



SOLUBILIZATION AND REMOVAL OF PETROLEUM HYDROCARBONS BY A NATIVE MICROBIAL BIOMASS IN A BUBBLE COLUMN REACTOR

SOLUBILIZACIÓN Y REMOCIÓN DE HIDROCARBUROS DE PETRÓLEO POR BIOMASA MICROBIANA NATIVA EN UN REACTOR DE COLUMNA DE BURBUJA

R. Hernández-Martínez¹, S. Valdivia-Rivera², J. Betto-Sagahon³, A. Coreño-Alonso⁴, O. Tzintzun-Camacho⁵, M.A. Lizardi-Jiménez^{1*}

¹CONACYT-Instituto Tecnológico Superior de Tierra Blanca. Av. Veracruz S/N Esq. Hérores de Puebla, Colonia Pemex, C.P. 95100, Tierra Blanca, Veracruz, México.

²Instituto Tecnológico Superior de Tierra Blanca. Av. Veracruz S/N Esq. Hérores de Puebla, Colonia Pemex, C.P. 95100, Tierra Blanca, Veracruz, México.

³Universidad Politécnica de Puebla. Tercer Carril del Ejido "Serrano" S/N San Mateo Cuanalá. Juan C. Bonilla, C.P. 72640, Puebla, México.

⁴Universidad de Guanajuato. Lascuráin de Retana No. 5, Col. Centro, C.P. 36000, Guanajuato, México.

⁵Instituto de Ciencias Agrícolas, Universidad Autónoma de Baja California. Carretera a Delta S/N C.P. 21705, Ejido Nuevo León, Baja California, México.

Received: May 10, 2018; Accepted: July 12, 2018

Abstract

A native-microbial biomass obtained from a contaminated Atoyac River in Puebla, Mexico was evaluated to determine hydrocarbonoclastic capacities and solubilization strategies of Mayan crude oil. The biomass was able to use 13 g L⁻¹ of Mayan crude oil as a single carbon source in a bubble column reactor during a 14 day period. The Gompertz model was chosen as the best fit for the profiles of growth and hydrocarbon consumption. The microbial biomass achieved removal efficiencies of 70 ± 2% and a high maximum growth rate of $\mu_{max} = 0.76 \text{ d}^{-1}$. Hydrocarbon removal increased due to solubilization strategies, mainly emulsification. In particular, a maximum emulsification index (17.7 ± 0.4%) was reached at day 12 of cultivation, increasing the solubility and dispersion of hydrocarbons. Our work contributes to the understanding of the mechanisms of hydrocarbon-removal in multiphasic systems and reveals the potential use of this native-microbial biomass for bioremediation purposes.

Keywords: Native microbial biomass, hydrocarbons, emulsification, bioremediation, bubble column.

Resumen

Se evaluó biomasa microbiana nativa del contaminado Río Atoyac en Puebla, México, para determinar capacidad hidrocarbonoclasta y las estrategias de solubilización de petróleo crudo maya. La biomasa fue capaz de utilizar 13 g L⁻¹ de petróleo crudo Maya como única fuente de carbono en un reactor de columna de burbujas durante un período de 14 días. El modelo de Gompertz fue elegido como el mejor ajuste a los perfiles de crecimiento y consumo de hidrocarburos. La biomasa microbiana logró eficiencias de remoción de 70 ± 2% y una alta tasa de crecimiento máximo de $\mu_{max} = 0.76 \text{ d}^{-1}$. La degradación de hidrocarburos aumentó debido a estrategias de solubilización, principalmente la emulsificación. En particular, se alcanzó un índice de emulsificación máximo (17.7 ± 0.4%) a los 12 días de cultivo, aumentando la solubilidad y la dispersión de hidrocarburos. Nuestro trabajo contribuye a comprender los mecanismos de degradación de hidrocarburos en sistemas multifásicos y revela el uso potencial de esta biomasa nativa microbiana para fines de biorremediación.

Palabras clave: Biomasa microbiana nativa, hidrocarburos, emulsificación, biorremediación, columna de burbuja.

* Corresponding author. E-mail: malizardiji@conacyt.mx

Tel. +52 55-82-55-81-95

doi: <https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n1/Hernandez>

issn-e: 2395-8472

1 Introduction

Microbial removal is a major natural mechanism by which pollutants, such as petroleum hydrocarbons can be cleaned up from the environment and have been of great interest for recovering soil and bodies of water (Al-Mutairi *et al.*, 2008; Cerqueira *et al.*, 2012; Marín-Muñiz, 2016). The use of different biotechnological methods related to remediation have received special attention due to their low costs and environmentally friendly aspects, compared to standard chemical procedures, such as vitrification or incineration (Kuiper *et al.*, 2004; Penberthy and Weston, 2000).

