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Asphaltenes biodegradation under shaking and static conditions

H. Jahromi [a], M.H. Fazaelipoor [a,*], Sh. Ayatollahi [b], A. Niazi [c]

[a] Department of Chemical Engineering, Shahid Bahonar University of Kerman, Iran
[b] School of Chemical and Petroleum Engineering, Sharif University of Technology, Iran
[c] Institute of Biotechnology, Shiraz University, Iran

HIGHLIGHTS

- The ability of several bacterial species for asphaltenes degradation was documented.
- Asphaltenes degradation was quantified under shaking and static conditions.
- A bio-kinetic model was presented for asphaltenes degradation.

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ABSTRACT

In this study the biodegradability of asphaltenes was investigated using four bacterial consortia isolated from oil contaminated soils and sludge. The species in consortium 1 were identified as *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*. Consortium 2 contained *Citrobacter amalonaticus* and *Enterobacter cloacae*. Consortium 3 contained only one species identified as *Staphylococcus hominis*, and the species in consortium 4 were identified as *Bacillus cereus*, and *Lysinibacillus fusiformis*. Spectrophotometry at 281 nm wavelength was applied to quantify asphaltenes biodegradation. The biodegradation tests were performed in flasks with the initial asphaltenes concentrations of 2, 4, 10, 20, 30 and 35 g/L for the four consortia. Under shaking conditions the best results were obtained with the initial asphaltenes concentration of 35 g/L. With this initial concentration, consortia 1,2,3, and 4 were able to degrade 51.5%,43%, 21.5% and 33.5% of asphaltenes, respectively at 40 °C in two months. Under static conditions the best results were obtained with the initial asphaltenes concentration of 30 g/L. Under these conditions, consortia 1,2,3, and 4 were able to degrade 32%, 27%, 15%, and 24% of asphaltenes, respectively at 40 °C in two months. Kinetic studies showed that Tessier model could accurately describe asphaltenes biodegradation under shaking conditions. Kinetic parameters of the model were fitted by the method of Differential Evolution Optimization using a specific set of experimental data for each consortium. FT-IR analysis showed that alkene and alkyne functional groups were easily biodegradable while aldehydes resisted biodegradation.

1. Introduction

Asphaltenes, causing such problems as reduction in oil recovery and environmental contamination, are thought to be recalcitrant to biological transformations [1–5]. Asphaltenes are high molecular weight solids which are soluble in aromatic solvents such as benzene and toluene and insoluble in paraffinic solvents. The transformation rates for these large molecules are limited by low solubility and mass transfer rates in aqueous media [6]. Despite these facts, there is evidence in the literature for bacterial transformation of these complex, high molecular weight substrates. This is possible because these compounds contain carbon, hydrogen, sulfur, nitrogen and oxygen, which are necessary elements for microbial growth [7–11].

Some researchers extracted asphaltenes from crude oil and examined their biodegradability as a separated fraction. Pendrys isolated seven gram negative, aerobic asphaltenes degrading bacteria by an enrichment technique. The predominant genera of these isolates were *Pseusomonas*, *Acinetobacter*, *Alcaligenes* and *Flavobacterium*. A mixed culture of these bacteria could use asphaltenes as a sole source of carbon and energy [12]. Pineda et al. reported the utilization of asphaltenes as a sole source of carbon and energy by a microbial consortium isolated from Maya crude oil. The isolates were identified as *Corynebacterium* sp., *Bacillus* sp., *Brevibacillus* sp., and *Staphylococcus* sp. [13]. Tavassoli et al. reported 46% biodegradation of asphaltenes after two months, with the initial concentration

* Corresponding author. Address: Department of chemical engineering, Shahid Bahonar University of Kerman, Kerman, Iran. Tel.: +98 341 2262916; fax: +98 341 2118298.
E-mail addresses: fazaelipoor@uk.ac.ir, fazaelipoor@yahoo.com (M.H. Fazaelipoor).

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Table 1
The results of biochemical tests for microbial identification.

Isolate	Gram stain	H2S production	Utilization of urea	Utilization of rhamnose	Utilization of sucrose	NO_2 production	Reduction of N_2	Catalase	Hydrolysis of gelatin
Pseudomonas aeruginosa	–	–	–	–	+	–	+	+	+
Pseudomonas fluorescens	–	–	–	–	+	+	+	+	+
Citrobacter amalonaticus	–	–	–	+	–	+	+	+	–
Enterobacter cloacae	–	–	–	–	+	–	+	+	+
Staphylococcus hominis	+	+	+	–	+	+	–	–	–
Bacillus cereus	+	–	–	+	–	+	+	+	+
Lysinibacillus fusiformis	+	+	+	–	+	+	–	–	+

Table 2
Species obtained from the original samples.

