TRANSACTIONS ON SCIENCE AND TECHNOLOGY

Biodegradation of Sabah Light Crude Oil by Locally Isolated *Candida tropicalis* RETL-Cr1 and *Pseudomonas aeruginosa* BAS-Cr1

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Abstract
Increases in demand of petroleum hydrocarbon across the world inevitably contributed to the oil pollution in marine environment. Biodegradation is a proven cost effective approach for treatment of polluted marine environment. This study was performed to assess the biodegradation of Sabah Light Crude Oil by locally isolated microorganisms, *C. tropicalis* and *P. aeruginosa* in simulated seawater condition. Efficiency comparison and rate of biodegradation between single strain and consortia were investigated in shake flask trials. Utilization of 5% (v/v) crude oil as sole carbon source can support growth of bacteria up to 28 days. Consortia culture of *C. tropicalis* and *P. aeruginosa* has the highest degradation of 50% while single culture was 40% and 30% respectively. GC-MS analysis showed degradation of *n*-alkane in crude oil after four weeks of incubation. Present consortia culture has the potential as potent petroleum hydrocarbon degrader in the marine environment due to its specific ability to metabolize hydrocarbons.

Introduction

There is an increasing concern towards water pollution with regards to hydrocarbon pollution in marine environment day by day. Hydrocarbons are essential components of crude oil. Numbers of research have shown that most potential bacteria for petroleum hydrocarbon degradation have been isolated from areas contaminated with oil (Chaerun *et al*., 2004). The most important genera based on frequency of isolation for hydrocarbon degraders in aquatic environment are of *Candida* (yeast) and *Pseudomonas* (bacteria) species.

In many reports, bacteria such as *Pseudomonas aeruginosa* (Ainon *et al*., 2010; Latha & Kalaivani, 2012) has been identified as more efficient crude oil degraders than yeast. On the contrary, there is appreciable information that yeasts are better crude oil degraders than bacteria (Chaillan *et al*., 2004). However, both *Candida tropicalis* and *Pseudomonas aeruginosa* are proven microorganisms with known potential to be able to degrade petroleum hydrocarbons (Ijah & Antai, 2003; Piakong *et al*., 2009).

Biodegradation is defined as a biologically catalyzed reduction process of complex chemicals. It is also referring to the metabolic ability of microorganisms usually performed by a variety of bacteria,

fungi and yeasts. The microorganisms oxidize the hydrocarbons to fatty acids and then to acetates. Some of the acetates are further metabolized to carbon dioxide and water producing energy. The energy is then utilised to build cell materials from acetates and salts. Biodegradation by microorganisms is more favourable as these modify crude oils in beneficial ways and the end products are of environmentally safe. Therefore, this study aims to determine the biodegradation ability of locally isolated microorganisms in degrading Sabah Light Crude Oil.

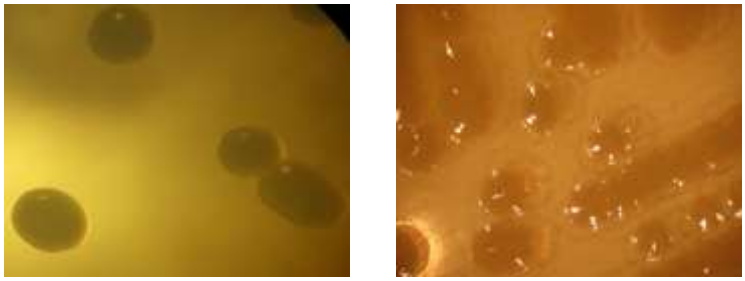
Methodology

Sabah Light Crude Oil

Sabah Light Crude Oil obtained from Sabah offshore terminal was classified as light crude oil based on the gas chromatography profiles (GC-MS) with *n*-alkanes ranging from C_9 to C_{38}.

Locally Isolated Microorganisms

C. tropicalis and *P. aeruginosa* was locally isolated from petrochemical wastewater treatment plants. Previous isolation, characterization and identification had been done by (Piakong *et al*. 2004) *C. tropicalis* coded RETL-Cr1 (Figure 1a.) is a cream colour with round configuration, smooth margin, convex elevation and the colony of a very small size. Meanwhile, *P. aeruginosa* coded BAS-Cr1 (Figure 1b) is greenish colour, wrinkled configuration, wavy margin, raised elevation and the colony give fluorescent effect.



(a) (b)

Figure 1. Colony morphology of (a) *C. tropicalis* RETL-Cr1 (b) *P. aeruginosa* BAS-Cr1 (Stereo microscope (a) x20 magnification (b) x12 magnification)

Culture Medium

Culture media used in this study were Ramsay agar and broth and preparation was according to (Ramsay *et al*. 1989). The concentration of Sabah Light Crude Oil was expressed in volume per volumes basis (v/v). The amount of crude oil supplied was 5% (v/v). Treated natural seawater 70% (v/v) was used to simulate marine condition in laboratory testing.

