration mg/L** | **Removal efficiency % after 3 weeks** |
|---|---|---|
| | **Crude oil** | **Crude oil** |
| MSM-monophasic | 511 | 79% |
| MSM-biphasic | 517 | 86% |
| DW-monophasic | 520 | 82% |
| DW-biphasic | 521 | 100% |
| MSM-monophasic | 1017 | 78% |
| MSM-biphasic | 1093 | 85% |
| DW-monophasic | 1100 | 81% |
| DW-biphasic | 1112 | 100% |
| MSM-monophasic | 2080 | 76% |
| MSM-biphasic | 2093 | 84% |
| DW-monophasic | 2112 | 80% |
| DW-biphasic | 2120 | 100% |
| MSM-monophasic | 5088 | 73% |
| MSM-biphasic | 5100 | 80% |
| DW-monophasic | 5090 | 80% |
| DW-biphasic | 5120 | 100% |




**(a)**




**(b)**

**Figure. 1** SEM images for the mixed culture; (a) before, (b) after acclimation process.

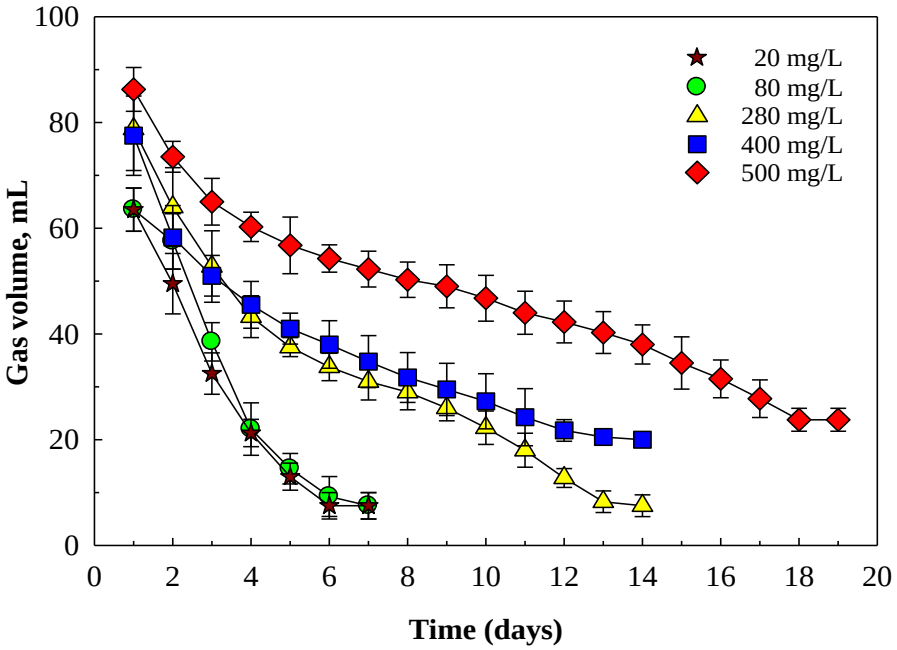



**Figure. 2** Biogas production profiles for the enrichment process at different initial concentrations of crude oil.

(a) (b)

