Microscopically observations for *Rhodotorula* sp biodegradation to crude and exhausted oil

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Abstract

In this present study Benghazi Lake or 23rd of July Lake is considered as the major environmental and ecological problematic as results of several contaminations. A Success isolated of *Rhodotorula* strain from Benghazi Lake by three tested media of solid and broth, it cultivated for 2-4 weeks. The results are shown that it has highly degradable of crude oil and their derivatives reached about of 70-80 % during 1-2 weeks, while it has degraded to exhausted fuel oil of 95% for 7-10 days. The observations of two media 1, and 2 had visibility differences, whereas the examination by light Microscope were closed similarity, due to the structure of slimy capsulated; it may be enhanced to the production of biosurfactant. A solubility test is confirmed that the ball shaped formation of medium 2 has structured of viscosity and biogases. So this study has shown that a novel strain of *Rhodotorula sp* is promising to use in the bioremediation for the degradation of petroleum, their derivatives and exhausted fuel oil.

Keywords: Exopolysaccharide (EPS), Crude Oil, Exhaust Oil, Potato Dextrose Agar (PDA).

1 Introduction

Benghazi Lake or 23rd July Lake is considered as a nice viewing to the tourist in the second largest city of Libya. Nowadays this lake is one of the major environmental and ecological problematic as results of several contamination sources such as industrial wastes, chemical wastes, hospitals wastes, plastics wastes, as well as petroleum or their derivatives has released amount of drain of pollutions to waters [http ://www. lenntech. com/ abstracts / 2265 / pollution – of – water – resources – from – industrial – effluents - a-case –study – Benghazi - Libya]. Indeed the most important influential problem that rises from these pollutants was contaminant by sewage sludge comes from different sites of origin [http://www.aljazeera.net/news/pages/ca27c7d7-bbf8-460e-bfbe-4b747bb4e919].

Therefore this lake is became as a harmfully causative agent have too many ecosystem and environmental problems somewhat effect to humans, animals, plants or other natural components of ecosystem (Elhassadi, 2008). Unfortunately the dangers pollutants to the people in such as that a releasing of worst odors, its similar to smell of spoiled eggs that indicated releasing to the poison gas of hydrogen disulfide. So almost those people have dominant accommodation on street Jamal Abd El- Nasser or around this area in Juliana region

extension of Benghazi Lake their health will be exposed in high risk by these pollutant circumstances (Abdulsamad and Elbabour , 2013). Although in recent years the process of bioremediation have applied by microorganisms to remove pollutants hence it depends on their growth rates, resist or adapt to the most environmental, biological, chemicals or physicals variables that those have exposed. Most researchers have looked for the best famous species general known such as bacteria, fungi or yeast (Minegishi *et al*., 2006). It could be grown at high growth rate and adapted to any unusual conditions, such as high temperatures, toxic chemical compounds e.g., petroleum, or its derivatives (de Cássia *et al*., 2007). So many of the previous studies confirmed that there are different types of bacteria for example : *Pseudomonas* sp, *Rhodococcus* sp, *Mycobacterium* sp, *Xanthomonas* sp, and *Alcaligenes* sp (Abari *et al* , 2012).

Foght *et al*., in 1989 have been reported on the utilization of crude oil by pure bacterial strains of *Acinetobacter calcoaceticus* RAG-1 and *Pseudomonas sp*. HL7b. Such yeasts strains of *Candida* sp, and *Rhodotorula* sp also have possesse the ability of biodegradations to the hydrocarbons e.g. petroleum and their derivatives due to the pathway of enzymatic feedback (Das and Chandran, 2011). In addition to Shailubhai *et al*. in 1985 have reported the degradation of oil sludge by pure strains of *Rhodotorula rubra* and *Pseudomonas aeruginosa*. Recent studied were reported by Haggag, and Bempelou in 2013 indicated that the a strain of *Rhodotorula glutinis* have ability to break down pesticides, and it used to as a biological control in the environment, and also has been a high production capacity for effective compounds as antibacterial agent and antifungal agent. Then the aim in this purpose study is searched for the solutions that help Libyan government to reducible levels of pollutions for Benghazi Lake by isolated some highly biodegradable organism for petroleum compounds, or their derivatives, and exhausted fuel oil.

2 Material & Methods

2.1 Samples Collection and Isolation

Samples are collected from different locations of Benghazi Lake, as shown in Figure (1); such as places of the flow sewage that opens into the Lake, areas within the Port take around of place at **anchor** of ships and samples collected from the center of the lake as well.

