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Process optimisation and parametric effects on synthesis of lipase immobilised carbonaceous catalyst for conversion of rubber seed oil to biodiesel

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ABSTRACT

Enzymatic transesterification for biodiesel synthesis have gained significant attention due to green and advantageous process over alkali and acid catalysed reactions. In this view, the present study elucidates the synthesis of carbonaceous catalyst support for immobilisation of lipase enzyme towards its use as heterogeneous biocatalyst in biodiesel synthesis. The study mainly emphasised on the optimisation of parametric conditions for catalyst synthesis process towards maximisation of adsorption capacity and catalytic activity in transesterification. The parametric effects on immobilisation process were studied to identify the significance of individual parameter using taguchi approach. The significance of each parameter was tested by ANOVA study and confirmed by estimating contribution factor. The analysis illustrates that initial concentration, pH and time affects the adsorption capacity significantly. However, the catalytic activity in transesterification is affected significantly by catalyst preparation conditions like temperature, pH and initial concentration. The optimised condition evaluated for catalyst preparation to achieve maximum adsorption capacity and biodiesel yield were: initial concentration 400 ppm, temperature 30 °C, time 120 min and pH 6. Thus, the study revealed that indigenously prepared carbon supported lipase enzyme at optimised conditions were effective in catalysing the methanolysis of rubber seed oil to achieve the renewable biodiesel fuel. The study has also shown that taguchi approach was effective in identifying the significant parameters affecting the immobilisation process and achieving optimised condition for cost-effective synthesis of catalyst to ultimately minimise the cost of biodiesel synthesis process.

1. Introduction

The increasing environmental hazards due to exhaustive consumption of fossil fuels have motivated the researchers towards development of various renewable energy sources. The continuous depletion of conventional energy sources and increasing energy demand needs the development of prominent renewable energy source to replace the mostly consumed conventional diesel. The fatty acid alkyl ester usually recognized as biodiesel has emerged as a probable alternative, but its higher cost limiting its application on a commercial scale. The biodiesel cost is mainly determined by the cost of feedstock and catalytic route chosen for synthesis. The first generation biodiesel was produced by using edible oils as feedstocks but food scarcity and cost of the edible oil restricted its application [1]. Thus, the focus is shifted towards inedible oils due to their abundant availability and lower cost. However, the use of inedible oils still not effective enough to curtail the cost of the biodiesel to compete with existing petro-diesel. The economical biodiesel synthesis is mainly depending on the catalytic route and catalyst chosen for the transesterification. Generally, the biodiesel is produced via two routes namely, chemical and enzymatic catalysis [2]. The chemical catalytic processes are faster and cost-effective as compared to enzymatic route due to lower cost of the alkali and acid required. However, the soap formation, waste water generation in washing steps and difficulties in product separation significantly contributes to the higher cost of biodiesel using alkali and acid catalyst. The enzymatic route is very effective in terms of getting highly purified product at room temperature, thereby minimising the energy consumption, waste water generation, and purification cost. Recently, lipase has gained significant attention due to its versatile applicability and effectiveness in the synthesis of biodiesel. Despite the appreciable catalytic activity of the

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lipase, its applicability on a commercial scale is constrained by its cost and stability [3].

The work using free lipase as a homogeneous catalyst for biodiesel production is carried out substantially. However, the cost of free lipase and inability to recover and its reuse significantly increases the cost of biodiesel. Therefore, the attention is paid to immobilisation or encapsulation of the free enzyme using different supports [4]. Arumugam et al. (2018) investigated the use of silica aerogel for the immobilisation of lipase and used in methanolysis of rubber seed oil. The immobilised catalyst shows higher activity to yield 93% biodiesel and appreciable stability in reuse until 10 cycles [5]. The use of polymeric material polymethacrylate amino-epoxide as a catalyst support was reported by Bonet-Ragel et al. (2015) in the transesterification of high FFA oil. The catalyst was effective in transesterification but support was treated with glutaraldehyde to increase the immobilisation capacity of the support [6]. Moreover, modified silica induced with Fe_3O_4 was used as a support for lipase immobilisation to enhance biocatalyst stability in the conversion of cellulose to fructose [30]. Wu et al. have investigated the use of gold nanoparticles for immobilisation of lipase and checked the catalyst stability to verify the effect of lipase to support ratio on catalytic activity [31]. Moreover, Sarno et al. have modified gold nanoparticles with Fe_3O_4 for its application as a catalyst in biodiesel synthesis. The modified support has shown appreciable activity with a maximum yield of 98.5% [32]. The catalyst supports discussed above are chemically synthesised and requires various chemicals and larger energy for synthesis. Thus, there is a need for the development of natural and green catalyst support which could be derived from the waste available abundantly.

