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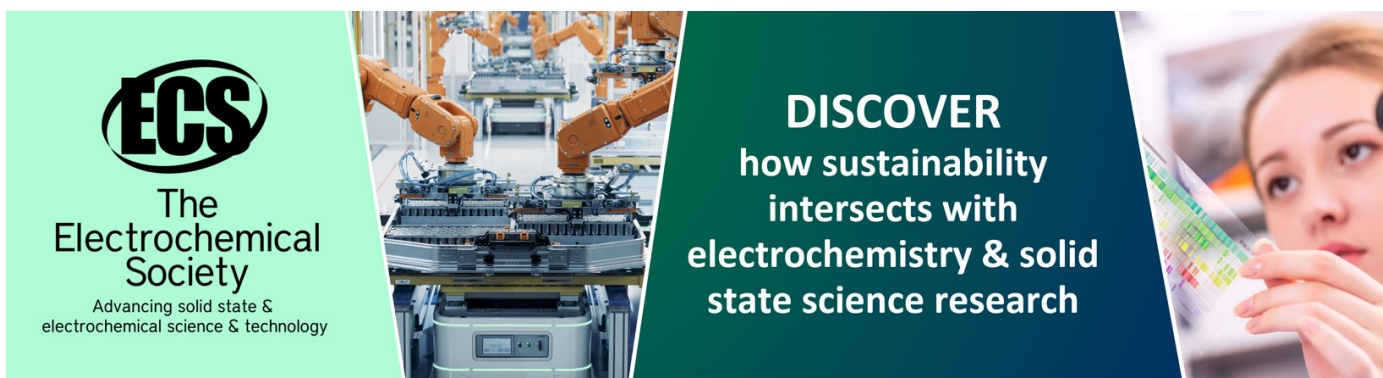
Kinetics studies and thermodynamics of oil extraction from immobilized microalgae cells of *Chlorella vulgaris*: The effect of the different matrix system

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Kinetics studies and thermodynamics of oil extraction from immobilized microalgae cells of *Chlorella vulgaris*: The effect of the different matrix system

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Abstract. This research article discusses the effect of different chemical matrices used for the immobilization of microalgae cells of *Chlorella vulgaris* on oil extraction in terms of oil yield, kinetic values, and thermodynamic values. The experimental procedures began with microalgae cultivation, immobilization of microalgae, microalgae harvesting, and oil extraction. For the immobilization method, the matrix systems involved were sodium alginate (SA), calcium alginate (CA), and sodium carboxymethyl cellulose (CMC). For the oil extraction process, heptane was used as an only solvent at different temperature and extraction time. The amount of oil extracted from the SA system was found to be the highest compared to other chemical matrix systems, which is 25.11 % at 348 K. The values of the reaction rate constants were found to increase with temperature and extraction time for SA and the activation energy was determined to be 39.6636 kJ/mol, which is lowest among the systems. Moreover, with the enthalpy value of 0.115 kJ/mol, the extraction process using the SA system is endothermic and was found to be industrially more feasible process compared to other matrix systems. Hence, the results proved that the immobilization of microalgae for biodiesel production is considerable for the future biofuel industry.

1. Introduction

Various studies across the globe have been made on the production of biodiesel by synthesizing microalgae and it has been considered as a potential alternative to satisfy the fuel demand for many sectors [1]. Microalgae are photosynthetic organisms that have the advantages of providing various bioactive compounds, such as essential fatty acids, carotenoids, chlorophylls, vitamins, and a range of high biotechnological compounds [2]. Microalgae also have been reported as a fast-growing organism with a high rate of carbon fixation compared to other terrestrial plants and they also give high biomass yield without competing for any arable land [3]. *Chlorella* sp is among the many other species of microalgae that have a higher capability of producing oils for the usage of biodiesel processing. Specifically, *C. vulgaris* is becoming researchers' favorite as it is easy to cultivate using a low energy input [4].

For the production of microalgal oil, the separation or detachment of algae from its growth medium plays a vital role since it was reported to be economically expensive, and to be precise this harvesting process accounts for up to 30 % of total production cost [5], [2]. Overcoming this issue, the usage of immobilized microalgae has picked up progressively considerations from the researchers and



industrialists. It is said to be requiring a lower input of energy to recover the immobilized microalgae by a simple separation method [3].

For the immobilization of microalgae cells, the method of gel entrapment is commonly considered, in which natural polysaccharides such as agars, carrageenan, and alginates are ideally utilized due to their low toxicity and high transparency [6]. Correspondingly, sodium carboxymethyl cellulose is also can be used for the microalgae immobilization since its permeability is higher due to its water content which is practical and advantageous for the biotechnological applications. The enhanced degradability of sodium carboxymethyl cellulose makes it a potential replacement for the polymer of petroleum origins and various uses [7].