Figure (1) described the location of 23rd July Lake in Benghazi City

2.2 Cultivated on Tested Agar Media

Before starting the laboratory work should be removed of large impurities parts by filtrations. The filtration of collected samples are cultured directly by sterile loop on three tested agar media, and then incubated on room temperature for 1-2 weeks. These media composition are as follows:

Medium (1): This consists of 1 liter of double ionized distilled water and added these components of Mg SO4. H_2O, 1 g/L ; $CaCl_2.5H_2O$, 0.02 g/L ; KH_2PO_4, 1 g/L ; Na NO_3, 1 g/L; and 1.5%, of agar and the final addition is crude oil (1%) or its derivatives.

Medium (2): It consists of agar (1.5%), double ionized of distilled water 1 liter and final addition is crude oil (1%) or its derivatives.

Medium (3): It consists of agar (1.5 %), double ionized of distilled water 1 liter and final addition is exhausted fuel oil (1%).

2.3 Study of Rhodotorula sp ***Growth Rate and Biodegradation*** **for Crud oil**

The same media compositions are used as in the previous without added the agar (1.5%). Sterilization the broth media by autoclave, the concentration inoculums of 10^6 - 10^8 (culture aged of 24 - 48 hours) were prepared and added the amount of 10 ml/100 ml for each of three broth media. After inoculated it, put three tested media broth in shaker of 150 - 200 rpm at room temperature for 1- 4 weeks. To be measured of all readings for the optical density by using Spectrophotometer (wavelength of 600 nm). Any microscopically observations should be recorded by using a camera.

2.4 Microscopically Examination

Microscopically examination using by Light Microscope and direct slide preparation are recognized the morphology of cells, colonies, colony color, buds and vegetative cells. It also should be used the camera for each Microscopically examination to observe the crude oil degradation steps during 24hours for the three tested media of batch culture.

2.5 Chemical Tests

2.5.1 Assimilation Sugar Test: By using broth basic media consists of reagent red with adding different types of 2% sugars such as sucrose, lactose, and glucose (Cheesbrough, 1984).

***2.5.2 Urease test*:** By using procedures in Cheesbrough (1984) .

2.5.3 Potato Dextrose Agar Cultured: it should be grown the colonies on PDA medium and studied the morphological characterization such as colony shape, color, size, and so on.

2.6 Solubility Test

This test is applied on ball shape that formed during cultivation batch for medium (2). By putting three balls on a clean slide and added drops of water, and crude oil, in respectively those are compared with a ball as a control (without any adding). Leave for more than half an hour to see the observations that it will be happened.

3 Results

3.1 Isolated of Rhodotorula strain on Three Tested Media

A successes isolated of *Rhodotorula* strain have ability to growth significantly on three tested media of solid or broth during 24-72 h. The colonies characterized were circle, orange bright color, with a smooth surface and wavy at the end of edge. On aerobically condition, this strain could be assimilated of glucose and non assimilation either for lactose or sucrose, beside that it could be breakdown of a urea by an enzyme of Urease (Tien *et al*, 2008, and Mokhtari *et al*, 2011). Microscopically examination of the cell shape was observed ovoid multinucleated vegetative cells connected by bud or more, as shown in Figure 2 (a-d). The yeast cell surrounded by a slimy capsulated may have been component of Exopolysaccharide (figure 2-d).