Different studies (Gesinde *et al.*, 2008; Lu *et al.*, 2009) have shown an enhanced hydrocarbon removal by using microorganisms and/or microbial consortia isolated from contaminated sites (aquatic or soil systems), because microorganisms are able to develop solubilization mechanisms when exposed to hydrophobic substrates. Solubilization refers to the increase in solubility of a substance upon addition of a biosurfactant (Lakra *et al.*, 2014) that can act as an emulsifying agent by decreasing the surface tension and forming micelles. The uptake of hydrocarbons by microorganisms in droplets is very common and frequently involves the synthesis of biological surfactants as emulsifying agents to produce hydrocarbon microdroplets from macrodroplets or non-emulsified oil-phase.

Furthermore, microbial cells can act similarly to the surfactant molecules to produce microdroplets, which can be reflected in the stability of the emulsion by Pickering effect (Fig. 1). These microdroplets, encapsulated in the hydrophobic microbial cell surface, are taken up by cells and degraded (Das and Chandran, 2011). Microdroplets cause an increase in the surface area of the oil-phase (Melgarejo-Torres *et al.*, 2017) but macrodroplets or free-hydrocarbon generally constitute the main reservoir of hydrocarbons (Denis *et al.*, 2017; Lizardi-Jiménez *et al.*, 2012; Medina-Moreno *et al.*, 2014). The development of specific adaptive mechanisms to the toxicity and low bioavailability of these substrates allows bacteria to modify their cell surfaces regarding their hydrophobicity to permit direct hydrophobic-hydrophobic interactions (Heipieper *et al.*, 2010).

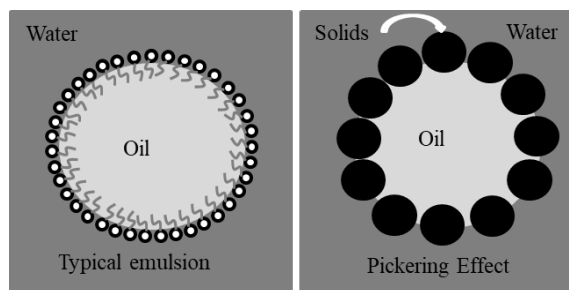


Fig. 1. Effect of emulsion stabilization: Pickering effect.

Therefore, the main challenges of bioremediation are focused on selecting the appropriate microorganisms that have developed the capacities for degradation and solubilization of hydrocarbons (Pacwa-Plociniczak *et al.*, 2016), and establish cultivation optimal conditions. Several biological methods have been used for the treatment of petroleum spills, including bioreactors, which reduce problems such as oxygen supply, temperature variations, and pH and nutritional requirements (Cappello *et al.*, 2016; Van Hamme *et al.*, 2003).

In particular, multiphase reactors such as airlift or bubble column bioreactors (BCB) are commonly used to cultivate oil-degrading microorganisms (Angeles *et al.*, 2017; Denis *et al.*, 2017; Tzintzun-Camacho *et al.*, 2014). The BCB is characterized by air flow that provides a low, uniform, shear stress, and low energy consumption, as compared to other conventional reactors like stirred tanks (Lizardi-Jiménez *et al.*, 2012).

Numerous studies in Mexico have reported bioremediation strategies of hydrocarbon contaminated sites (Angeles *et al.*, 2017; Lizardi-Jiménez *et al.*, 2012; Medina-Moreno *et al.*, 2014). However, scarce information is available about the cultivation of native consortia in pneumatic bioreactors although they are more efficient to degrade local crude oil or its local derivatives than microorganisms from other regions (Gesinde *et al.*, 2008).

The objectives of this study were to evaluate the hydrocarbonoclastic capacity of a native microbial biomass isolated from a contaminated site in the State of Puebla: Atoyac River, Mexico and to evaluate solubilization strategies that increase hydrocarbon bioavailability in a BCB, in order to contribute to the understanding of the mechanisms involved in hydrocarbon removal in multiphase systems, and reveal the potential use of this native microbial biomass for bioremediation purposes.

2 Materials and methods

2.1 Description of sampling areas

Liquid samples were obtained from the Atoyac River contaminated by an oil-spill in the year 2010. The sampling at Atoyac river was carried out near to the San Damián Bridge, in the municipality of San Martín Texmelucan, from the state of Puebla, Mexico located at 19°17'12" N and 98°25'35" W. Three samples (0.5 L) from different locations were collected randomly using sterile containers at a depth of one meter. In addition, the pH of the samples was measured and values around 7.0 at room temperature (20-24 °C) were observed. Finally, the samples were stored at 4 °C until use.

