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Synthesis of Eggshell-Lipase Immobilized Catalyst for Production of Biodiesel

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ABSTRACT:
This research work deals with the immobilization of commercial lipase on Eggshell powder to form a heterogeneous renewable catalyst for biodiesel production by utilising waste cooking oil. This will cause to reduce the cost and increase the yield of biodiesel production, it will also increase the thermal stability of the lipase, which will enhance the yield of biodiesel production. The specific activities of lipase were increased to 40 u. The parameters for the synthesis of ELC (Eggshell- Lipase catalyst) were optimised to achieve high activity of ELC, which leads to increase the thermal stability of lipase, which will enhance the rate of production of biodiesel. The lipase immobilised with eggshell, was stable at 48 – 50°C for a prolonged time, which led to increased yield of biodiesel production from 22 % to ~ 92 %.
Keywords*: Lipase, Eggshell, Immobilization, Transesterification, Biodiesel.*

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I. INTRODUCTION:

The Increase in world population and climate change has augmented environmental crisis and several adverse effects on human health[1]. Emitted gases, organics, and solid particulates from the varied industrial activities embracing are the leading cause for pollution of water, soil and air systems. Nitrogen oxides, sulphur oxides, evaporated hydrocarbons, carbon monoxide, and volatile organic compounds are the major polluting compounds in the air, the aquatic system, and the soil. These pollutants comprise the risk in their production sites and other places due to their mobility by air and water. Besides health issues, these pollutants participate in the global warming phenomena[2,3].

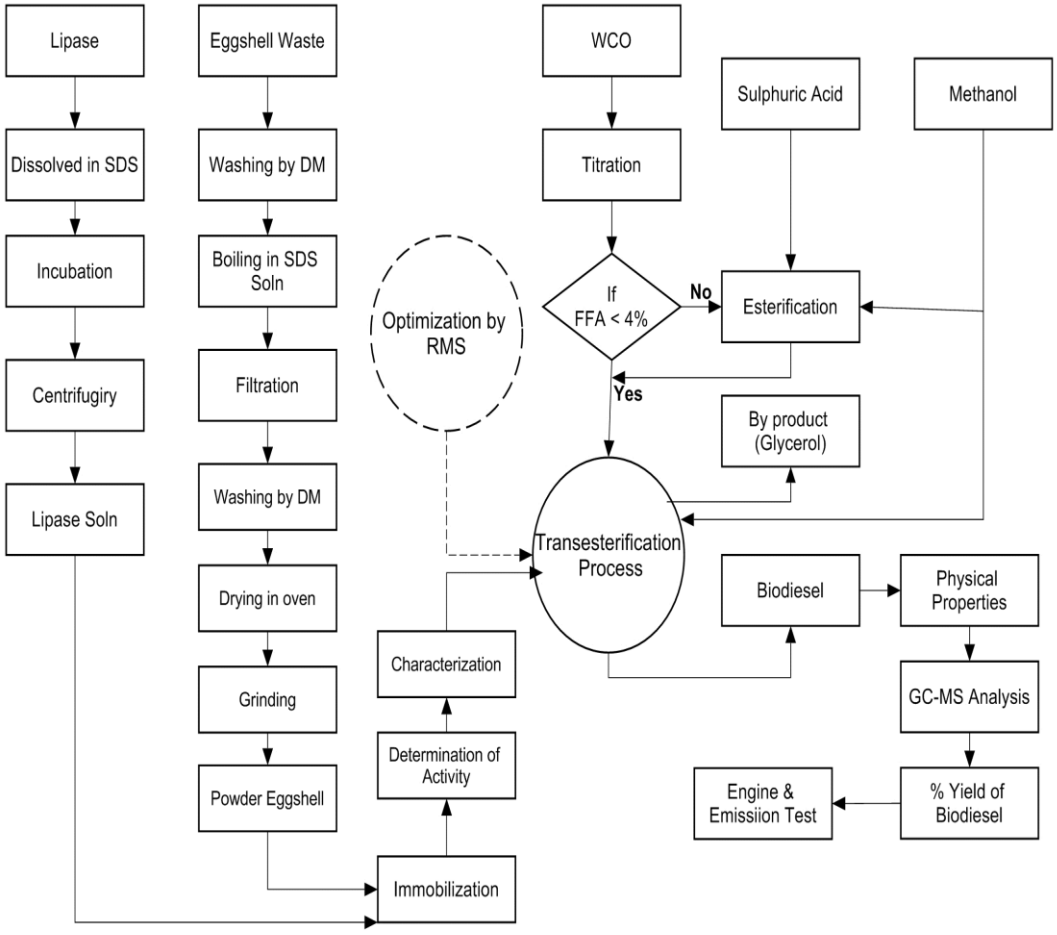
Biodiesel is an important alternative clean fuel with plant and animal sources composed of mono-alkyl esters derived from the esterified exchange of triglyceride (triple esters of fatty acids) esterification of free fatty acids with short-chain alcohol[4].One can refer to better lubrication, propagation of lesser pollutants such as carbon monoxide and sulphur dioxide, biodegradability, reduced accumulation of greenhouse gases in the atmosphere, complete combustion because of having 21% of the weight of oxygen in the given structure, and a reduction of the problem of global health as some of the advantages of biodiesel[5].

