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Enzymatic biodiesel production of microalgae lipids under supercritical carbon dioxide: Process optimization and integration

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ABSTRACT

Enzymatic biodiesel production in supercritical CO_2 (SC-CO_2) has recently received an increasing attention, as an alternative to the conventional chemical processes. In this study, enzymatic production of biodiesel from microalgal lipids was investigated in batch and integrated extraction-reaction systems. In the batch system, the effect of enzyme loading (15–50 wt%), temperature (35–55 °C) and methanol to lipid molar ratios (3–15:1) were studied, and response surface methodology was employed to optimize selected factors effect. The optimum transesterification yield of 80% was obtained at 47 °C, 200 bar, 35% enzyme loading, and 9:1 molar ratio after 4 h reaction in the batch system. The experimental results were also used to determine the kinetics parameters of the Ping-Pong Bi Bi model, with methanol inhibition, suggested to describe the reaction.

In the continuous integrated extraction-reaction system, the effect of methanol to lipids molar ratio was investigated, and enzyme operational stability and reusability were tested. Bed regeneration by *tert*-butanol washing was also assessed. The optimum methanol to lipid ratio was found to be 10:1. At this ratio, the enzyme was able to attain 78% of its original activity when reused for 6 continuous cycles, and the bed was successfully reused by washing with *tert*-butanol.

1. Introduction

Biodiesel, which is a mixture of fatty acids methyl esters, produced by transesterification of triglycerides (TGs) received considerable attention as a petroleum diesel replacer [1,2]. Using the conventional feedstock, which is vegetable oils, negatively affects the food security and increases the food prices. In addition, the high biodiesel cost is mainly due to the high raw material cost, which accounts for 60–75% of the total production cost [3–5]. These drawbacks have increased the need for the search for an alternative feedstock. Among possible alternatives, microalgae appear to be the most promising. They have high growth rate and lipid productivity compared to oil crops [6–9]. The oil yield from microalgae is reported to be 10 times higher than that obtained from best crop oil, namely palm oil [7].

Although high conversions have been reported from the alkali-catalyzed reactions in biodiesel production, the use of these catalysts possesses several environmental issues and requires feedstock pre-treatment to remove the free fatty acids (FFAs). In addition, such processes require complete biodiesel purification processes, which further increase the production cost [10,11]. Recently, enzymatic catalysis by lipase has received considerable attention as an alternative approach. Lipases are biocatalysts that work at mild operating conditions, which lower the overall energy requirement. They can esterify FFAs and transesterify TGs and therefore feedstock pretreatment would not be required. In addition, the recovery and reuse of the catalyst can be enhanced by using the enzyme in an immobilized form.

On the other hand, the use of immobilized lipases suffers from inhibition and mass transfer limitations that decrease the reaction rate. The use of an organic solvent has been suggested to improve the diffusivity of substrates toward the enzyme active sites and the products away from them. Several organic solvents have been successfully tested [12–16]; however, they all raise environmental concerns and require separation from the reaction medium for their re-use and product purification.

Supercritical CO_2 (SC-CO_2) on the other hand, has received increasing attention in biocatalysis applications as a green reaction

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medium. SC-CO_2 can modify the lipase formation and enhance its catalytic activity and stability [17,18]. It has also many useful features such as being inert, non-flammable, inexpensive, available in sufficient quantities, and CO_2 has moderate critical temperature and pressure with tunable properties. Compared to conventional reaction solvents, SC-CO_2 yields a faster reaction rate [19,20]. It offers easy product separation while maintaining the same advantages for lipase catalysis as organic solvents do, such as solubilizing the lipid and favoring esterification to hydrolysis. Another advantage of using SC-CO_2, over organic solvent, is that the produced biodiesel dissolved in SC-CO_2 and does not require glycerol separation unit, as found in conventional techniques, since the solubility of glycerol is low in SC-CO_2 and is left in the reactor.

Promising results have recently been reported for the use of lipase with SC-CO_2 in the production of biodiesel from FFA by esterification. However, limited work has been done on the transesterification of TGs.

Recently, several studies tested the use of continuous substrates flow in a reactor, packed with immobilized lipase. These types of continuous reactors are popular due to their easy operation and product separation, continuous removal of inhibitory products and re-use of the enzyme [21]. In addition, bed regeneration could be achieved by washing with a proper solvent, such as *tert*-butanol. The continuous production process using SC-CO_2 and immobilized lipase has been reported by many investigators. Dalla Rosa et al. [22] investigated a single continuous reactor to produce ethyl esters from soybean oil. More recently, Ciftci and Temelli [23,24] investigated the conversion from corn oil in a similar system. Lubary et al. [25] and Rodrigues et al. [26] used similar reaction process for ethyl ester production from milk fat and sunflower oil, respectively. In all these studies, the substrates mixture were initially mixed in an agitation system and then fed into the enzyme bed by a high pressure pump. Therefore, the economic feasibility of such processes may not be obvious due to the high pumping cost, despite the positive effect on reducing the inhibition caused by short alcohols and easy product separation. The use of SC-CO_2 for lipid extraction from mictroalgae may however be justified, especially if the biomass leftover, after lipid extraction, is to be used in pharmaceutical application. A combined continuous process, however, of extracting lipid from the biomass using SC-CO_2 and the use of the extracted lipid for biodiesel production in SC-CO_2 in one integrated system would then be economically be feasible. In this way, the attractive advantages of performing the reaction in SC-CO_2 medium will be gained, avoiding at the same time the disadvantage of high pumping cost. Authors of this work have developed a continuous process for lamb fat conversion to biodiesel, where the fat that was extracted from lamb meat was already dissolved in SC-CO_2, and was fed directly to the enzymatic bioreactor to produce biodiesel without the need of expensive pumping.