2.2 Cultivation of native microbial biomass

Microbiota obtained from samples of Atoyac river water were cultivated in a mineral medium composed of NaNO₃, 6.75 g L⁻¹; K₂HPO₄, 2.15 g L⁻¹; KCl, 1.13 g L⁻¹; MgSO₄·5H₂O, 0.54 g L⁻¹, adjusted with 13 g L⁻¹ of Maya crude oil (Medina-Moreno *et al.*, 2005). A BCB was used with a volume capacity of 1.5 L (Fig. 2). A cylindrical vessel reactor was made of Pyrex glass (0.14 m diameter; 1.0 m height). Air was sparged by a stainless steel L-shaped diffuser of 1/4 inch internal diameter (7 orifices; 1.0 mm diameter), air superficial velocity of 1 cm s⁻¹, measured using a rotameter (Cole-Parmer, USA). The native microbial biomass was inoculated from the water samples at 10% (volume/volume) and the pH was adjusted to 6.5 with HCl.

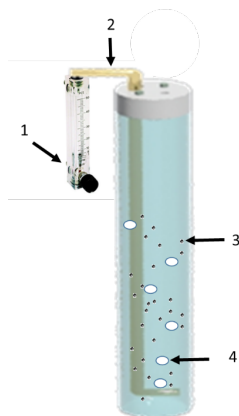


Fig. 2. A schematic of a Bubble column bioreactor (BCB) system marked with 1) air flow control, 2) air diffuser, 3) crude oil droplets and 4) air bubbles.

The bioreactor was operated and incubated in batch-culture at 18 °C for 14 days. The cultures were developed by triplicate. In addition, a control bioreactor was operated under abiotic conditions (containing mineral medium, Maya crude oil and filtered air) to determine the oil fraction released by evaporation.

2.3 Determination of suspended solids and total residual hydrocarbons

Determination of suspended solids and residual hydrocarbons was performed using gravimetric analysis. For determination of suspended solids, the samples (10 mL) were centrifuged (J2-HS, Beckman, USA) at 4000 × g for 30 min at 4 °C. After centrifugation, the generated phases were separated: biomass, supernatants, and petroleum. For determination of suspended solids, the biomass was washed with an isotonic solution and centrifuged. The process was repeated until the oil phase disappeared after centrifugation, in order to diminish possible inert solids that could be attached to the biomass. Subsequently, the biomass was transferred to a porcelain container and heated in an oven at 105 °C to constant weight (Duo Vac, Lab-line Inc. Instruments, USA). In addition, recovered oil was separated and weighed to determine the residual hydrocarbons (Medina-Moreno *et al.*, 2014). It is important to mention that the fraction of volatilized hydrocarbons was evaluated under abiotic conditions (2 ± 0.6% of initial concentration of Maya crude oil released by evaporation).

2.4 Modelling of growth kinetics

Several studies have reported mathematical models that describe microbial growth of oil-degrading consortia isolated from highly-polluted soil (Medina-Moreno *et al.*, 2013) and minimally-polluted underwater sinkholes (Denis *et al.*, 2017), and these models are used to predict the growth pattern of microbial populations under controlled conditions. These models are mainly empirical or semi-empirical in nature. One of the most popular models is the Gompertz function (Zwietering *et al.*, 1990). The reparametrized Gompertz model (Eq. 1) was used to adjust the growth profile of the native microbial biomass to predict its hydrocarbonoclastic capacities using hydrophobic substrates in a BCB.

$$Y = a * e^{-e^{-(b-c*t)}} \quad (1)$$

where Y is a function of relative cell concentration at time t in days, e is Euler number which approximately equals 2.71828, while a , b and c are variables that can be related to biological parameters such as the specific maximum growth rate (μ_{max}), $\mu_{max} = ac/e$, and the time of the adaptation phase or lag (λ) phase, $\lambda = (b - 1)/c$ (Zwietering *et al.*, 1990). In the present work, data obtained with the Gompertz model were adjusted, using Matlab software (Student license, R2017b version), in order to predict the growth pattern of the microbial biomass under the studied conditions.

2.5 Emulsifying activity index

Emulsifying activity of supernatants was evaluated through the emulsification index (E24). Samples of supernatants (4 mL) were mixed with petroleum (6 mL) and mechanically stirred for two minutes to promote emulsion formation, and then stability of the emulsions formed was evaluated, maintaining the samples for 24 h at room temperature. Subsequently, E24 was calculated as the ratio between height of the emulsion and total height multiplied by 100 (Cooper and Goldenberg, 1987). All determinations were conducted in triplicate from samples taken from the BCB at 0, 3, 5, 8, 10, and 14 days.