Vegetable oils and animal fats are prospective sources for the production of biofuel and hydrocarbons[6,7], and the utilization of used edible oil would be especially desirable since this approach would not divert resources from the food supply. Cracking vegetable oil for liquid hydrocarbon production is feasible with micro porous and mesoporous production of biodiesel from various sources have been reported[7]. Pyrolysis conversion of edible waste oil[9-12], Palm oil[13], oil palm wastes[14], fishing wastes[15], shark liver oil[16], *Posidonia oceanica* (L.) and frying oil wastes[17], waste clay oil[18], cooking and non-edible oil[19] suggest the possible utilization of oils as the primary source of biodiesel production. In this study, waste cooking oil was used as the substrate for biodiesel production utilizing eggshell immobilized lipase (ELC) as a catalyst. A study on optimization brought about highly stable ELC, which enhanced biodiesel production. The flow sheet for production of biodiesel with lipase-eggshell catalyst is represented in Fig.1.It is consists of various stages like source of WCO, Eggshell , lipase , preparation of reactant solution, optimization of process parameters by RSM and condition for production of biodiesel under esterification reaction. Transesterification reaction was carried out between WCO and methanol under the presence of lipase-immobilized catalyst. The produced biodiesel was characterized by physical and chemical properties observation. The GC-MS analysis was carried out to determine the presence of FAME percentage and percentage yield was calculated using amount of FAME present in the production. The yield of biodiesel was observed as 92 % by transesterification reaction with methanol and WCO in the presence of lipase –eggshell immobilized catalyst , which is tremendous improvement in the yield of biodiesel compared with production of biodiesel with other combination of subtract with eggshell catalyst[20] .The engine and emission test was carried out for produced biodiesel with lipase-

eggshell catalyst, there is negligible amount of greenhouse gases were generated, it is too low compare to generation of greenhouse gases by fossil fuel .

Flow sheet for production of biodiesel:

Lipase was purchase from SISCO Pvt. Ltd, prepare the lipase solution in SDS solution. The eggshell was collected from SIST & IGCAR cafeteria, washed, boiled, filtered, dried and grind to get eggshell powder. The lipase was immobilized in eggshell powder, determine the activity of the immobilized lipase-eggshell catalyst and characterized the catalyst. Transesterification reaction was carried out with waste cooking oil, which FFA <4%. The biodiesel produced, which undergo physical and GC-MS analysis to know the presence of FAME and yield of biodiesel. The process parameters were optimized by using RSM, Engine and emission test was carried out to confirm the application of the biodiesel in commercial use. Every steps samples were collected and analysed to ensure the performance of the product and to take feedback to control the parameters to justify the behaviour of the sample. The flow sheet of the process is given in Fig.1.



Flow Sheet For Production Of Biodiesel With Lipase-Eggshell Immobbilized Catalyst

Fig.1 Flow sheet for production of biodiesel with lipase-immobilized catalyst

II. EXPERIMENTAL:

Materials and methods

Chemicals and reagents

Lipase (Activity 40 u): purchased from SISCO Pvt Ltd. Chemicals and reagents with an analytical grade high purity used for further studies were obtained from Himedia Labs Pvt Ltd. NaOH ,SDS , HNO_3, DM water , H_2SO_4,olive oil , sodium buffer solution gum Arabic emulsion, and other chemicals were purchase from Uma scientific industries.

Preparation of Lipase

1.0 g of lipase was dissolved in a sufficient amount of sodium buffer solution (50 mM),(pH - 7), centrifuged for 10 minutes at 3200 ×g, and at -4 ℃ temperature. Supernatant was taken out, and the precipitate was added in a sufficient amount of sodium buffer solution and again repeated the centrifuging at same parameter for 10 min,

continued and repeated the same procedure 4 to 5 times so that milky precipitate converted into white colour. i.e., all the lipase protein had extracted into solution form. The obtained lipase was used for further studies.