It is essential to know the nature of the reaction involved in a process of production. Kinetic and thermodynamic studies of the oil extraction are gaining aggressive attention from researchers since it has an important role in the realization of a feasible industrial process [8], [9]. Considerations of the thermodynamic parameters of the extraction process are essential for the conclusion of the process is spontaneous or not. Adding to it, the physicochemical nature was indicated by the enthalpy value. The enthalpy value indicates whether the reaction is endothermic or exothermic whereas the entropy value will determine the reaction reversible or not [10].

In the present research work, it is aimed to investigate the effect of different chemical matrices used to cultivate the immobilized microalgae cells of *C. vulgaris* on the determination of kinetic and thermodynamics data of the oil extraction. Additionally, the yield of oil production from the immobilized microalgae was also compared between the different chemical matrices while the best chemical matrix also been chosen.

2. Materials and methods

2.1. Materials

The Microalgae strain used in this research was *Chlorella vulgaris* obtained from the Culture Collection of Algae and Protozoa (CCAP), Scottish Marine Institute, United Kingdom. Microalgae *C. vulgaris* was cultured in Bold Basal Medium (BBM) with 3-fold nitrogen and vitamins.

Four types of BBM were used in this study were BBM (I), BBM (II), BBM (III), and BBM (IV). The chemical compounds of BBM (I) contained 75.0 g of NaNO_3 , 2.50 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 7.50 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 7.5 g of $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, 17.5 g of KH_2PO_4 , and 2.5 g of NaCl . The chemical compounds of BBM (II) contained 0.75 g of Na_2EDTA , 97.0 mg of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 41.0 mg of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 5.0 mg of ZnCl_2 , 2.0 mg of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, and 4.0 mg of $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$. BBM (III) and BBM (IV) comprised of 0.12 g of vitamin B1 (thiamin hydrochloride) and 0.10 g of vitamin B12 (cyanocobalamin), respectively.

Heptane (C_7H_{16}) is the only chemical used for oil extraction in this research work. Chemicals used for the immobilization studies were calcium chloride (CaCl_2), sodium carbonate anhydrous (Na_2CO_3), sodium alginate (SA), calcium alginate (CA), and sodium carboxymethyl cellulose (CMC). All the chemicals were purchased from Merck (Germany), Sigma-Aldrich (USA), Oxoid (UK), and R&M Chemical (UK).

2.2. Medium Preparation

BBM (I) and BBM (II) were freshly prepared and autoclaved for 15 min at 121 °C to avoid any contamination. The most concentrated solution was BBM (IV). Therefore, 1 mL of the concentrated solution was added to 99 mL of sterilized distilled water to make up a 100 mL volume of new BBM (IV). After that, BBM (III) and BBM (IV) were transferred slowly into a 250 mL of Schott bottle using a sterilized syringe and was filter sterilized through a 0.2 μm membrane. The culture medium was prepared by adding 10.0 mL of BBM (I), 6.0 mL of BBM (II), and 1.0 mL of each of BBM (III) and BBM (IV) to 1 L of sterile distilled water [11]. Then, the culture medium was prepared.

2.3. Culture stock preparation

The aseptic technique was applied to the microalgae culturing and media preparation to avoid any contamination and was conducted in the laminar flow. The stock culture of *C. vulgaris* was seeded into 2 L Schott bottle filled with sterilized distilled water containing BBM (I) until BBM (IV). Two fluorescent lamps were used to expose the culture for continuous illumination and continuously aerated within 10-14 days by providing air at constant pressure.

2.4. Preparation of immobilized beads

All the necessary apparatus together with distilled water were autoclaved at 121°C for 15 minutes for sterilizing the apparatus to avoid contamination. Firstly, 2.5 mL of BBM (I), 1.5 mL of BBM (II), 0.25 mL of BBM (III), and 0.25 mL of BBM (IV) were added into a conical flask which was containing 250 mL of distilled water to prepare the culture medium. 3 mL from the medium were added into an empty 100 mL beaker, together with 0.06 g of sodium alginate. 10 mL of microalgae stock which was prepared earlier was added into the same 100 mL beaker. At the same time, 2 g of calcium chloride was weighed and added into 100 mL of distilled water to form a calcium chloride solution. The aqueous solution of sodium alginate and microalgae stock were dropped slowly using micropipette into calcium chloride solution. Every drop of the aqueous solution formed beads in the calcium chloride solution. After the hardening of beads, all the beads were transferred into a 250 mL conical flask which was containing the BBM medium. The medium with beads was aerated for 8 days of cultivation [6],[12]. The above methods were repeated with different combinations of matrix systems including sodium alginate, calcium alginate, and calcium carboxymethyl cellulose.