2.6 Statistical analysis

All determinations were conducted in triplicate and data were processed using NCSS Statistical Software (NCSS 2007, version 1). One-way ANOVA was used to compare mean values with $\alpha \leq 0.05$. In addition, differences between mean values were determined by Tukey-Kramer Test, $\alpha \leq 0.05$.

3 Results and discussion

3.1 Cultivation of microbial biomass

Profile of microbial growth during culture time was determined through concentration of suspended solids, which include microbial biomass and extracellular metabolites (Fig. 3). The microbial biomass used the Mayan crude oil as a sole source of carbon and energy, showing rapid growth during the period ranging from day 3-8, after that there were no significant differences in the microbial growth.

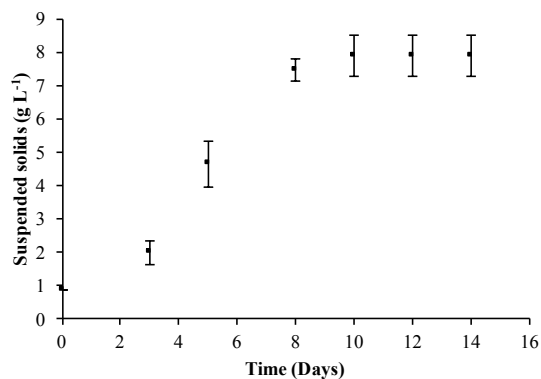


Fig. 3. Growth profile of a native microbial biomass during the Mayan crude oil degradation in a bubble column bioreactor (BCB). Each value is the mean of three replicates.

In particular, the stationary phase began approximately at 8 days of culture, probably at this stage there remained a fraction of hydrocarbons that were difficult to degrade, as previously reported by Jackson (1997). In order to determine growth kinetic parameters, the Gompertz model was adjusted for microbial growth as seen in Fig. 4. As a result, a specific rate of maximum growth (μ_{max}) of 0.76 d^{-1} and a lag phase (λ) of 2.1 days were obtained. Similar studies with pneumatic bioreactors used for hydrocarbon removal, a bacterial consortium using diesel and hexadecane ($\mu_{max} = 1.09 \text{ d}^{-1}$ and $\lambda = 1.66 \text{ d}$) (Lizardi-Jiménez *et al.*, 2012) were observed.

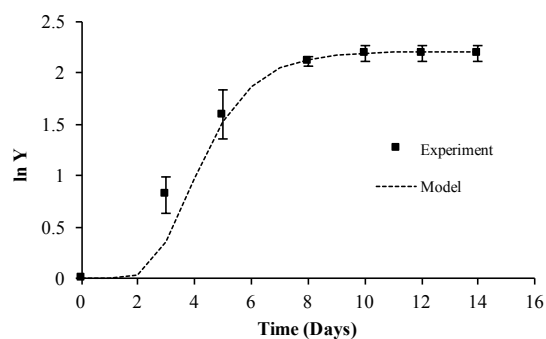


Fig. 4. Gompertz model used to adjust the data of growth profile of native microbial biomass during the Mayan crude oil degradation in a bubble column bioreactor. (■) Real data, (---) Model data. Each value is the mean of three replicates. Y experimental is natural logarithm of ratio of Suspended Solids to Initial Suspended Solids ($\ln [SS/SS_0]$).

In the present study, the parameters μ_{max} and λ were lower than those reported by Lizardi-Jiménez *et al.* (2012). These results could be explained because Mayan crude oil contains higher molecular weight fractions compared with diesel and n-alkanes (Díaz-Ramírez *et al.*, 2003). The hydrocarbons of petroleum differ in their availability to microbial attack; hydrocarbons removal is generally considered in the following order: linear alkanes, branched alkanes, small aromatics, cycloalkanes, polycyclic aromatic hydrocarbons (Pacwa-Plociniczak *et al.*, 2011). Therefore, the results obtained in this work showed that the microbial biomass was able to use hydrocarbons as a sole source of carbon and energy, however, a fraction remained in the BCB, presumable corresponding to those hydrocarbons difficult to metabolize, such as aromatics and asphaltenes.

3.2 Consumption of hydrocarbons

The concentration of residual hydrocarbons was quantified during culture. As shown in Fig. 5, a rapid decline in hydrocarbon removal was observed during the first 8 days of culture. Interestingly, this period of quick consumption of hydrocarbons was related to the increase in microbial growth. Particularly, the microbial biomass was able to degrade $70 \pm 2\%$ of the initial concentration of hydrocarbons. The results obtained in this study were higher than removal efficiencies obtained by Sugiura *et al.* (1996) and Silva *et al.* (2008). These authors reported removal efficiencies of 60% and 57% using a mixture of crude oil by a microbial consortium and *Pseudomonas auroginosa*, respectively.