Immobilisation of Eggshell with lipase

Lipase solution (50 ml) thus obtained was transferred to the 250 ml of a conical flask incubated under shaking condition at 37 ℃ , 125 ×g for 2 h. After shaking, the mixed content was stored for 36 h at 4 ℃. The content was filtered to separate the eggshell from the lipase solution; lipase solution gets adsorbed on the eggshell and forms bonds between them, unbounded lipase washed with sodium buffer solution (50 mM, pH - 7). Scanning electron microscopy (SEM), FTIR, XRD and BET analysis were carried out to characterise immobilised lipase with the eggshell.

Optimisation of process parameters

Effect of pH ranged from 4-9, temperature 20-60°C, time for the adsorption, and the number of cycles was considered for optimisation studies. Enzyme thus obtained at the respective optimum condition was evaluated for the activity, revealing the optimum parameter for the eggshell immobilised lipase enzyme preparation. The process parameters like temperature, amount of catalyst, time, and methanol /WCO molar ratio by RSM (Response surface methodology). The Designed optimized process parameters were compared with actual process parameters and concluded regression coefficient of correlations and was determined the fitness of the data for the project.

Lipase enzyme activity

Lipase activity was identified using the Parry Jr et al.1966[20], a method using 10% olive oil mixture in 10% gum Arabic emulsion. The reaction mixture contained 3 ml of the substrate, 2.5 ml of deionized water, 1 ml of 0.2 M Tris-HCL buffer (pH 7.5) and 1.0 ml lipase sample evaluated. The reaction was conducted for 2 h at 37°C in a shaking water bath. The reaction mixture was then supplemented with 10 ml ethanol. The amount of oleic acid was determined by titrating the hydrolysis yields with 0.05N NaOH using the phenolphthalein indicator. The quantity of enzyme catalysingthe establishment of one micromole of oleic acid in 2 h at 37°C and pH 7.5 was taken as one lipase activity unit.

Production of Biodiesel

Collected waste cooking oil from the university cafeteria was subjected to heat treatment at 60°C for the removal of impurities and moisture content. Followed by heat treatment, free fatty acids content was determined. In this procedure, a known volume of sample (10 g) dissolved in isopropyl alcohol (50 ml) with NaOH, 0.1 N NaOH, heated at 60℃, then cooled at 27°C, titrated with 0.1 N NaOH with phenolphthalein indicator. The FFA was calculated at the neutral point of reaction by titration formulae, the FFA was obtained as 2.4221wt %, which was less than 4.0, a similar result was obtained as the presence of FFA as 2.41 wt % by Hsiao et al.2021[21] in WCO. If the amount of FFA < 4, the transesterification reaction was economical; otherwise, the esterification reaction would be uneconomical.

Transesterification Reaction in the presence of immobilized lipase – eggshell catalyst

Earlier reports reveal that the immobilized lipase -eggshell catalyst would be stable at 50°C for maximum yield beyond this temperature. This study conducted a transesterification reaction at 20-65°C[21], for a time duration of 1h to 5h, in the presence of immobilized lipase 1% w/w for maximum yield of biodiesel. The yield of biodiesel was calculated by using the formulae.

$$\text{The percentage yield of Biodiesel} = \frac{mass of Biodiesel}{mass of WCO} \text{x } 100$$

The percentage yield of biodiesel production was also verified by calculating the following formulae.

$$\text{Yield of Biodiesel} = \frac{(mass of biodiesel \times \% FAME)}{(mass of WCO)} \text{x } 100$$

Purification of Biodiesel

The separation of Biodiesel from the reaction mixture contains various impurities like water, free fatty acids, glycerol, acid or other contaminants. Filtration of the oil fractions was done to different immobilized lipase. After filtration, the filtrate was centrifuged, the collected heavy oil fractions containing biodiesel were dried at 100°C for 4 h in a hot air oven to vaporize more moisture content.

III. RESULTS AND DISCUSSION:

Biodiesel is a biodegradable and eco-friendly non-synthetic petroleum-based biofuel. Various oil sources, such as edible and non-edible oils, have been used as foodstuffs for biodiesel production[22,23]. The endlessly rising price of edible vegetable oils makes them too costly as a long-term biodiesel source. Though waste vegetable oils are considered environmental pollution, they can be used as a potential raw material for biodiesel production and are cheap and readily available. Biodiesel was produced in the presence of a catalyst using the transesterification of vegetable oil. The catalyst can be homogeneous, heterogeneous, nanoparticles, or enzymatic. Homogeneous catalysts are considered more effective than their heterogeneous counterparts because

