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# Using marine microalgae strains for oil removal from oil-water emulsion remediation

Sulaiman Al-Zuhair, Ebin George, Mutasim Nour\*

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## Abstract

Emulsified oils are considered major environmental hazards. Biological treatment of these contaminants, using bacteria, is very effective, but the produced biomass does not have any obvious value. If oils can be removed by microalgae, the produced biomass in this case can be readily used to produce lipids that can be used for biodiesel production. The ability of two marine strains, namely *Nannochloropsis sp.* and *Tetraselmis sp.* to grow in water containing emulsified oil has been tested. The effectiveness of the selected strains to utilize the organics as a carbon source and reduce their concentration has also been assessed. The oil removal efficiency and cells growth rates were evaluated. Under the tested condition, the two strains were able to grow and reduce the oil concentration equally well, with *Nannochloropsis sp.* performing slightly better. The positive outputs from this work benefit the energy and wastewater treatment sectors..

**Keywords:** Microalgae; Oil-water emulsion; Wastewater treatment; *Nannochloropsis sp.*; *Tetraselmis sp.*

## Introduction

The aquatic system is subject to contamination by organic pollutants from a variety of sources. Oils that are emulsified in water are considered one of the most challenging contaminations. The biological treatment of emulsified oils, using bacteria, has gained a lot of attention and its effectiveness has been widely proven. However, despite their high efficiency in emulsified oil removal, the release of these bacteria could cause diseases in plants and depletion

of fish stocks, and may also have the potential to cause diseases to humans (Christenson and Sims, 2011). In addition, the grown bacteria do not have any obvious value. If emulsified oil can be removed by microalgae, the produced biomass in this case can be readily utilized to produce lipids that can be used for biodiesel production. It has been reported that microalgae are capable of producing ten times more oil than the best oil crops (Demirbas, 2007). Furthermore, microalgae are capable of growing in saline water, which reduce the fresh water loading, and allows it to be used to remove emulsified oil in saline water, like seas and oceans. Nevertheless, to the best of the knowledge of the investigators, no work has been reported on using microalgae for the bioremoval of emulsified oil droplets.

In this work, the oils removal efficiencies and the growth rates are evaluated using two marine microalgae strains, namely *Tetraselmis sp.* and *Nannochloropsis sp.*, grown in saline water.

## Materials and Methods

### *Strains, culture media and reagents*

Palm cooking oil was purchased from local market. All other chemicals were purchased from Sigma-Aldrich, USA. Marine strains, *Tetraselmis sp.* and *Nannochloropsis sp.*, were obtained from a local marine research center in Umm Al-Quwain, UAE. The strains were grown in F/2 medium (32 ppt salinity), which consists of ( $\mu\text{M}$ ); 880  $\text{NaNO}_3$ , 36  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ , 106  $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ , 1 ( $\text{ml L}^{-1}$ ) of; vitamine B12, biotin vitamin and thiamine vitamin solutions, and 1 ( $\text{ml L}^{-1}$ ) of trace metal solution that consisted of ( $\mu\text{M}$ ); 0.08  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.9  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , 0.03  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , 0.05  $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.04  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ , 11.7  $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$  and 11.7  $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$  (Guillard and Ryther, 1962). The prepared media, excluding vitamins, were sterilized in an autoclave (Hirayama HV-50, Japan) at 121 °C for 15 min and cooled to room temperature prior to use. The microalgae suspensions were prepared by growing the two strains in F/2 medium for two weeks, prior to be used in the experiment.

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### Sulaiman Al-Zuhair

Chemical and Petroleum Engineering Department, UAE University, 15551 Al Ain, UAE

**Ebin George, Mutasim Nour\***

School of Engineering & Physical Sciences, Heriot-Watt University, Dubai, UAE

\*E-mail: Mutasim.Nour@hw.ac.uk

The oil emulsifying reagent was prepared, as described by Al-Zuhair (2006) by dissolving 8.95 g NaCl and 0.2 g  $\text{KH}_2\text{PO}_4$  in 200 mL demineralized water with 270 mL glycerol. Under vigorous stirring, using ultrasonicator (Q-Sonica, USA) at 1000 W, 3.0 g gum Arabic were added to the mixture. The mixture was then transferred to a 500 mL measuring flask and dematerialized water was added to bring the volume to 500 mL.

#### Experimental set-up

The growth medium was prepared by mixing 50 mL of emulsifying reagent, 0.08 mg oil and 200 mL growth medium in 500 mL Erlenmeyer flasks. The mixture was then thoroughly mixed using the ultrasonicator. Once the oil is totally emulsified, and a stable solution is formed, 20 mL of microalgae suspension was added. The initial oil concentration would then be  $0.3 \text{ mg L}^{-1}$  (roughly 300 ppm). The flasks were placed in temperature controlled incubator shaker (Innova 40 benchtop) at 275 rpm and  $30^\circ\text{C}$ . To enhance heterotrophic growth, and direct the microalgae to utilize the phenol as a sole carbon source, the cultures were not subjected to excessive lighting, and the lighting was limited to that used to illuminate the lab. To confirm the reproducibility of the data, the experiments were run in duplicates, and the presented results are the average values of the two runs.