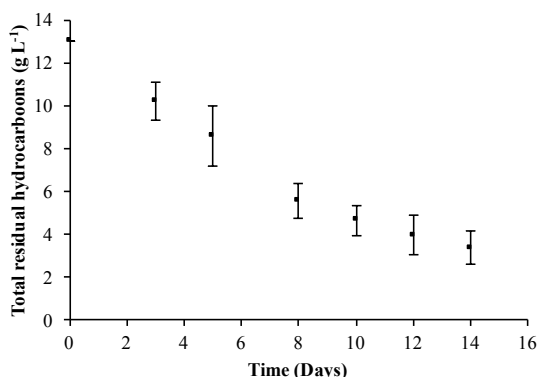


Fig. 5. Kinetic of residual hydrocarbon concentration by a native microbial biomass during the Mayan crude oil degradation in a bubble column bioreactor. Each value is the mean of three replicates.

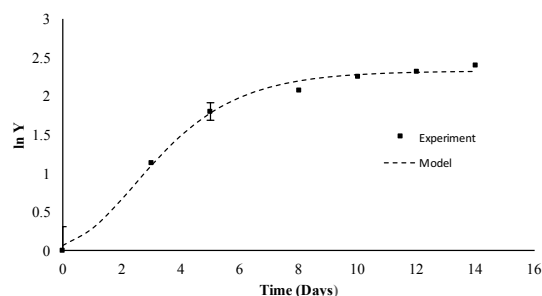


Fig. 6. Gompertz model used to adjust the data of hydrocarbon consumption by a native microbial biomass in a bubble column bioreactor. (■) Real data, (—) Model data. Each value is the mean of three replicates. Y experimental is natural logarithm of ratio of Total Residual Hydrocarbons to Initial Total Residual Hydrocarbons ($\ln [TRH/TRH_0]$).

Conversely, the removal efficiencies obtained in the present study were similar to results reported by Tzintzun-Camacho *et al.* (2014) using a microbial consortium and hexadecane as a source of carbon, reaching a removal efficiency of 69%. A possible explanation for the differences observed could be found in the work of Maroto-Arroyo and Rogel-Quesada (2002); they indicated that the transformation rate of pollutants is influenced by various factors, such as nutrient type, pH, temperature, and hydrocarbon chemical structure. Medina-Moreno *et al.* (2005) demonstrated that it is not possible to overcome levels of removal higher than 75% (assuming a total removal of aliphatics and aromatics).

In order to determine the kinetic parameters for the Maya crude oil removal, the Gompertz model was adjusted to the residual hydrocarbon concentration kinetic data (Fig. 6). As result, a specific rate of maximum consumption (q_{max}) of $0.44 \pm 0.09 \text{ d}^{-1}$ and $\lambda_q = 0.48 \pm 0.06 \text{ d}$ was obtained which was lower than the rate obtained during the exponential growth phase. These results confirm that the microbial biomass is able to degrade hydrocarbons faster during the first days.

The starting point of hydrocarbon consumption began at 34 h of culture time (λ_q). However, remaining hydrocarbons were observed at the end of the culture time, probably due to their degradation difficulty. A possible explanation for the residual hydrocarbon concentration observed was that it is composed of the most recalcitrant compounds to degrade, such as cycloalkanes or asphaltenes.

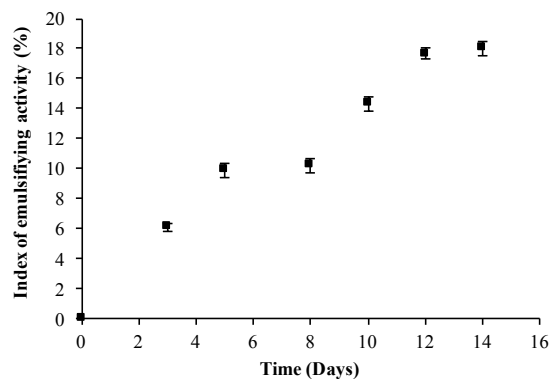


Fig. 7. Kinetic of emulsifying activity of a native microbial biomass during the Mayan crude oil degradation in a bubble column bioreactor. Each value is the mean of three replicates.

3.3 Emulsifying activity

In order to determine strategies of hydrocarbon solubilization, the E24 index was evaluated in this study. According to results, the isolated microbial biomass was able to produce emulsifying agents (Fig. 7). At day 3, the culture showed emulsifying activity resulting in an emulsification index of $6.1 \pm 0.25\%$, while a maximum emulsification index ($17.7 \pm 0.4\%$) was reached at day 12 of cultivation and was maintained up to 14 days (Fig. 7). In addition, the culture showed an apparent color change of the crude oil (Fig. 8) due to the biosurfactant production to solubilize the hydrocarbons. At 0 days, the oil phase was observed as immiscible small droplets dispersed in the liquid phase (culture medium).