**Figure. 3** The enrichment process; (a) before acclimation, (b) after acclimation.

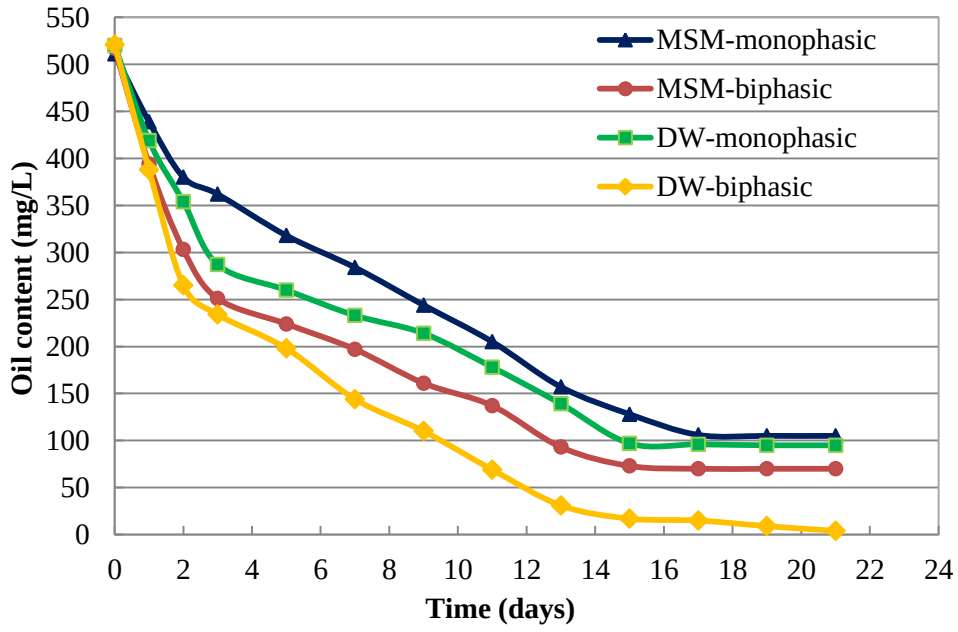



**Figure. 4** Oil content gradient in the 4 bioreactors at 500 mg/L concentration of crude oil.

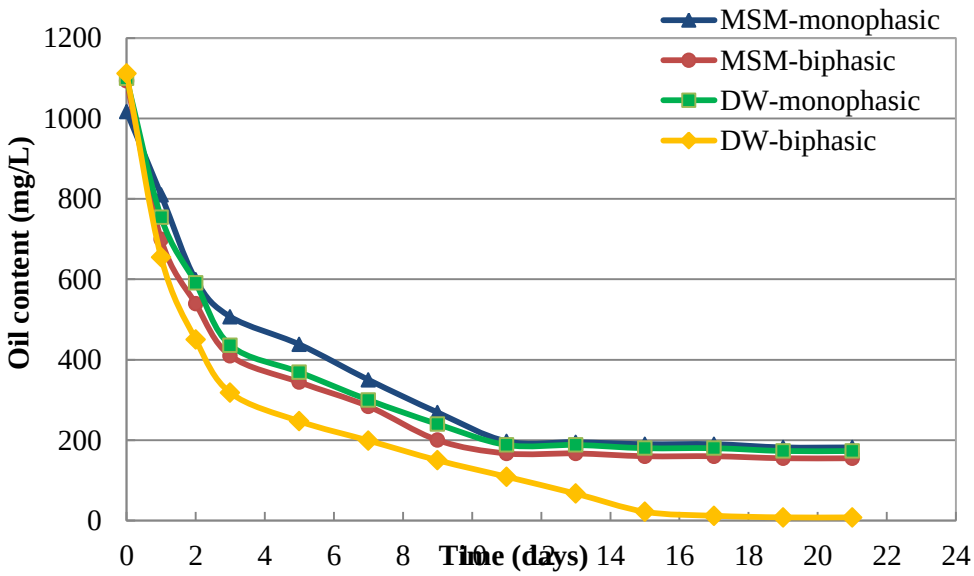



**Figure. 5** Oil content gradient in the 4 bioreactors at 1000 mg/L concentration of crude oil.