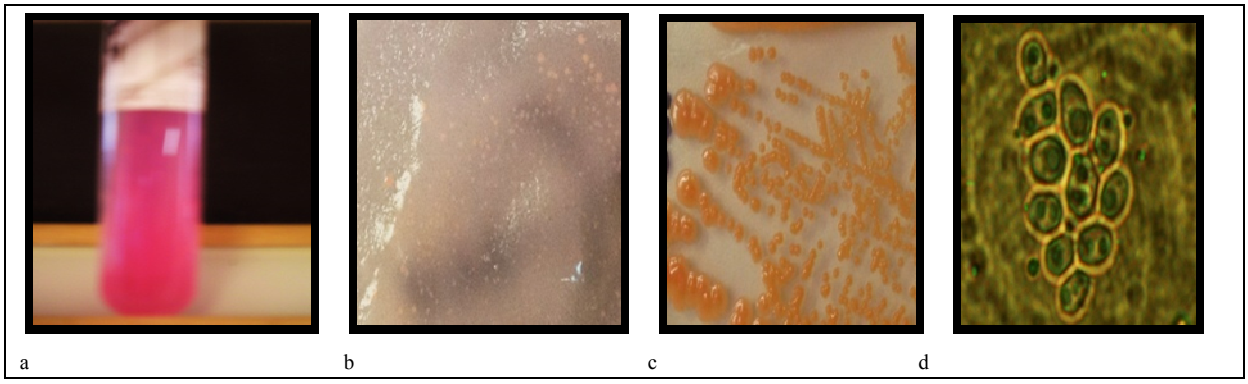
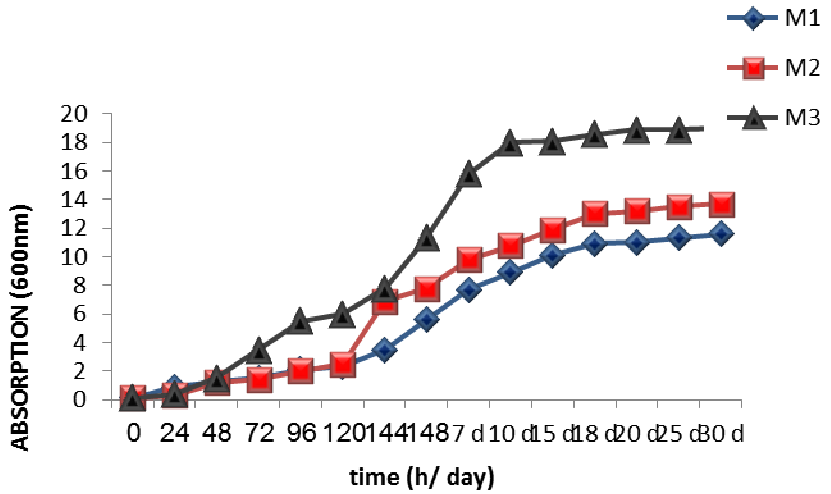


Figure (2): it`s showed that **a** ; *Rhodotorula* sp urease test positive. **b**; *Rhodotorula sp.* grown on agar medium (1) during 24-48h. **c**; *Rhodotorula sp.* grown on 48h on PDA, **d**; *Rhodotorula* sp. capsulated cell by light microscope .

3.2 *Growth Rate and Biodegradation to petroleum and exhausted oil*

The optical density and degradation of petroleum compounds and exhausted oil measurements showed that the lag phase of the culture in both media 1and 2 were very slow slope at 120 hrs of the culture age , as in figure (3). The exponential phase for both tested media (1and 2) extended to about 15 days; in this phase it already degraded the amount of crude oil compounds. The degradation rate was about 70-85%.of final batch culture aged which is in stationary phase, as in figure (4). Whereas, a lag phases in a medium 3 was a fastest slope from 48-72 hrs of the batch culture age. An exponential phase was become about 7days, while the stationary phase entered after 10 days. The *Rhodotorula* have to be the highes t degradable rate of exhausted oil, it reaching more than 95% through in 10 days.



Figure(3): Growth Curve of *Rhodotorula* strain cultivated of three tested media

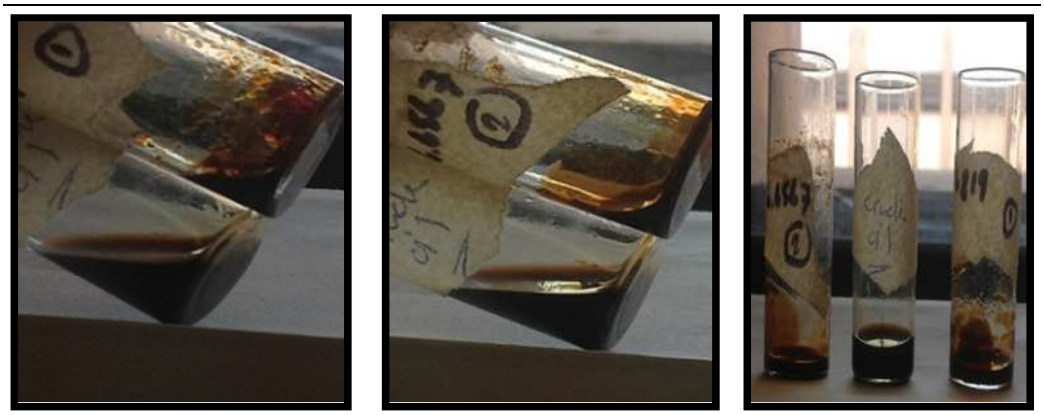


Figure (4): it showed the final cultivation of Crude oil degradation for 1&2 media.