However, an emulsion was observed between the two liquid phases (culture medium and oil) after 3 days of culture. González and Ukrainczyk (1999) reported that microorganisms with hydrocarbonoclastic capacity have the ability to produce surfactants, in order to emulsify the hydrocarbons and thus improve their availability in the medium. In addition, the insoluble carbon sources like vegetable oils, motors oils, diesel, and hydrocarbons when present, increase the removal efficiency and biosynthesis of biosurfactants (Martínez-Trujillo *et al.*, 2015).

Biosurfactants act as solubilizing, dispersing, and emulsifying agents, improving the availability of substrates hydrophobic for microorganisms, and consequently the removal of these types of compounds (Vasileva-Tonkova *et al.*, 2008). Interestingly, our findings showed a direct relationship between the emulsifying activity and hydrocarbon removal in

the BCB, that is, hydrocarbon removal increased when the E24 index was high. Our results suggest that the biosurfactant biosynthesis began during the exponential phase but was maintained until the growth stationary phase was attained. According to literature, the biosurfactants are produced as primary metabolites, having a direct relationship with biomass production (Khopade *et al.*, 2012).

However, several strategies can be used by the microorganisms for the consumption of hydrocarbons, regardless of biosurfactant production. For example, Kim *et al.* (2002) have reported two possible mechanisms for hydrocarbon consumption in liquid phases: direct contact and consumption of the emulsified forms. In this study, a direct relation between consumption with hydrocarbon emulsification is shown. On the other hand, Gong *et al.* (2015) reported that the remediation of hydrocarbon spills using bacteria depends on the correct emulsification and stability of the emulsion in the medium. In addition, it is important to mention that in our work, there is a direct relationship between the emulsification and concentration of suspended solids, which can be reflected in the stability of the emulsion by Pickering effect produced by the cells of microbial biomass that act as solid particles, and consequently giving efficient removal of hydrocarbons.

Based on the results of the emulsification index, probably the droplets of hydrocarbons dispersed into the culture medium were taken up by the microorganisms for biosurfactant production during the first 3 days of culture. Then, the biosurfactants acted as emulsifying agents to produce an emulsion between the two liquid phases (culture medium and hydrocarbons).

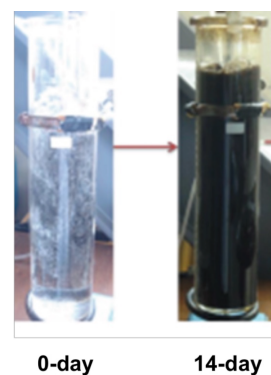


Fig. 8. Changes of coloration during the cultivation of a microbial biomass during the Mayan crude oil degradation in a bubble column bioreactor.

The results obtained from this work contribute to an understanding of microbial strategies to solubilize and remove petroleum hydrocarbons by a native microbial biomass in a bubble column reactor that can be applied as inoculant in bioremediation techniques. Our findings demonstrated the hydrocarbonoclastic potential of selected consortia from a hydrocarbon-contaminated site. In addition, the emulsification of the hydrocarbons of Mayan crude oil is a key mechanism for the use of this substrate as a carbon source. Future studies could be oriented to produce or scale up oil-degrading microbial consortia production, using multiphase systems such as bubble column reactors. Moreover, biodegradation assays of hydrocarbons under lab conditions may not adequately reflect field conditions when in situ bioremediation is considered. As a consequence, it is important to consider for future investigations, the geographic locations, nature and movement of pollutants, competition, aeration, temperature, and nutrients.

Conclusions

The results presented in this study contribute to an understanding of the mechanisms of hydrocarbon-removal in multiphase systems by a native-microbial biomass using Mayan crude oil. The emulsification of the Mayan crude oil was fundamental to allow this substrate to be used as a carbon source, obtaining a removal efficiency of 70%. The Gompertz model was used to determine the growth kinetic parameters for obtaining a maximum growth specific rate (μ_{max}) of 0.76 d^{-1} and a lag phase (λ) of 2.1 days. Based on the hydrocarbonoclastic capacities of the native-microbial biomass, it was revealed as effective and economical for bioremediation purposes. In addition, the model system used in the present investigation could therefore be studied in detail, in order to improve the removal levels, focusing on the evaluation of operating conditions in multiphase systems.

Acknowledgements

This work was supported by the National Council of Science and Technology (288099 INFR-2016-1); and Cátedras-CONACYT (ITSTB 694).