2.5. Harvesting of immobilized beads

After eight days of cultivation, the immobilized culture was harvested and dried to obtain the microalgae biomass. All the beads were collected first and then were washed through distilled water. 4.32 g of sodium carbonate anhydrous was weighed and poured into 216 mL of distilled water in a beaker to prepare the sodium carbonate anhydrous solution. Every five microalgae beads were dissolved in 2 mL of sodium carbonate anhydrous solution in another 250 mL beaker. After that, the beads together with sodium carbonate anhydrous solution were dried in an oven with a set temperature of 70°C until it was completely dried. The complete dried microalgae biomass was obtained after 48 hours of drying in an oven.

2.6. Oil extraction

Oil was extracted from the microalgae biomass using the solvent extraction method. Microalgae biomass (0.07 g) from the immobilization systems were used for the extraction of oil. The biomass was mixed with 5.5 mL of distilled water in a test tube and sonicated (sonicator Fisher brand FB15051) for 5 min to lyse the cells. Then, 12 mL of heptane was added to the microalgae biomass for the extraction process, and the process was carried out from the temperature 318 to 348 K with an interval of 10 K. Every set of experiment was conducted for 5 hours of mixing time with one-hour interval. Every one hour the test tube was taken one by one and centrifuged to separate the oil from the solvent by layer. The rotating speed of the centrifuge was set at 3000 rpm and it was done for 5 minutes. It had been observed that there were two layers formed after the centrifugation where the bottom layer was the oil and the other layer was the solvent used and the bottom layer was carefully transferred into the vial using a micropipette [12]. The vial was then labeled and placed in an oven for 24 hours at 70°C. After 24 hours, the mass of oil extracted was determined gravimetrically. The experiments were repeated twice to obtain the average value.

3. Results and discussion

3.1 Extraction kinetics

3.1.1 Effect of different chemical matrices on the yield of oil extraction. As mentioned earlier, heptane was used for the oil extraction process as a solvent and the extraction processes were conducted for five hours with an interval of one hour and at four different temperatures which are 318, 328, 338, and 348 K. Table 1 shows how the oil yield changes during the extraction from immobilized microalgae cells of *C. vulgaris* at different chemical matrices with increasing temperature and extraction time. As the temperature increases relatively with the extraction time, the amount of oil extracted using SA and SA + CA + CMC also increasing, which can be said that the higher the temperature, the higher the amount of oil extracted from microalgae. This is in line with the results obtained by Toda et al,[13] where the oil extracted from soybean using ethanol. These results justify the fact that an increasing temperature will increase the solubility of the oil in the solvent by decreasing the viscosity, of both the oil and solvent in the extract solution [14]. However, the amount of oil extracted from the immobilized microalgae cells that have been prepared using SA+CA and SA+CMC are not following the trend where the oil yields are fluctuating as can be seen in Table 1. The highest amount of oil extracted using SA+CA is at 65 °C and SA+CMC is at 55 °C, respectively. These fluctuations might result from other external factors.

Table 1. The oil extraction yield (%) from *C. vulgaris* biomass and the reaction rate constants at different temperatures and extraction time.

i. Oil extraction yield (%) for SA						
Temp (K)	Reaction rate constant (min ⁻¹)	Oil extraction yield (%)				
		60 min	120 min	180 min	240 min	300 min
318	8.66×10^{-4}	16.06 ± 3.06	16.78 ± 3.45	17.56 ± 4.35	18.36 ± 5.36	19.18 ± 6.31
328	2.45×10^{-3}	18.54 ± 3.23	19.47 ± 3.74	20.45 ± 5.06	21.44 ± 1.91	22.46 ± 2.32
338	2.62×10^{-3}	19.55 ± 0.66	20.51 ± 2.53	21.52 ± 1.45	22.54 ± 0.21	23.59 ± 0.14
348	3.51×10^{-3}	20.37 ± 1.94	21.51 ± 1.81	22.68 ± 0.23	23.87 ± 1.05	25.11 ± 1.94

ii. Oil extraction yield (%) for SA + CA						
Temp (K)	Reaction rate constant (min ⁻¹)	Oil extraction yield (%)				
		60 min	120 min	180 min	240 min	300 min
318	3.95×10^{-8}	18.29 ± 5.86	18.61 ± 5.92	19.00 ± 5.72	19.40 ± 5.70	19.84 ± 5.71
328	4.80×10^{-3}	18.19 ± 3.06	19.46 ± 2.80	20.77 ± 2.16	22.11 ± 1.47	23.51 ± 1.35
338	5.10×10^{-4}	21.66 ± 0.72	22.46 ± 0.12	23.29 ± 0.05	24.15 ± 1.06	25.04 ± 1.88
348	9.38×10^{-4}	20.86 ± 1.61	21.72 ± 0.92	22.62 ± 0.06	23.53 ± 0.14	24.49 ± 1.17

