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Biodegradation of waste water treatment containing petroleum hydrocarbon using Rotating Biological Contractor (RBC)

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Abstract — *The ability of biological treatment of produced water, that separated from petroleum hydrocarbons was evaluated using rotating biological contactor (RBC) reactor. A strain of Pseudomonas alkanolytica ATCC 21034 have been examined to degrade petroleum hydrocarbons separate in surface water. Some environmental parameters will influence on biodegrade of hydrocarbons to find the optimum conditions. Therefore, this study were investigated various parameters include: initial biomass concentrations, dodecane concentrations and number of stages rotating. Different tests was performed by varying initial biomass from (0.125, 0.250 and 0.4) mg/l. The appropriate conditions initial biomass for biodegradation were observed at 0.25 mg/l. Dodecane as substrate was studied at varied concentrations (0.5, 1 and 2%) v/v that equivalent as (221.2, 442.4 and 884.8 mg/l) respectively. Results showed the high biodegradation was obtained at 2% v/v. The size of biodegradation increased when higher dodecane concentrations were applied. A set of experiments have been carried out at varied number of rotating as a bioreactor (1- 4). Results exposed the quantity efficiency of biodegradation was more than 87.9% when the number of stages rotating was 4 stages.*

Keywords- *biological treatment; hydrocarbon dodecane; Pseudomonas alkanolytica; rotating biological contactor (RBC).*

INTRODUCTION

Production of oil and gas are very important processes for industrial. In contrast, produced water is considered to be one of the largest waste streams in the petroleum and gas industry. Oil and gas processes may be associated by huge production of contaminated water with pollutants, such as heavy metals, organic compounds, and dissolved/suspended solids [1, 2]. Therefore, one of the most important environmental pollution factors today is pollution caused by petroleum and petroleum derivatives. These cause a negative impacts on the human body [3, 4, 5]. This pollution can create environmental and economy harms. Biological treatment is an effective and economical approach that can be used in oil de-emulsification and wastewater treatment [6,7, 8]. It has been declared as innovative and promising biotechnology processes. Biodegradation of Petroleum hydrocarbon by bacteria, yeast, and fungi, which can grow using crude oil as a source of carbon and energy, have been reported by [9,10,11,12]. So far, conventional treatment processes are not sufficient to achieve the water quality requirements, advanced treatment processes are required as literature by [13,14]. Therefore, development the efficient bio-treatment processes for persistent chemicals in the environment including petroleum hydrocarbons is increasingly important in situ treatment for accidental release of petroleum and/or in wastewater treatment from industrial discharges. Several techniques and types of bioreactor for the biodegradation of hydrocarbons have been proposed. Rotating biological contactor (RBC) is a unique reactor adaptation of the attached culture process (biofilm process) [15, 16] . This reactor is a fixed biological film (biofilm) reactor system, in general, consists of a series of plastic circular disks (biodisk) mounted on a horizontal shaft and rotated perpendicular to the direction of the waste flow. Media in the form of flat disks mounted on a common shaft are rotated through especially contoured tanks in which wastewater flows on continuous basis. A laboratory scale study has been conducted to assess the efficiency of rotating biological contactor (RBC) to treat the synthetic wastewater from a petrochemical industry producing .Therefore, the aim of this study was examined the potential of RBC to carry on a sustainable treatment using a strain of bacteria. and evaluated the effect of various parameters affecting on biodegrade hydrocarbons.

2 Materials an d Methods

2.1 Microorganisms

Pseudomonas alkanolytica ATCC 21034 bacterial strain culture stock was obtained from a culture collection maintained at the (Chemical and Material Engineering Department-King Abdulaziz university, KSA). The culture stock was grown on marmul^{+} medium for 3 days using glucose as carbon source. The strain has the ability to use hydrocarbon oils as a sole carbon source and can also grow at high salt concentration as reported by [17]. It is a gram-positive bacterium that

exhibited the following physiological characteristics: yellow colored colonies on nutrient agar, presence of motility and spore formation and producing coenzyme A utilizing hydrocarbons [18].

2.2 Culture media

The medium used in this work was marmul^{+}, a synthetic similar to the water produced from one of the wells in the Middle East [19, 20]. The composition that used in this work is listed in Table 1. During the experiments the pH solution medium was adjusted at 7± 0.2 with a solution of NaOH (1N).