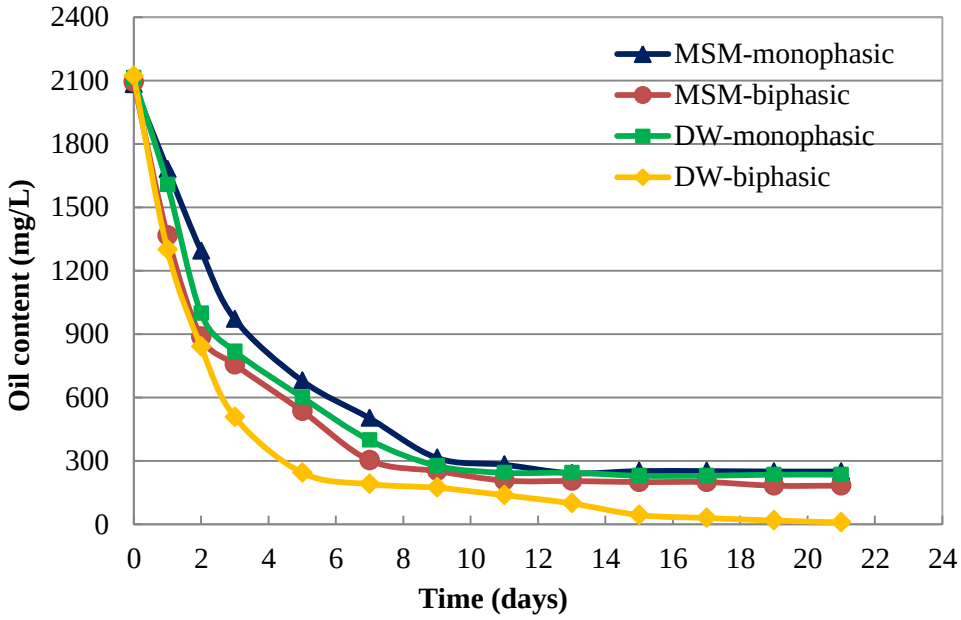



**Figure. 6** Oil content gradient in the 4 bioreactors at 2000 mg/L concentration of crude oil.

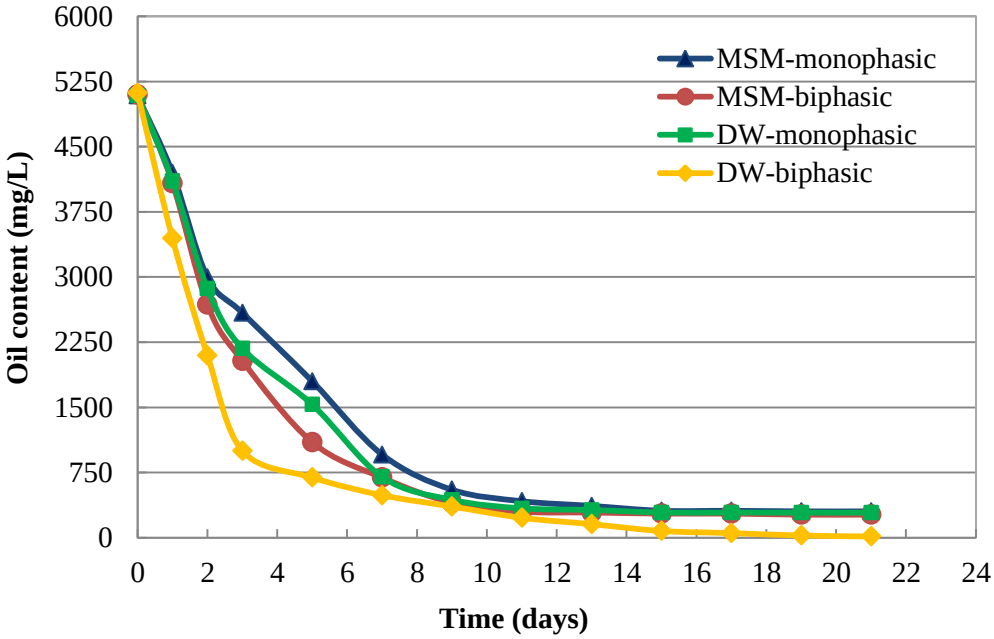



**Figure. 7** Oil content gradient in the 4 bioreactors at mg/L initial concentration of crude oil.