iii. Oil extraction yield (%) for SA + CMC

Temp (K)	Reaction rate constant (min^{-1})	Oil extraction yield (%)				
		60 min	120 min	180 min	240 min	300 min
318	3.77×10^{-3}	14.64 ± 1.24	15.64 ± 2.10	16.68 ± 2.14	17.75 ± 2.43	18.87 ± 1.31
328	4.09×10^{-3}	17.34 ± 4.15	18.48 ± 4.49	19.65 ± 4.05	20.87 ± 3.29	22.13 ± 2.30
338	6.60×10^{-3}	16.71 ± 3.87	17.90 ± 3.67	19.12 ± 2.44	20.36 ± 1.10	21.65 ± 2.19
348	1.41×10^{-2}	14.11 ± 0.85	15.67 ± 5.11	17.27 ± 3.60	18.89 ± 3.54	20.54 ± 2.34

iv. Oil extraction yield (%) for SA + CA + CMC

Temp (K)	Reaction rate constant (min^{-1})	Oil extraction yield (%)				
		60 min	120 min	180 min	240 min	300 min
318	2.60×10^{-17}	12.26 ± 0.59	12.38 ± 0.51	12.54 ± 0.49	12.74 ± 0.35	12.96 ± 0.29
328	1.25×10^{-16}	12.60 ± 0.57	12.72 ± 0.66	12.88 ± 0.58	13.05 ± 0.52	13.25 ± 0.49
338	1.58×10^{-3}	10.97 ± 2.16	11.63 ± 1.27	12.33 ± 0.69	13.05 ± 0.09	13.81 ± 0.21
348	6.12×10^{-4}	12.56 ± 0.55	13.20 ± 0.32	13.87 ± 0.98	14.56 ± 1.61	15.31 ± 1.50

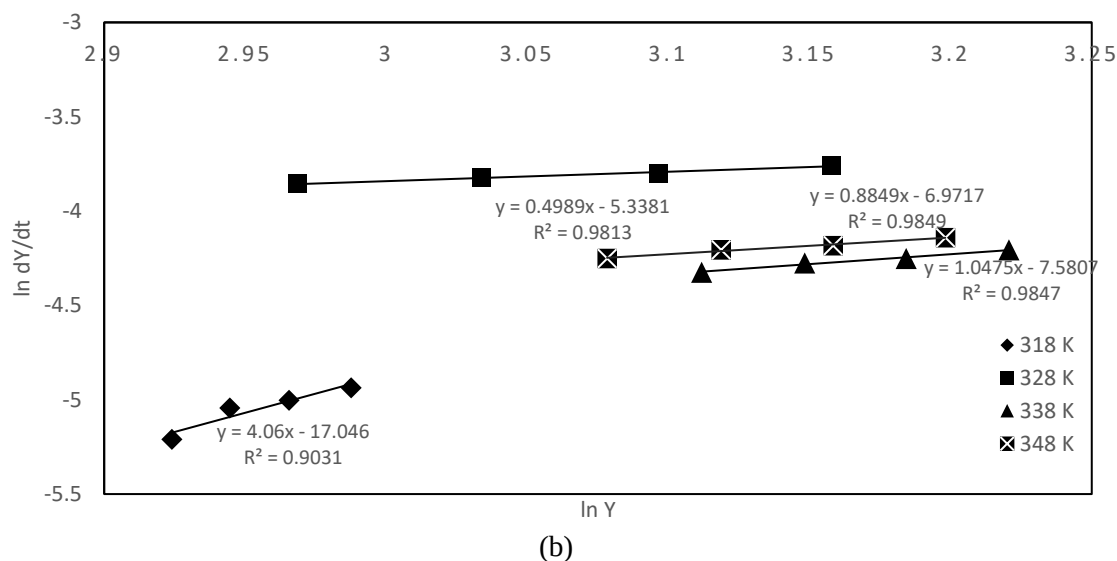
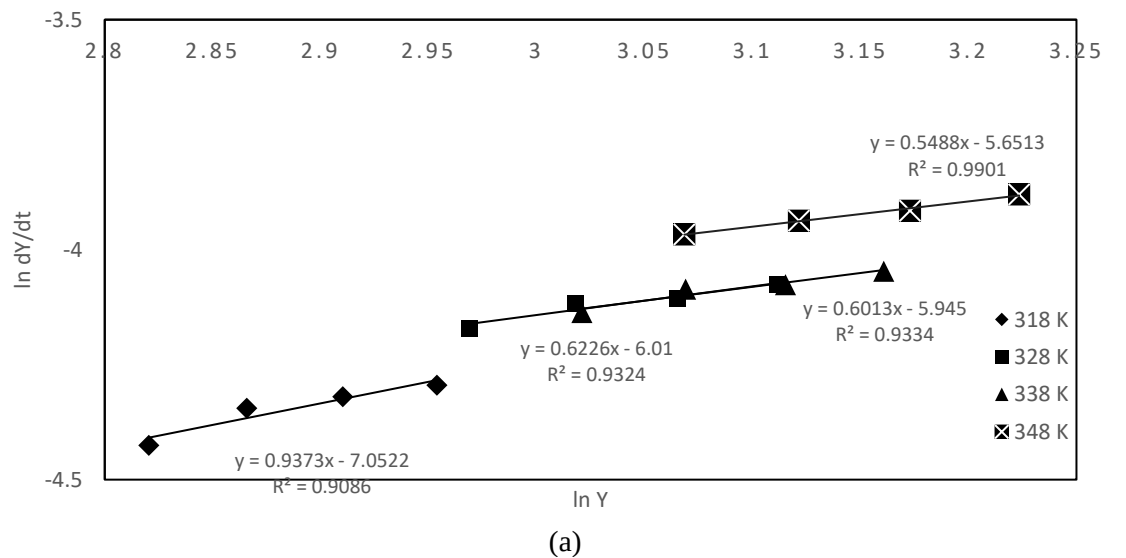
Based on Table 1, it can be seen that the amount of oil extracted from immobilized *C.vulgaris* for the SA system is the highest, which is 25.11 % at 348 K followed by SA + CA, SA + CMC, and SA + CA + CMC systems corresponding to 25.04 at 338 K, 22.13 at 328 K, and 15.31 % at 348 K, respectively. Notably, the highest yield for every chemical matrix was achieved when the extraction time longer which is 5 hours. Oil extraction from SA was found to be the most effective extraction process than the combined matrices in terms of extraction yield. This might be due to the physical properties of the chemical matrices that reducing their ability for the solvent extraction. This agreement is in line with the findings of Rushan et al, [15] where the highest yields were achieved for the 0.3:1 volume ratio of SA. Moreover, the author also reported that when using the SA system alone for the microalgae immobilization might cause the chlorophyll produced is higher compared with the combinations of chemical matrices [15].