Table 1: Composition of culture media marmul^{+}

Stock solution No.	formula	Quantity (g)
1	Na_2HPO_4	3.15
2	KH_2PO_4	1.75
3	$MgSO_4.7H_2O$	0.20
4	$(NH_4)SO_4$	2.0
5	$CaCl_2$	0.05
6	$FeSO_4.7H_2O$	0.001
7	H_3BO_3	0.010
8	$MnSO_4.7H_2O$	0.010
9	$ZnSO_4.7H_2O$	0.070
10	$(NH_4)_6 Mo_7O_4. 4H_2O$	0.010
11	$CuSO_4.5H_2O$	0.050

2.3 Reactor configuration

The rotating biological contactor (RBC) system is a model process for wastewater treatment in which the large amount of biomass permits short contact time, maintains a stable system under variable loading and produce an effluent meeting secondary treatment standards [23]. In this work a laboratory scale (RBC) was designed to treat wastewater of the petroleum hydrocarbons as illuminated by Figure 1. This (RBC) reactor consisted of a semicircular trough (volume 26.25 l). The tank was divided into four stages of equal volume. The discs are submerged in the wastewater to about 40% of their diameter and are rotated by power supplied to the shaft. Physical properties of this system is listed in Table 2.

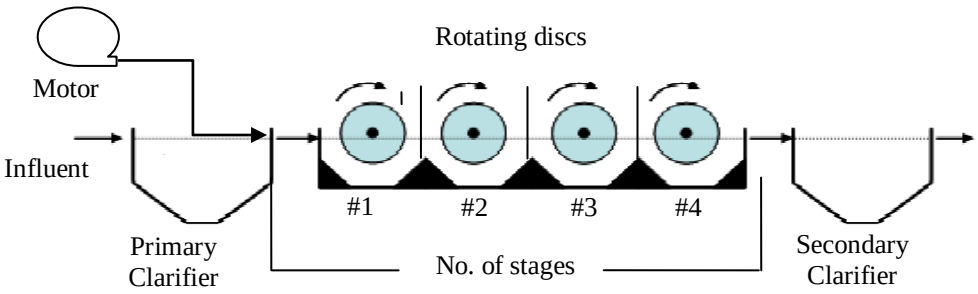

Fig. 1 Rotating biological contactor system.

Table 2: Physical properties of the rotating biological contactor

Length	1 m
height	0.5 m
Total volume	26.25 L
Number of Stages (rotating)	4
Effective volume	10.5 L
Rotational speed	20 ppm
Diameter of each disc	23 cm
Thickness of the strip	1.5 mm
Spacing between flat discs	3 mm

2.4 Hydrocarbon (dodecane)

Dodecane is one of medium-chain n-alkanes ($C_{12}H_{26}$) with low solubility in water (0.0037 mg/l at 25°C). It has been used as a representative hydrocarbon for liquid alkanes in various hydrocarbon mixtures [21]. Moreover, dodecane represents 42% of the petroleum pollutants [22]. In this work dodecane commercialized by Sigma- Aldrich and was chosen as the sole carbon source to simplify the study.

2.5 Experimental procedure

The suspension was prepared from marmul+ medium using Tween 20 (surfactant) and dodecane as hydrocarbon in a volume of 1:30. The suspension was prepared by introducing the dodecane as drops, into the concentration mixed marmul+ /surfactant solution and the mixture was stirred at least 3 hours to ensure homogeneity. The reactor was fed of suspension and strain [24] with different concentrations of dodecane (0.5%, 1% and 2% v/v). The chemical oxygen demand was monitored and analyzed every two days according to standard methods to detect the equivalent of carbon consumed and water quality. All experiments were achieved within 7 days using this strain.

2.6 Analysis methods

The cell of biomass concentrations were estimated by optical density spectrophotometer (OD), that measuring the optical density at the 660 nm wavelength. The composition of the hydrocarbon biodegradation was analyzed using Chemical Oxygen Demand (COD) which estimated the amount of oxygen needed to consume the organic and inorganic materials. The (COD) can be used as optimum functional for carbon degradation of the wastewater treated with experimental and commercial activated carbons were digested at 150 °C for two hours and then subjected to analysis by a colorimetric method (HACH- DR-3900) recommended by the standard method for examination of water and wastewater [25]. The test method consisted of the use of HACH test vials containing dichromate reagent as a chemical oxidant for measuring COD in the range of 100–1500 mg/l . Also. To study the initial biomass, and number of rotating. The experimental temperature was maintained at room temperature. Initial biomass 0.125, 0.250 and 0.50 g/l were evaluated. The Initial hydrocarbon concentrations of 0.5, 1 and 2 % (v/v) respectively, number of rotating of RBC of 1, 2, 3 and 4 stages.