Activated carbons (AC) are proved to be an effective catalyst support for increasing the stability and activity of the free lipase. The use of AC for the immobilisation of lipase was reported earlier but its use still not practised explicitly. Kabbashi et al. used the amino functionalised AC as a support and employed as a catalyst in the hydrolysis of jatropha oil with 78% yield [7]. Naranjo et al. also studied the use of coconut shell derived AC as a support for Candida antarctica B lipase and used as a catalyst in the transesterification of palm oil. The catalyst was highly effective in giving 100% yield using isobutanol as a reactant. However, it was observed that the catalytic activity is decreased with lower molecular weight alcohols [8]. Brito et al. have used yellow mombin fruit stones derived AC from for immobilisation of lipase. The results stated that the catalyst showed high hydrolytic activity with appreciable stability and concluded that AC is very effective as a catalyst support [9]. The greater surface area and effective porous structure of the AC make it prominent support as compared to other chemically synthesised materials.

Lipase is generally immobilised by various routes such as physical entrapment, cross-linking, adsorption, *de novo* approach and multipoint strong attachment [10,33]. The adsorption is the simplest method of immobilisation due to lower cost and negligible losses in catalytic activity and selectivity of the lipase. The lipase is usually got binds on the surface by low energy relations such as hydrophobic interactions or van der Waals forces, ionic bonding and hydrogen bonding [11]. The selection of support is highly influenced by surface properties such as surface area, pore volume, shape, pore distribution, nature of the surface, surface charge and cost of the material. Renewable carbon resources like biomass and polymers can yield quality AC that provides flexibility in tuning and tailoring the surface properties within minimal expenses thus proves their prominence as a catalyst support for immobilisation of lipase [12,34].

In this context, the present work is focused on the use of waste biomass as a precursor for the development of greatly porous and cost-effective AC to be used as a catalyst support for lipase immobilisation. To the best of our knowledge, no research has been reported on the use of lipase immobilised AC as a catalyst in the conversion of rubber seed oil into biodiesel. The study also provides an insight into parametric effects on the adsorption capacity of the AC and its respective catalytic activity in transesterification reaction. The optimisation and identification of the significant parameters within minimum number of experimental runs are also studied using taguchi approach for attainment of the cost-effective process. The development of catalyst at optimised condition using flamboyant pods and its utilisation in the transesterification of inedible rubber seed oil to give higher catalytic activity makes the process cost-effective and eco-friendly. Thus, the study identified that the developed AC could be used as effective catalyst support for immobilisation lipase towards its utilisation in biodiesel synthesis.

2. Materials and methods

2.1. Materials

Refined rubber seed oil used as a feedstock for biodiesel synthesis is purchased from local market of Burdwan, West Bengal, India. *Delonix regia* or flamboyant pods selected as a feedstock for AC synthesis is gathered from the native locality of Wardha, Maharashtra, India. Lipase is chosen as a catalyst in enzymatic transesterification and bought from SRL laboratories, India. All other reagents like borax, glacial acetic acid, sodium acetate trihydrate, sodium bicarbonate, sodium hydroxide, which was used for buffer synthesis are purchased from Merck, India. Analytical grade methanol used as a reactant and purchased from RANKEM, India. Supelco, 37 FAME mixture is used as a standard for GC analysis and purchased from Sigma. Deionised water is acquired from Arium 611 DI ultra-pure water system (Sartorius A.G., Gottingen, Germany).

2.2. Methods

2.2.1. Preparation of AC

The flamboyant pods used as a raw material is collected and washed with water to get rid of dust and impurities. The precursor is then sun dried for 5 days followed by oven drying at 110 °C for 5 h to ensure the complete moisture removal. The dried pods were chopped and sliced to small fragments and given for carbonisation in a cylindrical muffle furnace. The carbonisation was carried out at 500 °C for 1 h and subsequently activated using superheated steam at optimised activation condition of activation temperature 350 °C and activation time 1.5 h [13]. The obtained activated carbonised char was grinded to reduce the particle size in the range 50–100 μm. The prepared AC was stored in the plastic container to keep it moisture free and subjected to characterisation.

2.2.2. Immobilisation of lipase on AC

The developed AC was used as support for immobilisation of lipase through adsorption. The immobilisation process was optimised using taguchi approach by varying four parameters namely, initial concentration, temperature, time and pH at three different levels. The L9 orthogonal array approach was chosen for the design of experiments as tabulated in Table 1. The stock solution of 200, 400 and 600 ppm lipase solution were prepared in three buffers of pH 4, 6 and 8. A 10 ml of each solution was taken and mixed with 0.5 g of AC and kept at varying conditions of time and temperature in orbital shaker cum incubator at 150 rpm. The immobilisation by adsorption process was carried out following the operating conditions given in Table 1 to evaluate the adsorption capacity of AC and catalytic activity of the AC supported enzyme. After achieving the stipulated time the mixtures were filtered using Whatman filter paper to separate the AC. The immobilised AC obtained after filtration was allowed to dry at room temperature overnight and directly used as a catalyst in the enzymatic transesterification of rubber seed oil.

2.2.3. Protein estimation after immobilisation

The filtrate obtained after filtration was centrifuged at 5000 rpm for