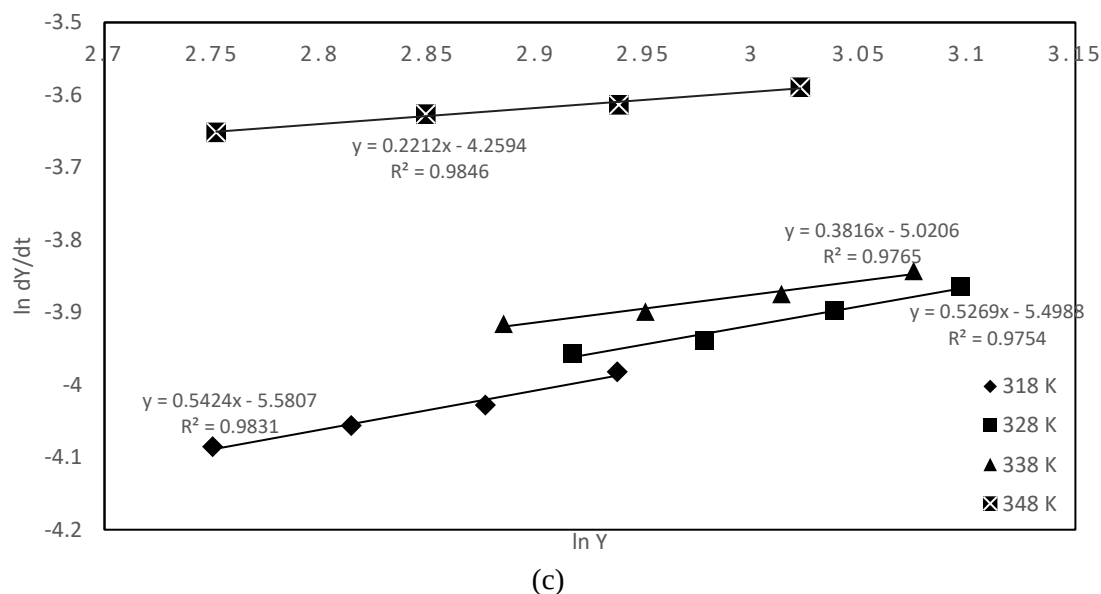
On the other hand, the combinations of the SA with other chemical matrices yielded a lesser amount of oil. This observation was similar to Lam & Lee, [6] where the author stated the single matrix has higher efficiency on oil recovery. A possible cause for this trend may be due to the slight changes that happened to the polarity and viscosity of the chemical matrices that have a direct impact on oil extraction, as compared to the that when using the pure SA matrix. However, Abu Sepian et al, [12] reported that the combination matrix systems SA+CA+CMC of volume ratio 1:1 has a higher percentage of oil yield at 44.43 %. Therefore, the combination of matrix systems still can be considered for the preparation of immobilized microalgae cells while implying appropriate proportions of media to achieve a high amount of oil extracted.