Shake Flasks Trial

C. tropicalis RETL-Cr1 and *P. aeruginosa* BAS-Cr1 was inoculated in the culture medium containing crude oil 5% (v/v), natural seawater 70% (v/v) and Ramsay broth. The cells were incubated in an orbital shaker at 30°C and 200 rpm for 24 hours. 10% of inoculums with desired optical density (OD) of 0.5 – 0.7 was then transferred to 250 ml Erlenmeyer flask and incubated with the same conditions where cell growth was monitored by measuring absorbance at 600 nm using Cecil 1011 Spectrophotometer, UK.

Hydrocarbon Analysis

The biodegradation rate and efficiency of single and consortia were determined after 28 days of incubation using gravimetrically method (modified APHA 5520 B) and by gas chromatography (Perkin Elmer model Clarus 500; Column: Elite-MS 30m x 0.25mm I.D., 0.25µm df; initial temperature 35°C; carrier: Nitrogen; rate 4°C/min; detector 280°C; injection volume: 1µL).

Result and discussion

In this result, the rapid growth of bacteria was experienced in consortia cultures compared to single culture of *C. tropicalis* RETL-Cr1 and *P. aeruginosa* BAS-Cr1 with highest OD measurement of 0.8, 0.7 and 0.6 respectively. The growth curves for both single and the consortia cultures did not experience loss of viability within 24 hours incubation as single and consortia were able to use crude oil as the sole source of carbon and energy as suggested by (Khan *et al*. 2013).

The biodegradation efficiency and rate of crude oil removal (Figure 2) after four weeks of incubation shows the consortia culture exhibits the highest percentage of total crude oil degradation up to 50%. Meanwhile single *C. tropicalis* RETL-Cr1 and *P. aeruginosa* BAS-Cr1 are 40% and 30% respectively. In similar investigation by (Minoui *et al*., 2008), efficiency of oil biodegradation by isolated depends on the crude oil concentration as the species can tolerate the crude oil concentration below 5% in their medium culture, but the optimum concentration was considered about 1% to 2%. The present work was conducted with 5% crude oil which could partly lead to a reduced ability of microbes in degrading crude oil.

Biodegradation rate of both single and consortia of *C. tropicalis* RETL-Cr1 and *P. aeruginosa* BAS-Cr1 (Figure 3) has a linear relation with the microbial growth across incubation period of four weeks. (Sepahi *et al.* 2008) suggested that the utilization of petroleum hydrocarbons as a substrates or sole carbon source by strains is evident by the increase in the microbial density. The percentage of crude oil removed in first week was the highest, thereafter the degradation percentage started to reduce. This was an agreement with the study by (Chorom *et al*. 2010) that the crude oil degradation rate decreased with increasing time.