3.3 *Microscopically Examination of Crude oil on Tested media*

The most important observations that appeared in a medium (1) were aggregates to air bubbles that those most likely to foam or soap bubbles which have floating and arrangements on the surface of glass edge, as shown in Figure (5). The medium texture was a viscosity, this viscosity increases with the time period and cultivation. Otherwise a huge different observations of a medium (2) that it seemed to be aggregate of many ball shaped, with brownish color floating on the surface and it has a viscosity texture of a medium. it also has a viscosity inside the ball contents. These ball will be exposure by similar happened to the soap bubbles during put the cover slide due to the gases content, as shown in Figure 6 (a and b).

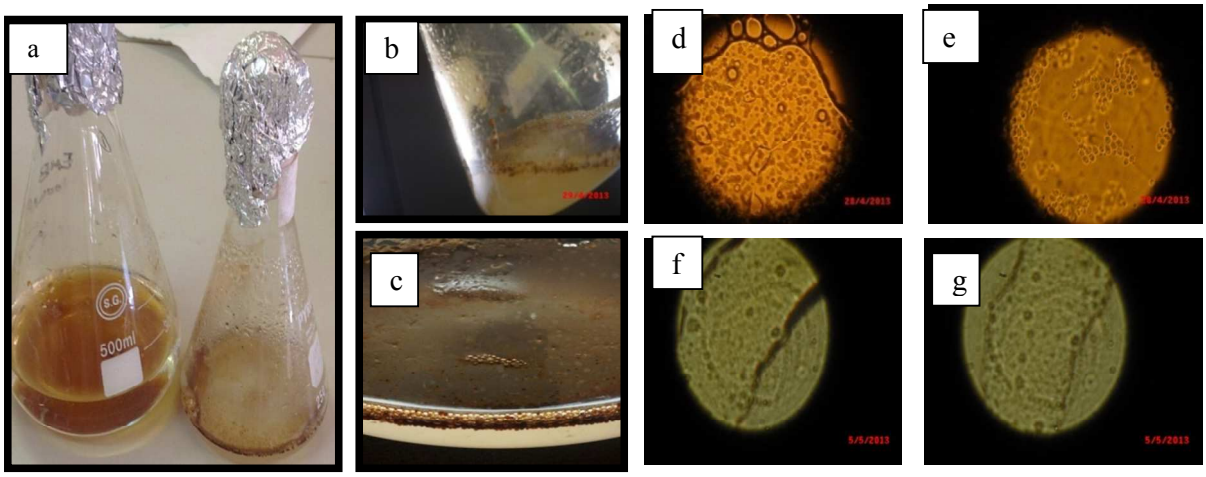


Figure (5): a-c; in medium (1) heavy aggregations of air bubbles with produced dark brown viscously substances c; camera zoom in high power 10X times microscopically observations of a medium (1): d-g; *Rhodotorula sp.* grown after 24h, Capsulated formation and starting to degrade the crude oil.

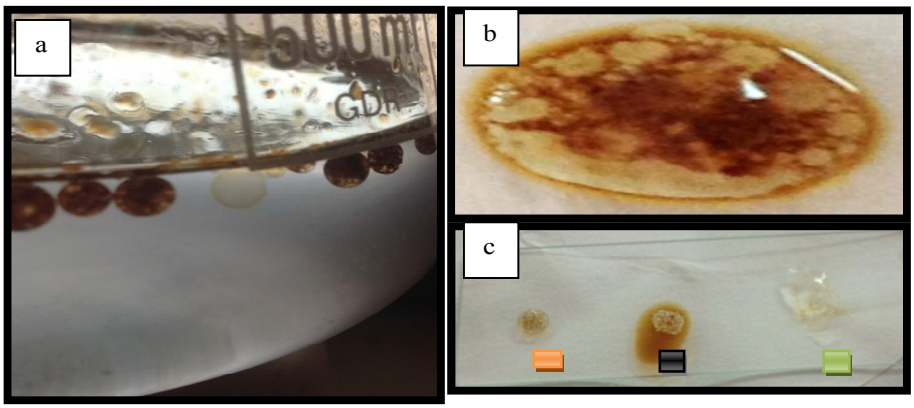
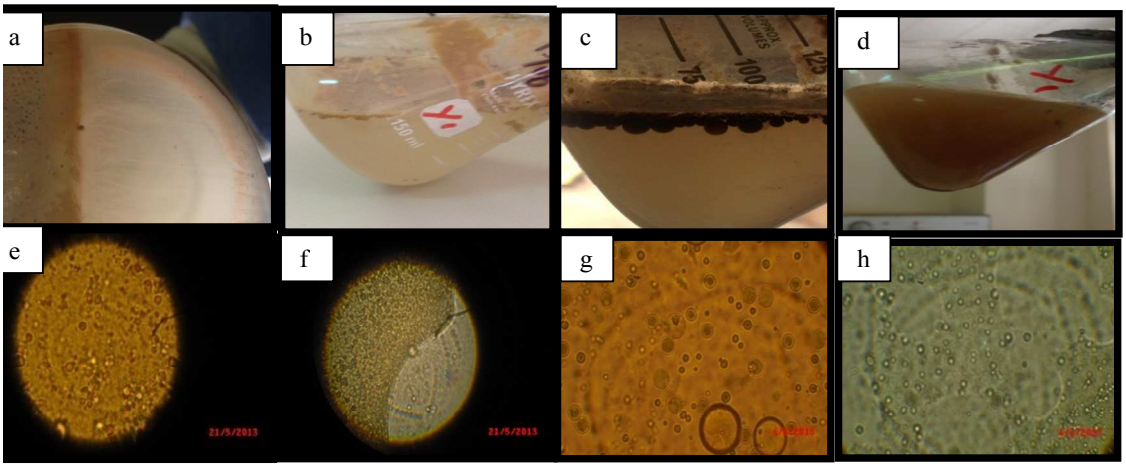


