Kinetic Studies of Oil Extraction from Microalgae *Chlorella vulgaris*: Free Cell Culture and Immobilized Microalgae

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ABSTRACT: This study was aimed to determine the best solvent composition for the extraction of oil from free and immobilized microalgae cells of *Chlorella vulgaris*. Three different solvent systems have been used to evaluate the maximum oil extraction from the free-cell culture of *Chlorella vulgaris*. The most effective solvent was determined based on the kinetic study. Among them, the heptane solvent system achieved highest rate of reaction (k=5.90×10^{-2} min^{-1}) at 75 °C. The result shows that the extraction of oil from free cell culture was more effective than that from immobilized microalgae using heptane based on the kinetic study. The kinetics study revealed that the reaction rate constant and activation energy were strongly correlated with temperature and extraction time. Thus, this study finding shows that heptane is an environment-friendly alternative solvent for extracting oil from both free cell culture and immobilized microalgae cells of *Chlorella vulgaris* due to its lower energy requirement and low toxicity

Keywords: *Chlorella vulgaris; Free Cell Culture; Immobilization*

1. INTRODUCTION

The utilization of microalgae has emerged as a promising feedstock for biofuel production in the last few decades [1]. However, the main limitation of microalgae biomass is the high cost of production. Also, it should be noted that harvesting and separation of biomass may represent up to 20–30% of total production costs. Recently, immobilization techniques have become a promising alternative due to their simplicity and cost-efficient [2]. The immobilized microalgae can be harvested by a simple sieving method without using high energy, making the microalgae biomass handling easier and feasible to be implemented on a commercial scale [3]. Besides, the selection of efficient lipid extraction solvents also crucial for ease of operation and scaling up for industrial application. Several factors are considered, such as low cost, non-toxic, high efficiency, and a low boiling point, when choosing a suitable solvent.

Therefore, heptane is selected as one of the solvents besides commonly used solvents (ethanol and methanol) due to its non-polar properties, low-viscosity high stability, low greasy residual, and low corrosiveness [4], [5]. The oil yield, reaction rate based on the kinetic study, activation energy and economic feasibility of both free cell culture and immobilized microalgae has been evaluated and compared in this current context.

2. METHODOLOGY

The experimental procedures initiated with the cultivation of free cell and immobilized microalgae for 14 days and 10 days, respectively. Bald Basal Mediums were added for both of cultures. After the cultivation periods, the free cell culture was harvested using centrifugation method and the *C. vulgaris* biomass was obtained in a powder form after it was freeze-dried for 24 hours. Meanwhile the immobilized microalgae cells were harvested by adding it in a sodium carbonate anhydrous solution and drying it for 48 hours at 70 °C.

Solvent extraction method was selected to extract the oil from both free and immobilized microalgae cells. The extraction processes were conducted for 5 hours of extraction time (interval of 1 hour) at four different temperatures (45, 55, 65, and 75 °C). 0.01 g of dried biomass of free cell culture was into the capped test tubes together with 5.5 mL of distilled water. The extractions were carried out using heptane as solvent and were repeated with different solvent systems (heptane-ethanol and heptane-methanol with a ratio of 1:1 v/v) ratio. Meanwhile, 0.07 g of dried immobilized microalgae biomass was mixed with 5.5 mL of distilled water in the test tubes [5]. Using 12 mL of solvent, the extractions were conducted at mentioned temperature and time. Then, the sample was centrifuged at 4000 rpm for 2 minutes, resulting in three layers of the solution inside the tube. Microalgae oil was collected from the bottom layer, and the middle layer (solvent) and the upper layer (microalgae biomass) was then discarded. After 24 hours of drying, the mass of extracted oil was measured using a gravimetric method.

A simple cost estimation approach was used to determine the total cost of the solvents and harvesting cost. The cost of microalgae was not taking into account to provide an estimated non-dependent of biomass production costs.

3. RESULTS AND DISCUSSION

3.1 Effect of solvents on extraction kinetics of free cell

Based on the results obtained, it shows that the oil yield for heptane/ethanol is the highest among the three solvent system used at different temperature. The results obtained are similar to the study of Li et al. [6], which reported that a solvent mixture containing a polar and a non-polar solvent could extract a significant amount of oil.