References

- Al-Mutairi, N., Bufarsan, A., Al-Rukaibik, F. (2008). Ecorisk evaluation and treatability potential of soils contaminated with petroleum hydrocarbon-based fuels. *Chemosphere* 74, 142-148.
- Angeles, O., Medina-Moreno, S.A., Jiménez-González, A., Coreño-Alonso, A., Lizardi-Jiménez, M.A. (2017). Predominant mode of diesel uptake: Direct interfacial versus emulsification in multiphase bioreactor. *Chemical Engineering Science* 165, 108-112.
- Cappello, S., Volta, A., Santisi, S., Morici, C., Mancini, G., Quatrini, P., Genovese, M., Yakimov, M.M., Torregrossa, M. (2016). Oil-degrading bacteria from a membrane bioreactor (BF-MBR) system for treatment of saline oily waste: Isolation, identification and characterization of the biotechnological potential. *International Biodeterioration and Biodegradation* 110, 235-244.
- Cerqueira, V.S., Peralba, M.C.R., Camargo, F.A.O., Bento, F.M. (2012). Comparison of bioremediation strategies for soil impacted with petrochemical oily sludge. *International Biodeterioration and Biodegradation* 95, 338-345.
- Cooper, D.G., Goldenberg, B.G. (1987). Surface-active agents from two *Bacillus* species. *Applied Environmental Microbiology* 53, 224-229.
- Das, N., Chandran, P. (2011). Microbial degradation of petroleum hydrocarbon contaminants: An overview. *Biotechnology Research International* 2011, 1-13.
- Denis, B., Pérez, O.A., Lizardi-Jiménez, M.A., Dutta A. (2017). Numerical evaluation of direct interfacial uptake by a microbial consortium in an airlift bioreactor. *International Biodeterioration and Biodegradation* 119, 542-551.
- Díaz-Ramírez, I.J., Ramírez-Saad, H., Gutiérrez-Rojas, M., Favela-Torres, E. (2003). Biodegradation of Maya crude oil fractions by bacterial strains and a defined mixed culture

- isolated from *Cyperus laxus* rhizosphere soil in a contaminated site. *Canadian Journal of Microbiology* 49, 755-761.
- Gesinde, A.F., Agbo, E.B., Agho, M.O., Dike, E.F.C. (2008). Bioremediation of some Nigerian and Arabian crude oils by fungal isolates. *International Journal of Pure and Applied Science* 2, 37-44.
- Gong, H., Li, Y., Bao, M., Lv, D., Wang, Z. (2015). Petroleum hydrocarbon degrading bacteria associated with chitosan as effective particle-stabilizers for oil emulsification. *RSC Advances* 5, 37640-37647.
- González, J., Ukrainczyk, L. (1999). Organic chemicals in the environment. *Journal of Environmental Quality* 28, 101-107.
- Heipieper, J., Cornelissen, S., Pepi, M. (2010). Surface properties and cellular energetics of bacteria in response to the presence of hydrocarbons. In: *Handbook of Hydrocarbon and Lipid Microbiology*, (K.N. Timmis, ed.), Pp. 1615-1624. Springer, Berlin.
- Jackson, K. (1997). Policing the offshore environment. *Petroleum Review* 51, 153-155.
- Khopade, A., Ren, B., Liu, X.Y., Mahadik, K., Zhang, L., Kokare, C. (2012). Production and characterization of biosurfactant from marine *Streptomyces* species B3. *Journal of Colloid and Interface Science* 367, 311-318.
- Kim, I.S., Foght, J.M., Gray, M.R. (2002). Selective transport and accumulation of alkanes by *Rhodococcus erythropolis* S+ 14He. *Biotechnology and Bioengineering* 80, 650-659.
- Kuiper, I., Lagendijk, E.L., Bloemberg, G.V., Lugtenberg, B.J. (2004). Rhizoremediation: a beneficial plant-microbe interaction. *Molecular Plant-Microbe Interactions Journal* 17, 6-15.
- Lakra, J., Tikariha, D., Yadav, T., Das, S., Ghosh, S., Satnami, M.L., Ghosh, K.K. (2014). Mixed micellization of gemini and cationic surfactants: Physicochemical properties and solubilization of polycyclic aromatic hydrocarbons. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 451, 56-65.
- Lizardi-Jiménez, M.A., Saucedo-Castañeda, G., Thalasso, F., Gutiérrez-Rojas, M. (2012). Simultaneous hexadecane and oxygen transfer rate on the production of an oil-degrading consortium in a three-phase airlift bioreactor. *Chemical Engineering Journal* 187, 160-165.
- Lu, M., Zhang, Z., Sun, S., Wang, Q., Zhong, W. (2009). Enhanced degradation of bioremediation residues in petroleum-contaminated soil using a two-liquid-phase bioslurry reactor. *Chemosphere* 77, 161-168.
- Marín-Muñiz, J.L. (2016). Removal of wastewater pollutant in artificial wetlands implemented in Actopan, Veracruz, Mexico. *Revista Mexicana de Ingeniería Química* 15, 553-563.
- Maroto-Arroyo, M.E., Rogel-Quesada, J.M. (2002). Aplicación de sistemas de biorremediación de suelos y aguas contaminadas por hidrocarburos. *Geocisa. Div. Protección Ambiental de Suelos*, 297-305.
- Martínez-Trujillo, M.A., Membrillo Venegas, I., Viguera-Carmona, S.E., Zafra-Jiménez, G., García-Rivero, M. (2015). Optimization of a bacterial biosurfactant production. *Revista Mexicana de Ingeniería Química* 14, 355-362.
- Medina-Moreno, S.A., Huerta-Ochoa, S., Gutiérrez-Rojas, M. (2005). Hydrocarbon biodegradation in oxygen-limited sequential batch reactors by consortium from weathered, oil-contaminated soil. *Canadian Journal of Microbiology* 51, 231-239.
- Medina-Moreno, S.A., Jiménez-González, A., Gutiérrez-Rojas, M., Lizardi-Jiménez, M.A. (2013). Hexadecane aqueous emulsion characterization and uptake by an oil-degrading microbial consortium. *International Biodeterioration and Biodegradation* 84, 1-7.
- Medina-Moreno, S.A., Jiménez-González, A., Gutiérrez-Rojas, M., Lizardi-Jiménez, M.A. (2014). Hydrocarbon pollution studies of underwater sinkholes along Quintana Roo as a function of tourism development in the Mexican Caribbean. *Revista Mexicana de Ingeniería Química* 13, 509-516.
- Melgarejo-Torres, R., De la Concha-Gómez, A.D., Calva-Hernández, R., Lizardi-Jiménez, M.A. (2017). Study of variation in droplet size of