Figure (6) : a; the observation s of ball-shaped in medium(2). b; transferred the ball shaped on clean slide for microscopically examination c; Solubility test: Control; added crude oil; added water

Actually the direct Microscopically examination of both media (1), and (2) have showed the same observations of degradation processes of crude oil which it was starting during *Rhodotorula* strain was grown and multiplication exactly within 24-48 hours. Furthermore the main important role playing of these degradation processes were capsulated that can be produced in heavy amounts of slime materials it will be given a viscosity of the medium.

The observations of exhausted oil in a medium 3 were completely different, *Rhodotorula* strain could be multiplication and grown rapidly in this component, with formed creamy color, and more turbidity of the medium, the dissolving of exhausted oil amount compound approximately completely, it also that confirmed with a microscopically observations, as in figure(7).



3.5 Results of Solubility Test

The results of solubility were emphasized that there is no any dissolving to the products when put the drop of crude oil but it have disappeared the air bubbles formation after 30 minutes leave as shown in figures 6(c) and 8(A); this formation stile remained until more than 5 days. Whereas the microscopically observations of solubility test by adding water are going to more dissolving to the viscosity components than other tested with crude oil figure(8:A and B).

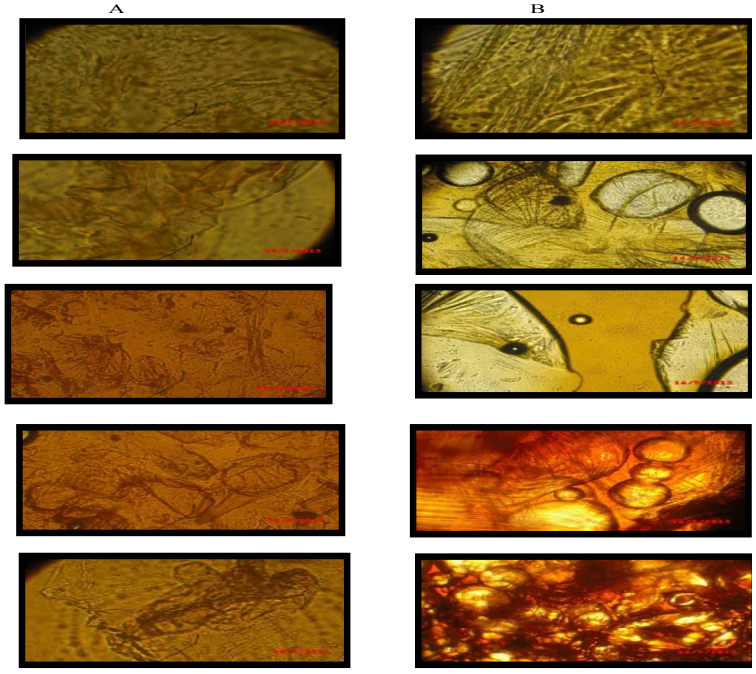


Figure (8): Microscopically observations of ball shaped forming in a medium 2, A; solubility test by crude oil dissolved to the gases and slime stile formation; B, direct test to a ball shaped formation in a medium 2 compared with" A", air bubbles and slime component with dark brown substances.