The yield of oil extraction was used to determine the reaction rate constant, k (min^{-1}). Using oil extraction yield and differential method, ln (dY/dt) versus ln Y is plotted. From the linear plots, the straight lines' intercept indicates constant reaction rate constant, k. The data show that yield increases with longer extraction times and higher temperatures.

In this research, the reaction rate constant, k, was chosen as an indicator to select the best solvent for the immobilization of microalgae. Therefore, in this study, heptane is chosen as a solvent to extract oil from immobilized *C. vulgaris* cells due to its high reaction rate compared to others as shown in **Table 1**.

Table 1: Values of reaction rate constant obtained for all three solvents used

Temp (˚C)	k (min^{-1}) H	HM	HE
45	8.15×10^{-4}	5.46×10^{-5}	1.70×10^{-3}
55	8.93×10^{-3}	1.91×10^{-2}	1.85×10^{-8}
65	4.74×10^{-4}	6.31×10^{-5}	5.85×10^{-7}
75	5.90×10^{-2}	3.37×10^{-2}	1.04×10^{-2}

3.2 Extraction kinetics of immobilized microalgae

The amount of extracted oil using heptane (selected based previous section) was determined, and it reveals as the temperature increased, the oil yield also increased. Figure 1 shows the plot of ln (dY/dt) graph versus ln Y. The ln (dY/dt) plots versus ln Y at different temperatures were linear, similar to free cell culture. Based on the values calculated from Figure 1, it can be noticed that the highest reaction rate constant, k, was obtained at 75 °C is 6.37581×10^{-06} min^{-1}.

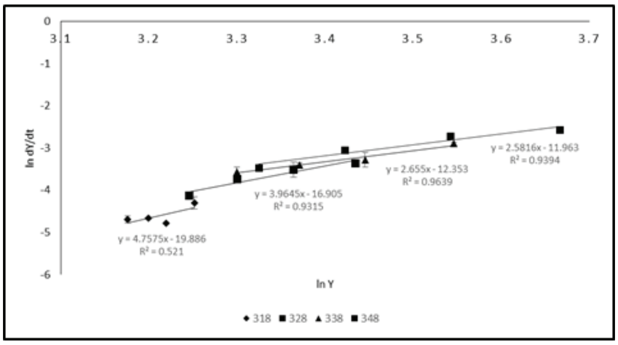

Figure 1: Graph of ln (dY/dt) versus ln Y at different temperatures

3.3 Determinations of activation energy

The activation energy is defined as the energy needed to convert the kinetic energy to potential energy for every reacting material mole [7]. The value of E_a for heptane, heptane-methanol, and heptane-ethanol are 9.8496 J/mol, 15.3485 J/mol, and 55.0370 J/mol, respectively. On the other hand, the activation energy for the oil extraction from immobilized cells yields up to 261834 J/mol. Therefore, the oil extraction occurred more accessible for free cell culture compared to immobilized cell culture.

3.4 Overall cost estimation

The cost for the current context was estimated by calculating the total solvent used and the cost of harvesting the both microalgae cells of *C. vulgaris*. The total cost for the extraction of oil using free and immobilized microalgae was approximately 67 USD and 66 USD, respectively, for the heptane solvent system. Hence, it can be said that solvent extraction using heptane for both free and immobilized microalgae cells of *C. vulgaris* is economically attractive and industrially viable given the less environmentally damaging properties.

4. CONCLUSION

The kinetic study showed that heptane had a higher reaction rate than methanol/hexane and ethanol/hexane. As a result, heptane is selected as a solvent to extract oil from immobilized *C. vulgaris* cells. However, heptane oil extraction of free cell culture yields the highest k value and the lowest activation energy when compared to immobilized microalgae cells. Heptane can be effectively extract the oil and the cost estimation was also very low. Therefore, this study's findings hypothesized that heptane is an environment-friendly replacement for extracting oil from free cell culture of *C. vulgaris* microalgae.

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