In this work, biodiesel was produced from microalgal lipids extracted from *Scenedesmus* sp. The lipids were extracted by SC-CO_2 and converted to fatty acid methyl ester (FAME) using Novozyme®435 and SC-CO_2 as reaction medium. A preliminary study on enzyme loading in the range of 15–50% was done to determine the optimum loading value. This enzyme loading was then used to carry out a parametric study on the reaction conditions that may significantly affect the yield, namely temperature (35–55 °C) and methanol to lipid (M:L) molar ratio (3:1–15:1), in a batch system. The experimental data were used to determine the kinetic parameters of Ping-Pong Bi Bi model, commonly used to describe the transesterification reaction. The data were also used to generate second order regression model, where the effect of the significant factors were determined statistically by analysis of variances (ANOVA) using response surface methodology (RSM). In addition, the packed bed reactor containing immobilized enzyme in SC-CO_2 has been tested in an integrated extraction-reaction process. The reusability and stability of the enzyme were investigated, which are significant for an economic production.

2. Theory and kinetic model development

For any reactor design and process scale up, the thorough knowledge of reaction kinetic is of great importance to get information about the rate of product formation. Several studies have been focused on the kinetics of enzymatic transesterification of lipids, where most of them confirmed that Ping-Pong Bi Bi with competitive alcohol inhibition best describes the reaction [3,16,27]. Ping-Pong Bi Bi model is a double displacement mechanism in which the first substrate, lipid, combines with the enzyme forming fatty acid-enzyme intermediate that forms the first product leaving behind a modified enzyme intermediate (E-X). This modified intermediate then joins with the second substrate, methanol, to produce another intermediate, which produce the second product and the enzyme returns to its natural state. Further information about the representation of the mechanism can be found elsewhere [27]. The rate expression of the transesterification reaction of the lipids based on this model with competitive inhibition by the methanol is given by Eq. (1):

$$v_i = \frac{v_{\max}[S][M]}{[S][M] + K_{m,m}[S] + K_{m,l}[M]\{1 + ([M]/K_{im})\}} \quad (1)$$

where v_i is the initial reaction rate, $v_{\max}$ is the maximum velocity of the reaction, [*S*] and [*M*] are the molar concentrations of lipid and alcohol, respectively, $K_{m,l}$ and $K_{m,m}$ are the apparent Michaelis–Menten constants for the lipid and methanol, respectively and K_{im} is the apparent methanol inhibition constant.

In Eq. (1), since the initial reaction rate is considered, the amount of produced glycerol is negligible, and its inhibitory effect can be eliminated. The classical studies used this model were performed by investigating the independent effect of both substrates concentration on the reaction rate. In such cases, one of the substrates concentration were fixed and changing the concentration of the other substrate, and vice versa.

Several simplifications to above model has been carried, and authors of this work had developed a simplified model, shown in Eq. (2) based on the initial mass concentrations rather than initial molar concentrations in term of the initial fixed lipid concentration [16]. The effect of methanol mass concentration on the reaction rate was investigated by transesterifying fixed initial quantities of lipids with various initial concentrations of methanol.

$$v_i = \frac{v_{\max}[M]^*}{[M]^* + K_{m,m} + K_{m,l}[M]^*\{1 + ([M]^*/K_{im})\}} \quad (2)$$

where $[M]^*$ is the initial mass concentration of methanol in g g^{-1}.

3. Materials and methods

3.1. Strain, chemicals, and enzyme

Dried biomass of *Scenedesmus* sp. microalgae was kindly provided by AlgaOil Limited, Philippines. The provided biomass was cultivated in an organic fertilizer (NPK, grade 14-14-14) and then sun dried. Liquefied CO_2 with a purity of 99.95% and zero air were supplied by Abu-Dhabi Oxygen Company, UAE. Ultra high purity helium was supplied by Air Product Company, UAE. HPLC grade methanol (99.9% assay), *n*-hexane (99% assay), *tert*-butanol (99% assay) and standards solution of high purity FAMEs that consist of; 4.0% myristic acid (C14:0), 10.1% palmitic acid (C16:0), 6.0% stearic acid (C18:0), 35% oleic acid (C18:1), 36.0% linoleic acid (C18:2), 2.0% of archidonic acid (C20:0) and behenic acid (C22:0) were purchased from Sigma–Aldrich, USA. Novozym®435 of 7000 PLU per