4 Discussion

The main important results in this present study particularly in this Lake have highly pollutant by microbial contamination. In fact this pollution indicated to flow of highly accumulative quantity of swage wastes through in Benghazi Lake so that it would be caused of dangerous epidemic infections to human(Elhassadi, 2008). It can also be caused harmful affected on animals, plants and their environmental components. Therefore, this present study give attention to Benghazi people especially who have doing activity in this lake ; for example they like to hunting or swimming should be prohibited and avoid it due to recorded high risk in polluted water (Abdulsamad and Elbabour, 2013) .

In addition to this work have interesting obtained of high rate of crude oil degradation by *Rhodotorula* isolated strain, according to results of both media (1 and 2), and it has got also very high rate degradable to exhausted oil in medium (3). This was nearly similar to the findings of many investigators concerning of *R. aurantiaca* grown with *Candida* that have highly degradation rate of petroleum and derivatives may reached to 93%, (de Cássia *et al*., 2007). Rizzo in 2008 also obtained the higher biodegradation levels by *Rhodotorula glutinis* and *Nocardia nova* reached of 98.8% with bioaugmentation in liquid medium. Although it did not has any reports in previous study that showed the ability of *Rhodotorula* species to degradable of exhausted oil or any other vehicles fuel, but some species in this genus such as *Rh. glutinis* and *Rh. rubra* could stile possibly be used for the removal or detoxification of diazinon residues on tomatoes (Bempelou, 2013).

Actually the growth curve of *Rhodotorula* sp indicated that have very slow slope of log phase in both media (1 and 2), this may be due to enzymes responsibility when initial degradable attack (Cappello *et al*, 2010). So, the Light microscopically observations showed that during cell growth of exponential phase the slime substances were produced increasing amount continue parallel to the timing of batch culture, this is agreed with Ibrahim *et al* in 2012 when emphasizes that the exopolysaccharide concentration paralleled the cell growth of *Rhodotorula glutinis* can be continued to increase after the onset of the stationary phase. So an increase in the viscosity of culture medium due to the increases accumulated in exopolysaccharide production(Abari *et al*, 2011). This is agreed with Goutx et al , in 1987 that indicated to some microorganisms cannot produce biosurfactant but is still able to degrade oil substrates effectively via formation of extracellular or cell membrane-bound bioemulsifiers e.g. exopolysaccharides in some bacterial strains also have ability to tolerate and degrade aromatic hydrocarbons because it has exopolysaccharides (EPS), biosurfactant and peroxidase(Abari *et al*, 2012).

This result also agreed with the previous study by Shailubhali *et al*., (1985) that established the bacteria *Ps. Aeruginosa* could be cultivated with *R. rubra* and it have production of biosurfactant due to the capsulated component of Exopolysaccharide and their ability to produce enzymes., because of the nature chemical component of the yeast cell wall, which is composed of 30-60 % sugars, 20 % of fat, and 30% of protein. And the components of these sugars are from Glucans and manans have ability to dissolve in water (Huang, 2008). Then the reason of ball shaped solubility in water due to the homogenous composition of many polysaccharides that mixture with other compounds. This is also evidence to the disappearance

of some viscosity composition through three days leave.

Furthermore the bioremediation of microorganisms to degrade of petroleum in the nature so far have variability applications inside the laboratory, because of there are homogenous adaptations between the environmental components with microorganisms. So there are too many complexity variable factors in somewhat mechanical, physical, chemical, and others have exposed influentially continuous on microorganisms those might be affected during degradations happened.

The experiment processes that have used in the laboratory, it's difficult to apply by exactly control conditions because of the ecological circumstances. Perhaps this strain has highly efficiency of degradation rate to petroleum and their derivative inside the laboratory that does not necessary meaning it will be occurred in the same percentage in the environment. Therefore as concluded the results there are many factors could be affected in ability of this degradation such as temperature, media composition, dissolving oxygen, rotation of shaker, as well as the microbial ability of enzymes that make degradation and its adaptation to their environmental changeable this is agreed with [Aber *et al,* 2012].

6 CONCLUSION

Consequentially one solution that we propose on the state of the attention and care of it is problem of pollutants to help our government in which to treated this Lake as soon as possible with backfilling the lake completely to avoid this problem and embankment the sewage sludge and emendation the recycle of swage. So *Rhodotorula strain* is promised as a highly biodegradable organism to use in further study of the bioremediation and biosurfactant production.

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