3. Results and discussion

3.1 Effect of initial biomass

The initial carbon concentration is an important role in the size and yield of biodegradation of hydrocarbons [26, 27]. The effect of the initial biomass concentrations of 0.125 g/l, 0.25 g/l, and 0.50 g/l were investigated in the biodegradation of petroleum hydrocarbons by RBC reactor. The absorbance optical densities (OD)S at 660 nm were 0.815, 1.361, and 0.95 for the 0.125, 0.25 and 0.50 g/l, respectively. Fig.2 illustrated the high growth was at 0.25 g/l. therefore, it was the

best condition for increase the biodegradation of hydrocarbon. Table.3 shows that the bacteria grew fairly well in the marmul+ medium with hydrocarbon suspension as a carbon source. These results reflect that a higher biodegradation efficiency, doubling time, and specific growth rate and most suitable conditions were observed when the biomass concentration size was 0.25 g/l. This result is in agreement with the results reported by references [13, 23] using other microorganisms and different carbon source.

Table 3: Effect of initial biomass concentrations by *Pseudomonas alkanolytica*.

Parameters	Initial biomass concentration g/l		
	0.125	0.25	0.50
Final pH	7.43	7.52	7.71
Specific growth rate, h^{-1}	0.0192	0.0541	0.033
Initial growth at Time = 0, day	0.032	0.061	0.056
Final growth at final Time	0.815	1.031	0.95
Duration biodegradation, days	7	7	7
% Efficiency of removal hydrocarbons	70.01	87.90	84.73

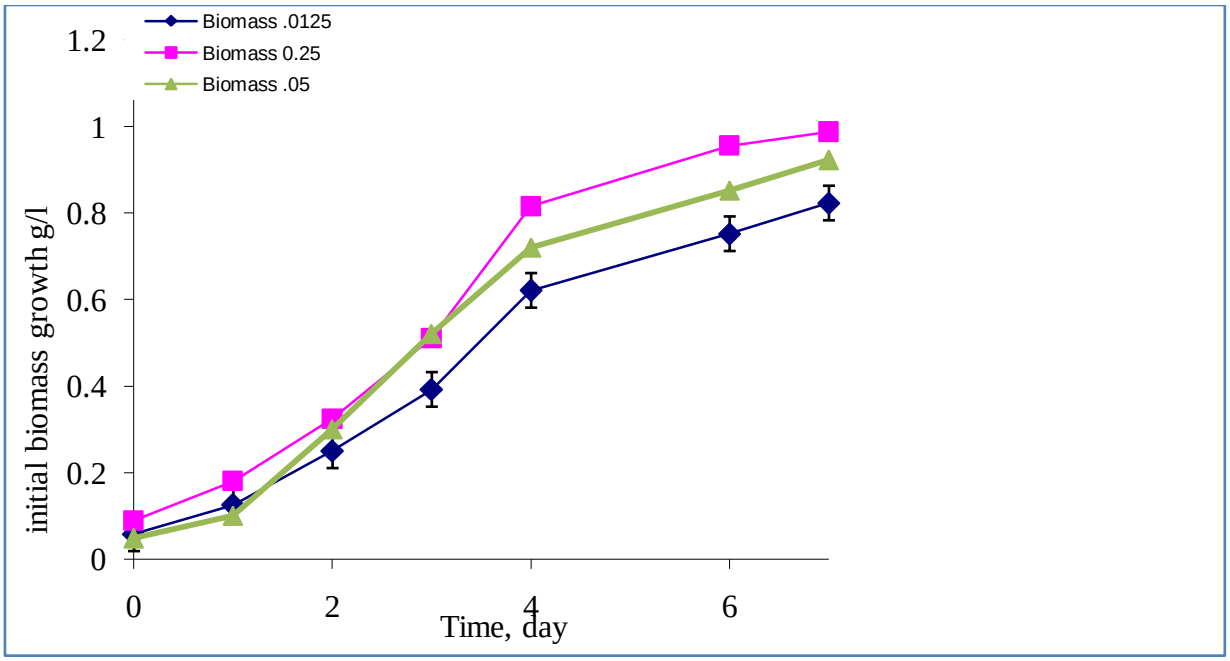

Fig.2 Effect of the initial biomass on the growth by *P. alkanolytica.*

3.2 Effect of a dodecane concentration

The kinetics and microbial growth yields are two much significant of biodegradation process parameters, which depend on substrate and biomass concentrations [28]. Experiments were carried out at different dodecane concentrations as a carbon source ranging (0.5, 1.0 and 2.0 % *v/v* that equivalent as 221.2, 442.4 and 884.8 mg/l respectively). Table 4 listed