3.1.2 Effect of different chemical matrices on reaction rate constant. Apart from the determination of yield of oil extraction, the current context also mainly focuses on the calculations of reaction rate constants, k . Being one of the key elements of kinetic studies, the reaction rate constant is defined as the requirement of time to get maximal oil from the extraction, and also the rate constants linearly increased with an increase in temperature [10]. The reaction rate constant can be determined by obeying the equation as follows:-

$$\frac{dY}{dt} = kY^n \quad \text{Eq. (1)}$$

where Y is the oil extraction yield (%), t is the time of extraction (min), k is the extraction rate constant (min^{-1}) and n is the order of the reaction. Theoretically, the term dY/dt will have a positive sign as the percentage of oil extraction increases over the temperature and extraction time [16]. Using the values in Table 1 and applying the differential method, plots of $\ln(dY/dt)$ versus $\ln Y$ at different temperatures were found to be linear according to Eq. 1. The reaction rate constants were determined from the intercept of the linear plot in Figure 1.





Seeing the k values in Table 1, there is an obvious trend of increasing values with the increasing temperature for SA and SA + CMC. This is because the increasing temperature tends to have a rise in the reactivity of the solvent, which boosts the extraction rate [10]. These results clearly state that the reaction rate constants depend strongly on temperature. This trend also reported by Priya et al, [17] where the rate of extraction increased concerning temperature. This trend confirms that the temperature variation will influence the solubility rate of microalgal oil. Hence, higher temperature permits rapid mass transfer giving way to a larger extraction rate. However, the cases are opposite for SA + CA and SA + CA + CMC where the values of reaction rate constants were fluctuating and not obeying the theory of reactivity. In other words, the combined matrices tend to have abnormal reaction nature due to their lower yield of oil extraction and should have to bring forward for future consideration.

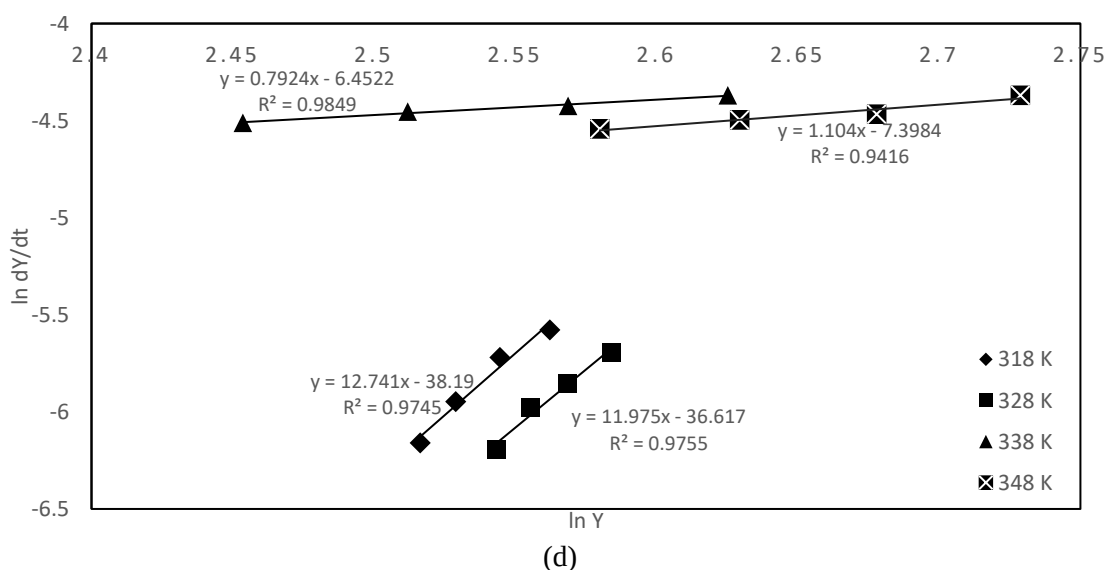


Figure 1. A plot of $\ln(dY/dt)$ versus $\ln Y$ for (a) SA; (b) SA + CA; (c) SA + CMC; (d) SA + CA + CMC

3.2 Activation energy

For a unit operation, it is crucial to have a chemical system with potential components for which activation energy is a must. Activation energy is the minimum energy required to begin an extraction process which is normally denoted by E_a . Equation 2 shows the association between the rate constant and extraction temperature as described by Arrhenius.

$$k = Ae^{-E_a/RT} \quad \text{Eq. (2)}$$

where k is the reaction rate constant (min^{-1}); A is the Arrhenius constant (s^{-1}); E_a is the activation energy (kJ/mol); R is the universal gas constant (J/mol.K) and T is the absolute temperature (K). A plot of $\ln k$ versus $1/T$ (Figure 2) yields a straight line with the slope representing the activation energy of extraction ($-E_a/R$) and an intercept as the Arrhenius constant ($\ln A$).