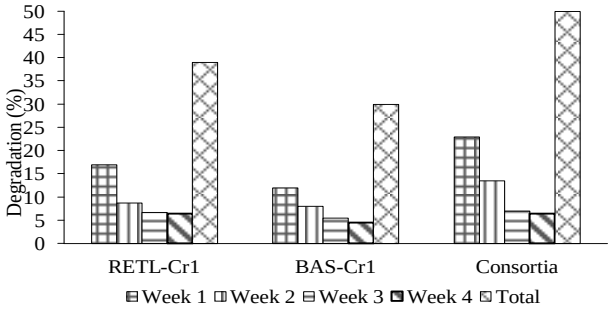
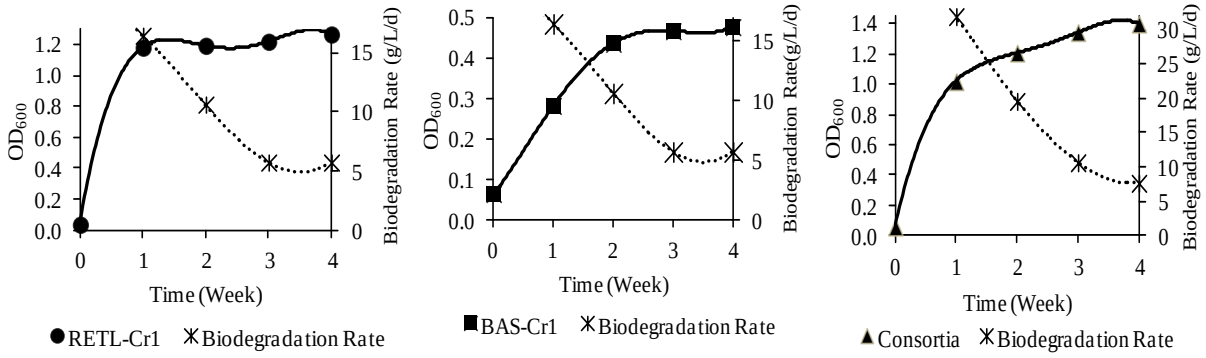
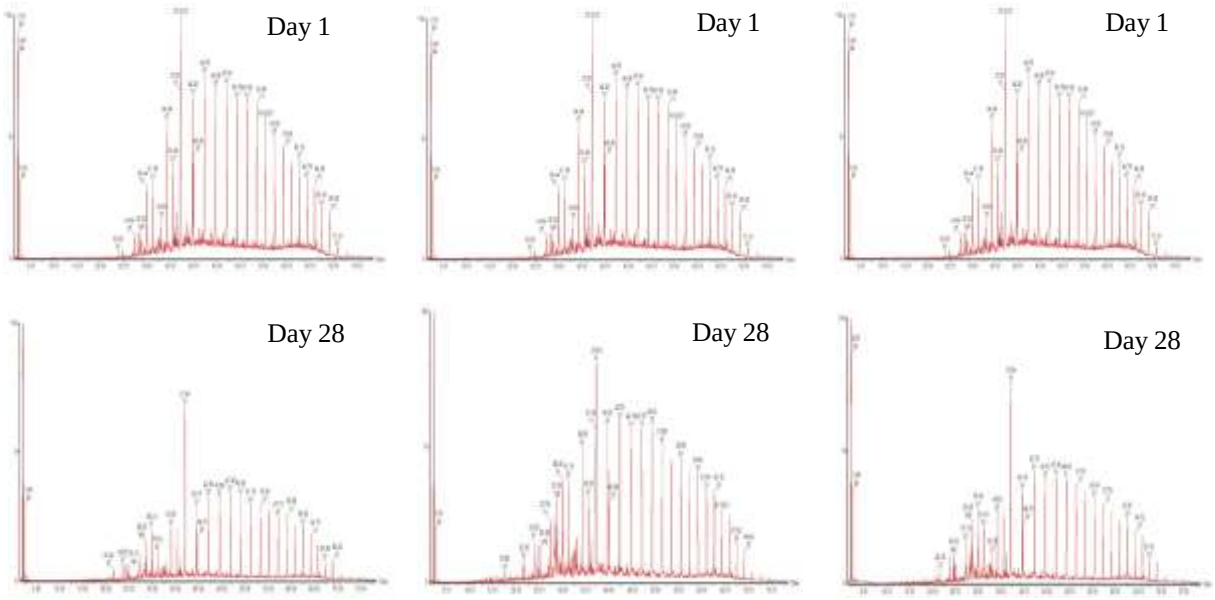


Figure 2. Biodegradation efficiency of crude oil in single and consortia cultures of *C. tropicalis* RETL-Cr1 and *P. aeruginosa* BAS-Cr1



(a) (b) (c)

Figure 3. Weekly growth and biodegradation rate of crude oil by single culture of (a) *C. tropicalis* RETL-Cr1 (b) *P. aeruginosa* BAS-Cr1 and (c) consortia cultures



(a) (b) (c)

Figure 4. GC profiles of (a) *C. tropicalis* RETL-Cr1 (b) *P. aeruginosa* BAS-Cr1 and (c) consortia cultures for crude oil biodegradation

Consortia cultures of *C. tropicalis* RETL-Cr1 and *P. aeruginosa* BAS-Cr1 shows almost 18 g/L/d, the highest rate of biodegradation, one-fold difference compared to 14g/L/d by single *C. tropicalis* RETL-Cr1 and about two-fold difference compared to 10 g/L/d by single *P. aeruginosa* BAS-Cr1. These findings were consistent with the study conducted by (Guru *et al*. 2013) that higher number of microbes presence in the media leads to a faster rate of biodegradation.

The GC-profile results (Figure 4) for both single and consortia cultures of *C. tropicalis* RETL-Cr1 and *P. aeruginosa* BAS-Cr1 exhibits significant decreased of peak heights and depletion of total area of chromatogram of various hydrocarbons after 28 days of incubation. A study conducted by (Vinas *et al*. 2002) reported that the reduction of hydrocarbon peak height and areas is strong evidence that the selected microbes in treatment cultures have the ability to utilize and degrade the hydrocarbon as sole carbon sources.

The reduction percentage of eight selected individual hydrocarbons (Figure 5) in this study was achieved by both single and consortia cultures consist of *C. tropicalis* RETL-Cr1 strain. The biodegradation of *n*-alkanes in crude oil in this study was relatively fast compared to study done by (Dutta *et al*. 2000) as previous study showed the microbial populations in seawater able to biodegrade 28% of the selected n-alkanes found in crude oil within 8 weeks of incubation period.