and presented the effect of carbon concentration on biodegradation by *P. alkanolytica* during RBC reactor. In this batch study for hydrocarbon degradation using *Pseudomonas alkanolytica*, the hydrocarbon concentration employed was found to range from 200 to 900 mg/L and the results demonstrate the values of substrate concentration with duration of biodegradation, carbon consumption and residual carbon concentration.as shown in the Table 4. Results observed the efficiency of carbon removal was 2% (*v/v*) with consumption of 87.9% , as illustrated in Figure 3. Experimental runs carried out with fixed room temperature and pH and 7.0±0.2 respectively.

Table 4: Effect of carbon concentration on biodegradation wastewater by *P. alkanolytica*.

Carbon concentrations (*v/v*) %	During of biodegradation, days	Carbon consumption %	Residual carbon concentration mg/l
0.5% ≈ (221.2 mg/l)	7	54.5	120
1.0% ≈ (442.4 mg/l)	7	77.2	100
2.0% ≈ (884.8 mg/l)	7	87.9	107

From the results the residual carbon concentration during the growth of *P. alkanolytica* in a batch (RBC) system decreased over time.

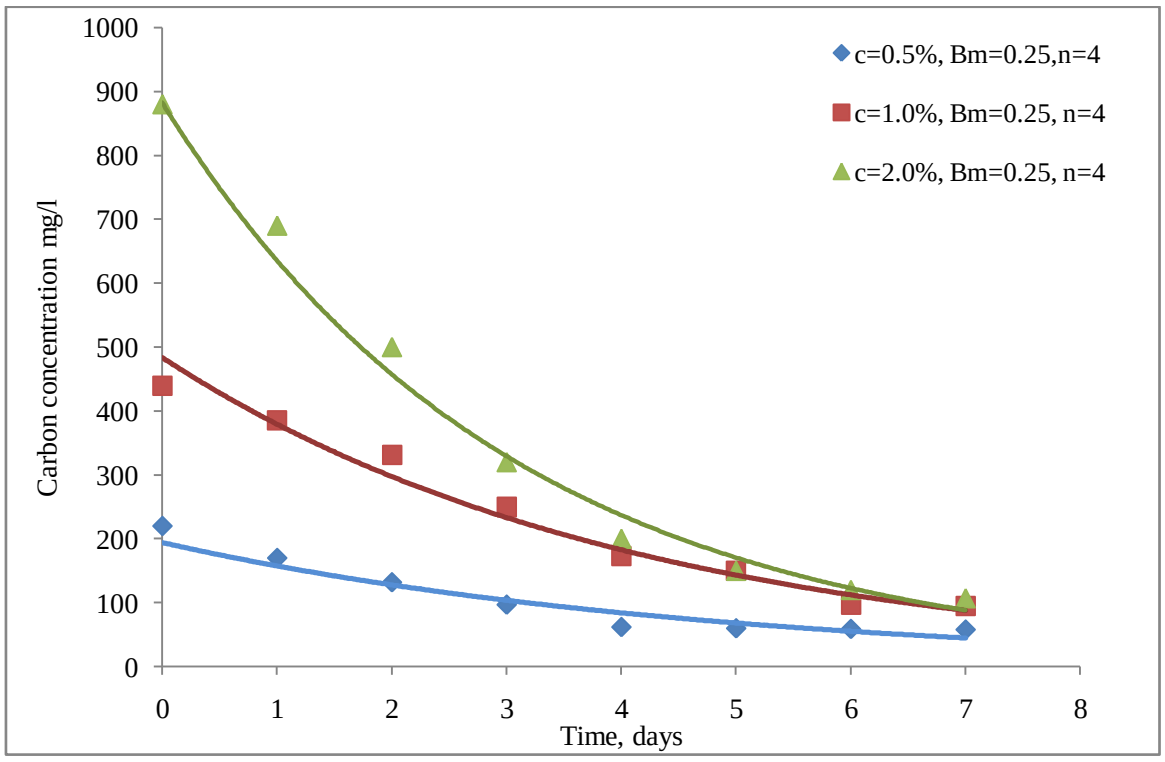

Fig. 3: biodegradation carbon concentration with time.