- an aqueous-diesel emulsion formed by an oil-degrading microbial consortium. *Journal of Petroleum Science and Engineering* 157, 951-957.
- Pacwa-Płociniczak, M., Płaza, G.A., Piotrowska-Seget, Z., Cameotra, S.S. (2011). Environmental applications of biosurfactants: Recent Advances. *International Journal of Molecular Science* 12, 633-654.
- Pacwa-Płociniczak, M., Płociniczak, T., Iwan, J., Zarska, M., Chorazewski, M., Dzida, M., Piotrowska-Seget, Z. (2016). Isolation of hydrocarbon-degrading and biosurfactant-producing bacteria and assessment their plant growth-promoting traits. *Journal of Environmental Management* 168, 175-184.
- Penberthy, J., Weston, R. (2000). Remediation of diesel and fuel oil hydrocarbons in high clay content soils: a field comparison of amendment performance conducted at the Mare Island naval shipyard. In: *Proceedings of the National Defense Industrial Association*, Pp. 27-30. Roy F. Weston, Inc, Santa Barbara.
- Silva, R.M.P., Pozo, M.I.C., de Oca, J.M.G.M., Rodríguez, A.Á., Viñas, M., Moreno, D.C. (2008). Aislamiento y selección de una cepa bacteriana degradadora de hidrocarburos a partir de suelos contaminados con petróleo. *Revista CENIC Ciencias Biológicas* 39, 44-51.
- Sugiura, K., Ishihara, M., Shimauchi, T., Harayama, S. (1996). Physicochemical properties and biodegradability of crude oil. *Environmental Science and Technology* 31, 45-51.
- Tzintzun-Camacho, O., Torres-Martinez, D., Gutiérrez-Rojas, M., Lizardi-Jiménez, M.A. (2014). Gas hold up on the cultivation of an oil-degrading bacterial consortium. *Environmental Engineering and Management Journal*, In Press.
- Van Hamme, J.D., Singh, A., Ward, O.P. (2003). Recent advances in petroleum microbiology. *Microbiology and Molecular Biology Reviews* 67, 503-549.
- Vasileva-Tonkova, E., Galabova, D., Stoimenova, E., Lalchev, Z. (2008). Characterization of bacterial isolates from industrial wastewater according to probable modes of hexadecane uptake. *Microbiological Research* 163, 481-486.
- Zwietering, M.H., Jongenburger, I., Rombouts, F.M., Van't Riet, K. (1990). Modeling of the bacterial growth curve. *Applied and Environmental Microbiology* 56, 1875-1881.