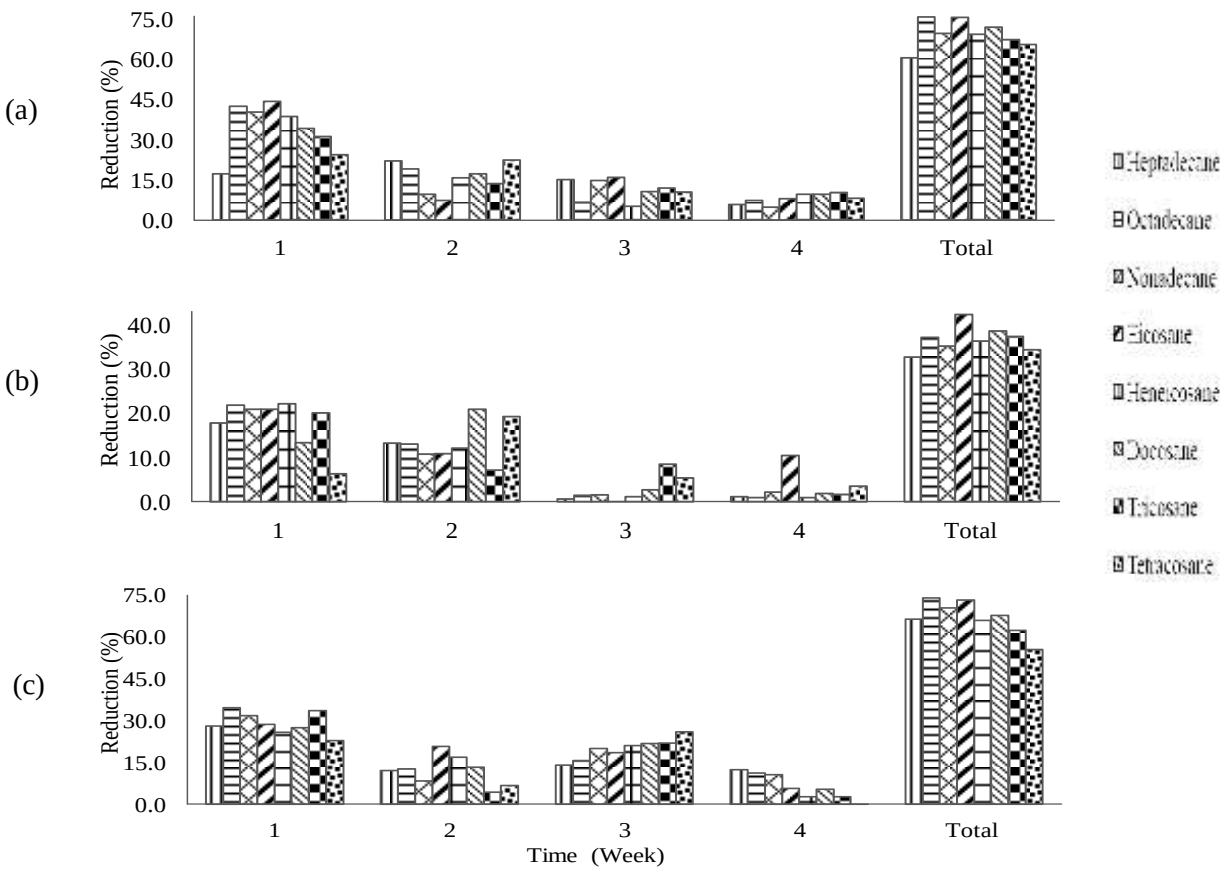


Figure 5. Percentage degradation of individual hydrocarbon present in crude oil by single (a) *C. tropicalis* RETL-Cr1 (b) *P. aeruginosa* BAS-Cr1 and (c) consortia cultures

Conclusion

The results described in this paper show that degradation efficiency of consortia culture of *C. tropicalis* RETL-Cr1 and *P. aeruginosa* BAS-Cr1 are 10 – 20% more efficient as compared to single culture of *C. tropicalis* RETL-Cr1 and *P. aeruginosa* BAS-Cr1. The biodegradation rate of consortia are 18 g/L/d, one-fold higher than single culture of *C. tropicalis* RETL-Cr1 and two-fold higher compared to single culture of *P. aeruginosa* BAS-Cr1. From the eight selective aliphatic hydrocarbon degradation, consortia culture also showed more than 60% of degradation efficiency. Thus, it can be indicated that higher potency of consortia culture of *C. tropicalis* RETL-Cr1 and *P. aeruginosa* BAS-Cr1 to become the petroleum hydrocarbon degrader in the marine environment.

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