3.3 Effect of a number of rotating

Rotating Biological Contactors is only aerobic treatment process that has both moving film of attached-growth biomass and suspended growth microbial systems. Therefore, RBC has the ability to provide high dissolve oxygen (OD) attentiveness in the bulk liquids due to diffusive transfer of oxygen from air into the exposed liquid film surface [29, 30, 31]. The effect of number of stages (rotating) on the rate of biodegradation of hydrocarbon was also investigated in this study using batch (RBC) system. The range of rotating studied was from 1 to 4. Results shows the volume of biodegradation of hydrocarbons increased when higher number of rotating (stages) were applied. The profile of removal

of carbon and residual carbon concentration during the course of growth of *P. alkanolytica* in a batch system is presented in (Fig. 4). Carbon removal increased with number of rotating during the experiment while the residual carbon concentration decreased over the number of stages.

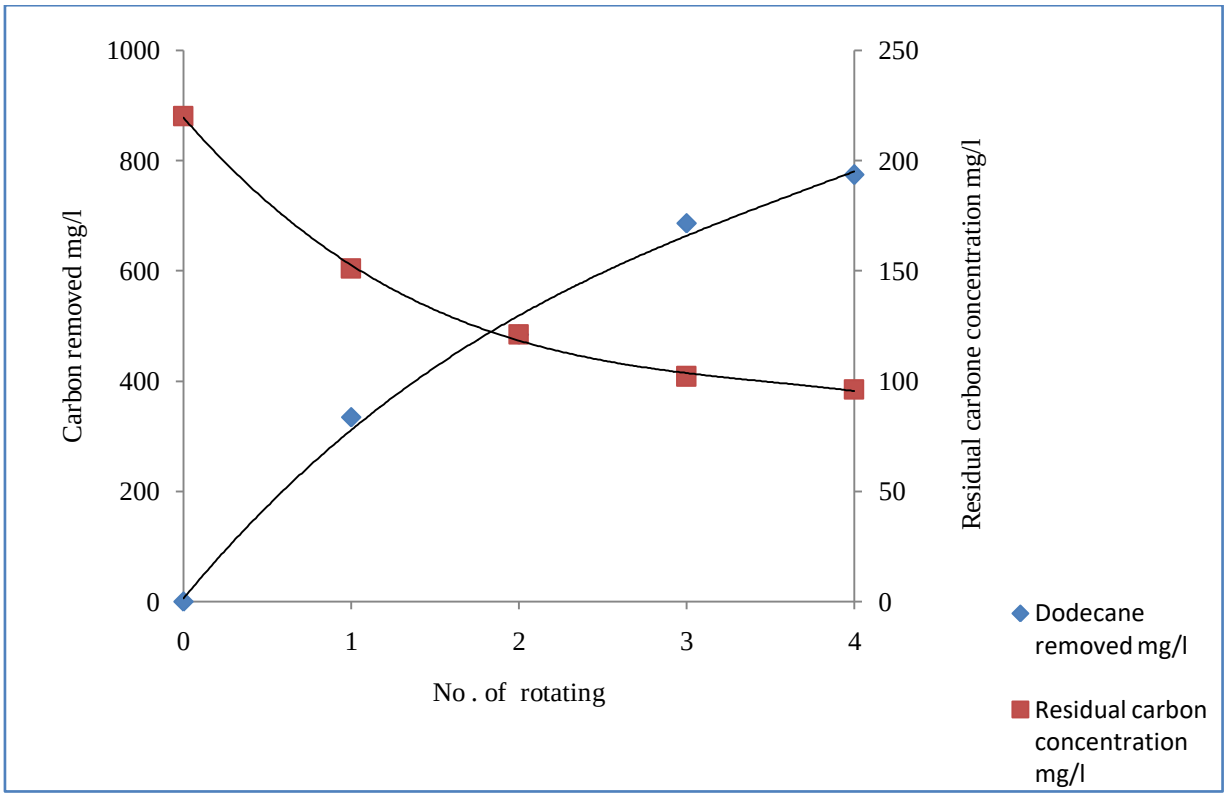

Fig. 4. Profile of removal of carbon and residual carbon concentration during the course of growth of *P. alkanolytica* with number of rotating.

4. Conclusion

This study was carried out to investigate some environmental factors effects on biodegradation of hydrocarbons using Pseudomonas alkanolytica by rotating biological contactors system. These parameters include, initial biomass, dodecane as hydrocarbon source and number of rotating. The most suitable conditions for initial biomass of biodegradation were observed at 0.25 mg/l. The high biodegradation of hydrocarbon when the dodecane concentration as source of carbon were examined at 884.8 mg/l that equivalent (2% v/v). The efficiency of biodegradation was more than 87.9% when the number of stages rotating was 4 stages.

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