Source	Consortium	Mocroorganism
Soil sample from Shiraz refinery	Consortium 1	*Pseudomonas aeruginosa* *Pseudomonas fluorescens*
Oily sludge from Shiraz refinery	Consortium 2	*Citrobacter amalonaticus* *Enterobacter cloacae*
Soil sample from Assaluyeh	Consortium 3	*Staphylococcus hominis*
Oily sludge from Assaluyeh	Consortium 4	*Bacillus cereus* *Lysinibacillus fusiformis*

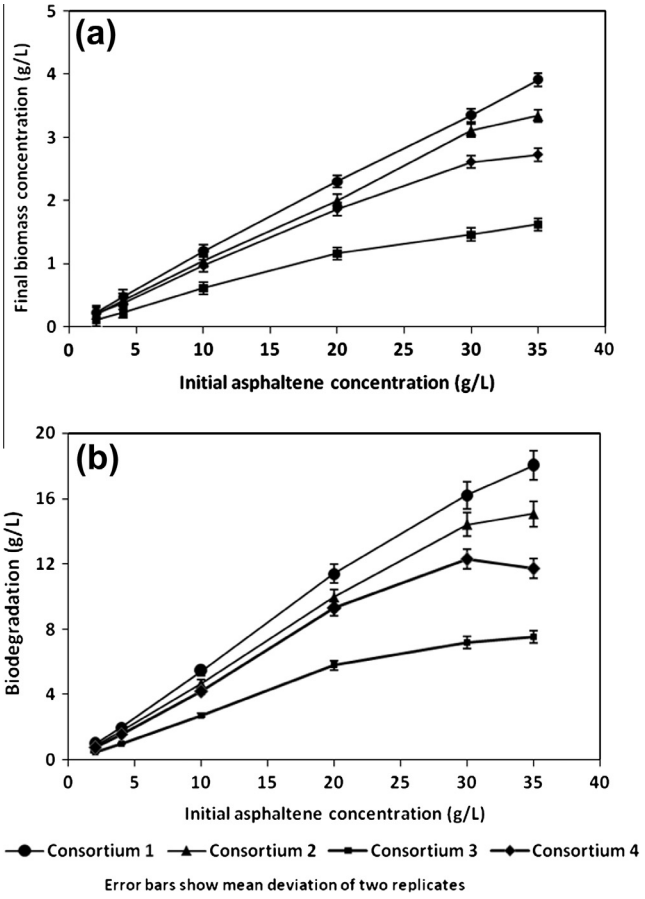

Fig. 1. (a) Final biomass concentration and (b) asphaltenes biodegradation after two months, under shaking conditions.

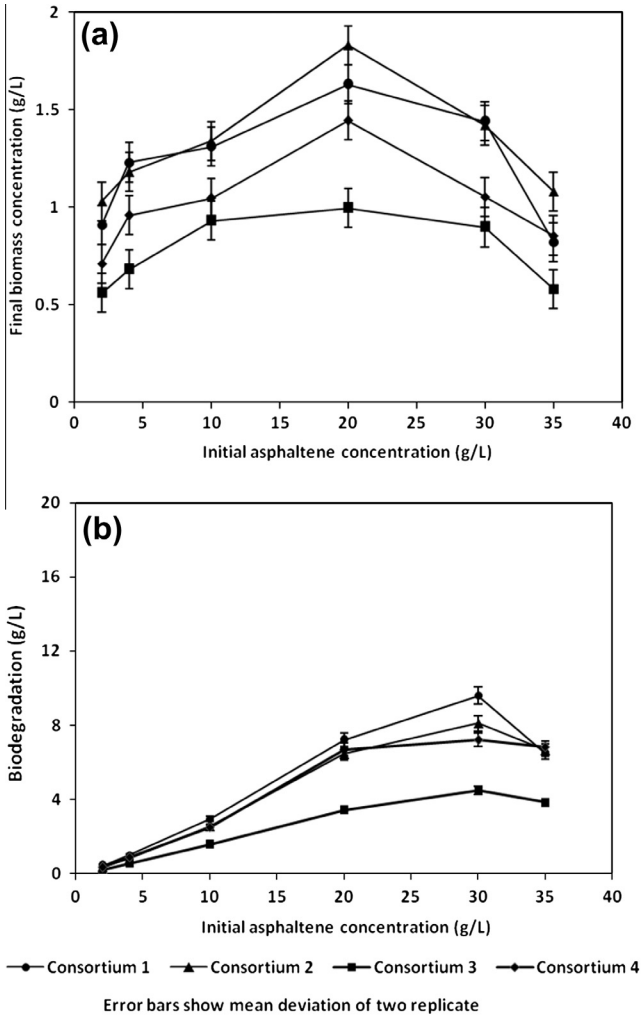

Fig. 2. (a) Final biomass production and (b) asphaltene biodegradation, both after two months under static conditions.

of 5 g/L. They reported the capability of the microorganisms, identified as *Pseudomonas* sp, *Bacillus licheniformis*, *Bacillus lentus*, *Bacillus cereus* and *Bacillus firmus*, to utilize asphaltenes as carbon and energy source. Furthermore, they concluded that asphaltenes biodegradation follows Tessier kinetics model [14]. In another development Lavania et al. isolated an asphaltenes degrading bacterial species identified as *Garciaella petrolearia*. Growth of this bacterium on asphaltenes resulted in viscosity reduction of up to 37% [15].