#### Biomass and oils concentrations determination

A 6 mL sample was withdrawn from the growth culture on a daily basis. The sample was centrifuged at 6000 rpm for 5 min using multispeed centrifuge (IEC CL31, Thermo Scientific, USA). The supernatant containing the water and the partially separated oil was carefully collected using a micropipette. To determine the biomass concentration, the settled cells were re-suspended in distilled water and the optical density was measured at 680 nm using a spectrophotometer (Shimadzu UV-1800 UV, Kyoto, Japan). The spectrophotometer was zeroed using distilled water. The growth was determined by dividing the optical density at any time by the initial optical density, measured on day 0.

The determination of oil content in the supernatant was based on the procedure described by Chen and Vaidyanathan (2012). 2.8 mL sample of the supernatant was mixed with 0.2 mL of phosphate buffer (0.05M and pH 7.4) and 17 mL of 25% Methanol in 1 N NaOH. The mixture was placed in boiling water and stirred continuously for 40 minutes to allow saponification of the oil. The solution was then left to cool down. Once the temperature dropped to room temperature, 12 mL was withdrawn and mixed with 18 mL of chloroform-methanol (2:1, v/v) solution, and the mixture was stirred thoroughly, using magnetic stirrer, for 10 minutes, to allow complete dissolving of the organics in chloroform. The mixture was then centrifuged at 6000 rpm for 10 min to separate the organic and aqueous phases. 4 mL was withdrawn from the bottom organic phase and mixed with 4 mL of 1M Triethanolamine-1N acetic acid-6.45%  $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$  (9:1:10) solution. The mixture was stirred thoroughly for 5 minutes, and then centrifuged again at 6000 rpm for 5 minutes. 2 mL sample of the bottom organic phase layer was placed in quartz cuvette and the optical density was measured in spectrophotometer at 260 nm. The spectrophotometer was zeroed using a blank sample that had undergone the same procedure except that the 2.8 mL oil-water emulsion was replaced with distilled water.

## Results and Discussion

The growth curves showing  $\ln(X/X_0)$  vs time of *Tetraselmis sp.* and *Nannochloropsis sp.* are shown in Fig. 1. Where, X and  $X_0$  are the biomass concentration at any time and at initial time, respectively.

The values shown in the figure are averages of runs done in duplicates, and the lines are connection between the experimental data to show the trend. Both strains showed a good growth, with insignificant lag phase.

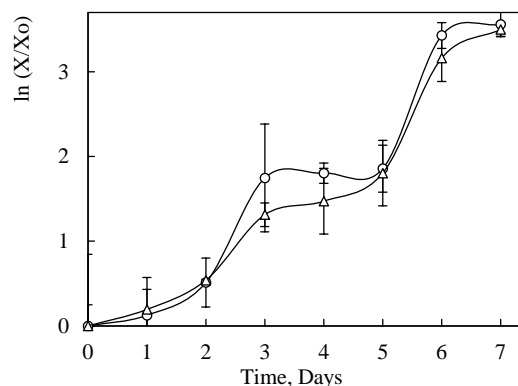


Figure 1: Growth curves of *Nannochloropsis sp.* and *Tetraselmis sp.* grown in oil-water emulsion.

To determine the effectiveness of the tested strain for oil degradation, the residual oil was estimated at different times, and the results are shown in Fig. 2.

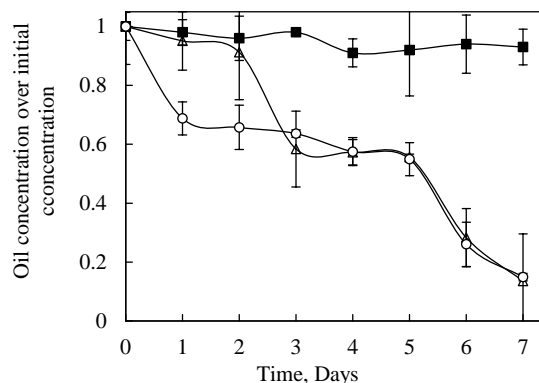


Figure 2: Drop in oil content in a media containing *Nannochloropsis sp.* and *Tetraselmis sp.*, compared to the  $\blacksquare$  Blank solution

It is clearly seen that both strains were able to remove most of the oil within the first seven days. It is also interesting to notice that the initial drop in oil content using *Nannochloropsis sp.* was faster than that using *Tetraselmis sp.*, which was also reflected on the initial faster growth rate of the former strain. After four days, both strains performed equally well, this also resulted in similar growth rates.

## Conclusions

It was found that the marine microalgae strains, *Nannochloropsis sp.* and *Tetraselmis sp.* were able to grow in water containing emulsified oil as the only available carbon source. The oil content dropped significantly in a period of seven days. This shows that the microalgae have the potential to be used in oil water emulsions found in saline water. The collected biomass has clear applications in biofuel, food and pharmaceutical industries.

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