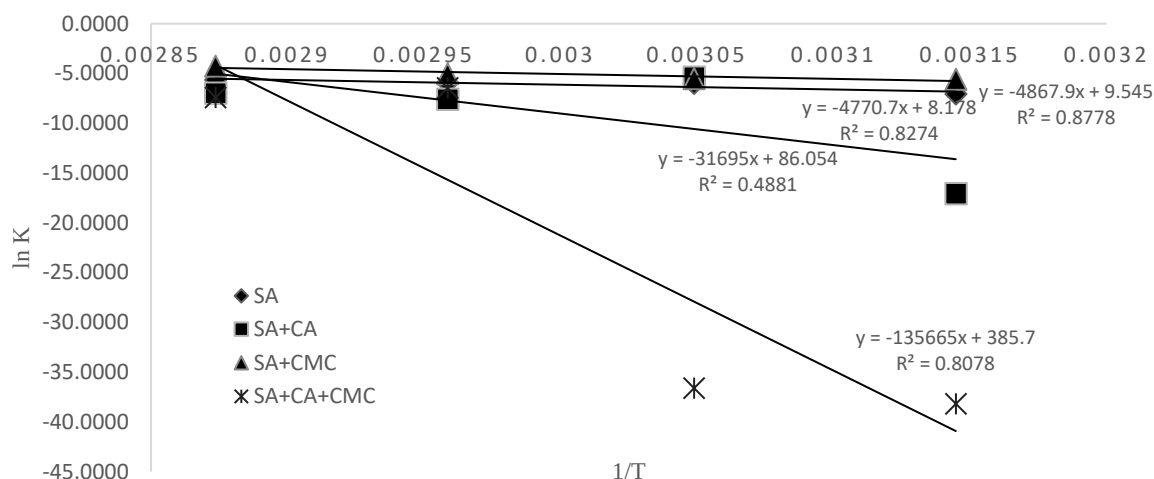


Figure 2. Activation energy calculation for different chemical matrices from the plot of $\ln k$ vs $1/T$ (K^{-1})

It is found that the oil extraction of the SA system has the lowest activation energy compared to other chemical matrices. The activation energy of SA was determined to be 39.6636 kJ/mol , followed by the combinations, SA + CMC, SA + CA, and SA + CA + CMC corresponding to 40.4717, 263.5122, and 1127.919 kJ/mol , respectively. Notably, the values of activation energy for the combinations of SA + CA and SA + CA + CMC reveals that the minimum energy needed to begin the extraction process was higher, and practically it is not suitable for the industries. The lower the value of activation energy, the lower the minimum energy input needed to begin the extraction of oil. This was also suggested by Mathiarasi & Partha, [10] where the activation energy for the oil extraction from Daturametel Linn oilseed is 25.8 kJ/mol . On the other hand, Priya et al, [17] also reported the same theory and the activation energy calculated is to be 10.0765 kJ/mol for the oil extraction from *C. vulgaris*.

3.3 Thermodynamic parameters

The current study also comprises the calculations of thermodynamics data for the reaction of oil extraction from immobilized microalgae cells of *C. vulgaris* for every chemical matrix. The thermodynamic parameters (ΔH , ΔS , and ΔG) were estimated using Equations 3 and 4.

$$K = \frac{Y_T}{Y_U} \quad \text{Eq. (3)}$$

$$\ln K = -\frac{\Delta G}{R} \frac{1}{T} = -\frac{\Delta H}{R} \frac{1}{T} + \frac{\Delta S}{R} \quad \text{Eq. (4)}$$

The slope of a straight line given by the plot of $\ln Y_T$ versus $1/T$ (Figure 3) represents the enthalpy change in extraction ($-\Delta H/R$). The enthalpy change for SA is 0.115 kJ/mol, followed by combinations of matrices, SA+CA, SA+CMC, and SA+CA+CMC, with the values of 0.094, 0.033, and 0.071 kJ/mol, respectively. The positive values obtained, indicate that all the reactions using different chemical matrices are endothermic. Hence, the extraction processes of all the chemical matrix systems absorb heat energy and external energy is required for efficient extraction. Ahmad et al [16], R. Mathiarasi, and N. Partha [10], and Priya et al [17] have claimed similar statements. In their works, the enthalpy change values are positive indicating their reactions are endothermic too.

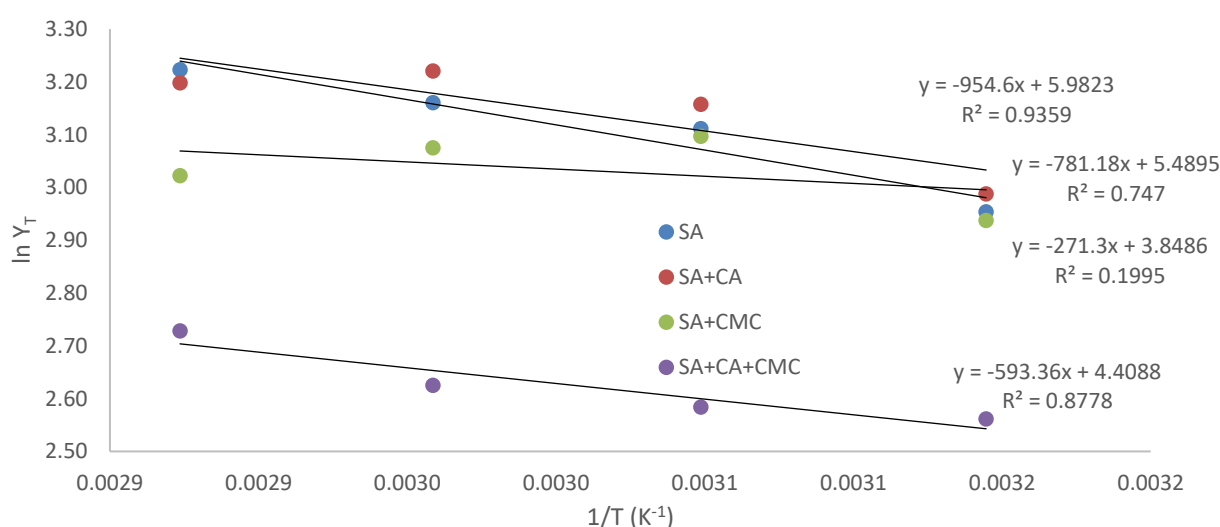


Figure 3. Enthalpy changes calculations for different chemical matrices from the plot of $\ln Y_T$ vs $1/T$ (K^{-1})

Other thermodynamic parameters such as entropy change, ΔS , and Gibb's energy, ΔG , and the equilibrium constant values for every chemical matrix systems are given in Table 3 for each temperature. Referring to Table 3, the negative values of ΔG and a decrease in free energy proving that the extraction process is thermodynamically spontaneous. Entropy ΔS is a state function and is a measure of disorder or randomness whereas entropy change shows whether the system can be returned to the initial state or not after the completion of the process. Here, the positive value of entropy change ($\Delta S > 0$) demonstrates that the process is irreversible and the spontaneity of the reaction more favorable. For the reactions using SA, it follows the theory where the values of free energy decreasing and entropy values are increasing though the positive value of entropy change ($\Delta S > 0$) at 328 K indicates that the process is irreversible, whereas the value of ΔS is negative for the $T < 318$ K. Thus, lower extraction temperature is not suitable for oil extraction from immobilized microalgae cells of *C. vulgaris*. These results are in line with the findings of Ahmad et al, [16].

Table 2. The equilibrium constant (K) and the thermodynamic parameters (ΔH , ΔS , and ΔG) for different chemical matrices

Chemical matrices	Enthalpy change, ΔH (kJ/mol)	Temp (K)	K	ΔG (kJ/mol)	ΔS (1/mol K)
SA	0.115	318	0.921	0.217	-0.321
		328	1.281	-0.674	2.406
		338	1.438	-1.020	3.357
		348	1.686	-1.512	4.675
SA + CA	0.094	318	0.984	0.042	0.162
		328	1.426	-0.967	3.235
		338	1.674	-1.447	4.560
		348	1.579	-1.322	4.068
SA + CMC	0.033	318	0.893	0.299	-0.838
		328	1.238	-0.583	1.877
		338	1.180	-0.465	1.471
		348	1.055	-0.156	0.543
SA + CA + CMC	0.071	318	0.479	1.944	-5.890
		328	0.495	1.916	-5.623
		338	0.527	1.798	-5.110
		348	0.620	1.383	-3.768

However, the combined matrix which is SA+CA+CMC yielded the opposite where the free energy is in positive values making it non-spontaneous while the entropy changes in negatives which indicates the reaction reversible and making the process not feasible. The other two combined matrices which are SA + CA and SA + CMC, the values of free energy and entropy changes are fluctuating, and the nature of the reaction couldn't be identified properly.

4. Conclusion

This study has investigated the efficiency of various chemical matrices that have been used for the preparation of immobilized microalgae beads on the study of kinetic and thermodynamic. In this process, oil extraction plays a vital role where kinetics and thermodynamic values reveal the nature of the reaction that can be forwarded to the industrial scale in the future. The results confirmed that the extraction process for SA was spontaneous and endothermic. In the terms of oil productivity, SA was proved to be the best and suitable chemical matrix where it yielded the highest amount. Hence, it can be concluded that the kinetic and thermodynamic data are an important determinant of the possible use of such raw material industrially. Also, it has been proven that oil extracted from immobilized *C. vulgaris* biomass has a high potential for biodiesel production.

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