of reduced mass transfer limitations and high conversion[10,24]. In this study, eggshell immobilized lipase was used as the catalyst for transesterification of waste cooking oil, which brought about high strength biodiesel.
BET analysis of immobilised lipase -eggshell catalyst was carried out to determine, surface area (m^2/g), pore volume (m^3/g) and pore width (m/g). Relative pressure of N_2: P/P0 Vs, Density.BET analysis reveals that the surface area of the ELC was found to increase (Fig. 2).The recorded pore width volume is pointed towards the mesoporous structure. This is due to distinct pores, which facilitate lipase diffusion into the oil matrix, affecting the transesterification process. Followed by the preparation of ELC, factors influencing the lipase activity was studied like initial eggshell lipase concentrations, pH, temperature and immobilization time(Fig. 3-5).Various studies reveal that the optimization of parameters for the production of biodiesel has been reported like biofuel from Jatropha oil using are cyclable anion-exchange resin[24],Integrated stochastic mixed-integer linear programming model[25], Cost optimisation[26], thermodynamic optimisation[27],response surface methodology for microalgae cultivation[28],production process[29], microwave-assisted hydrothermal carbonisation[30] and system-level energy consumption[31].

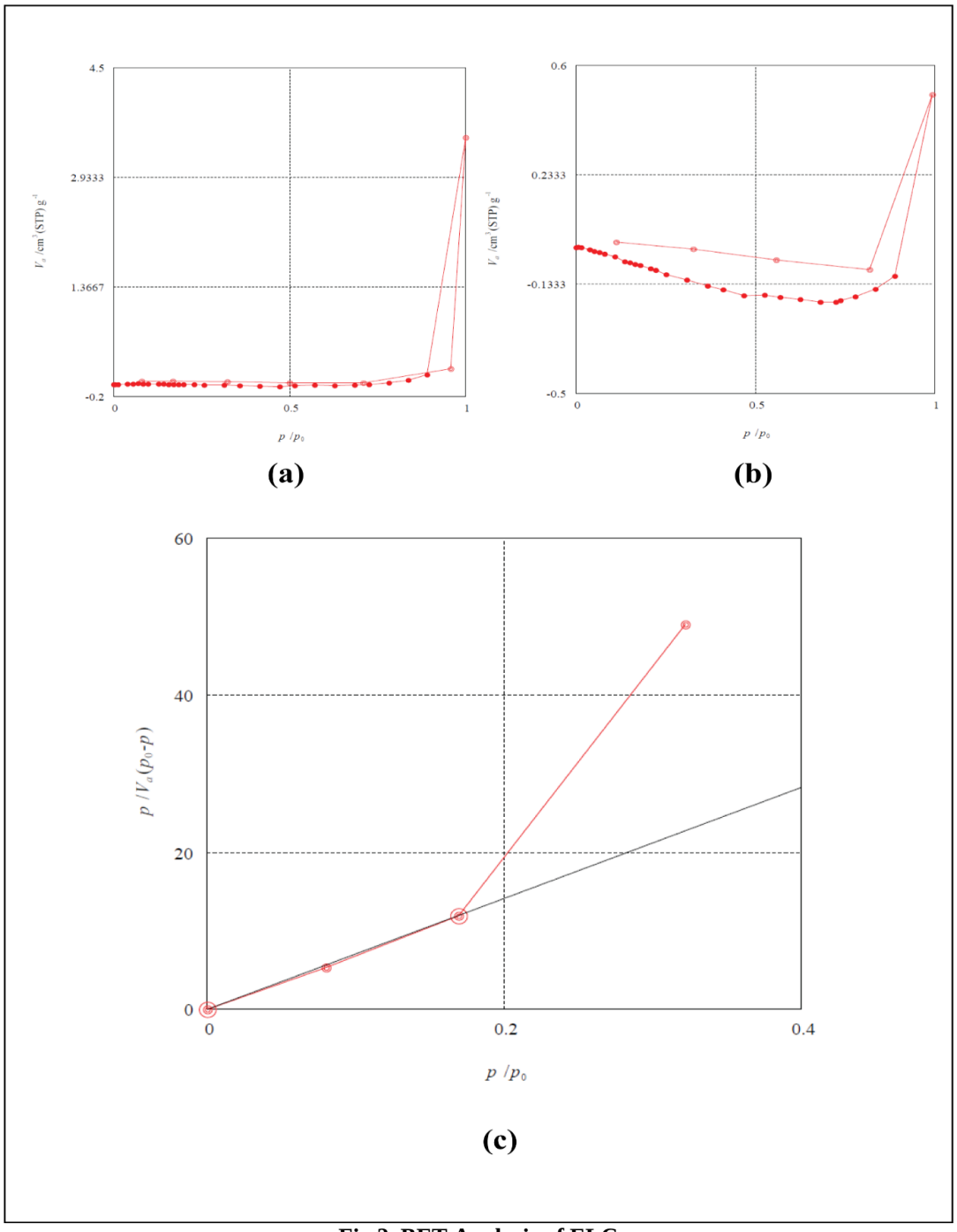


Fig 2. BET Analysis of ELC

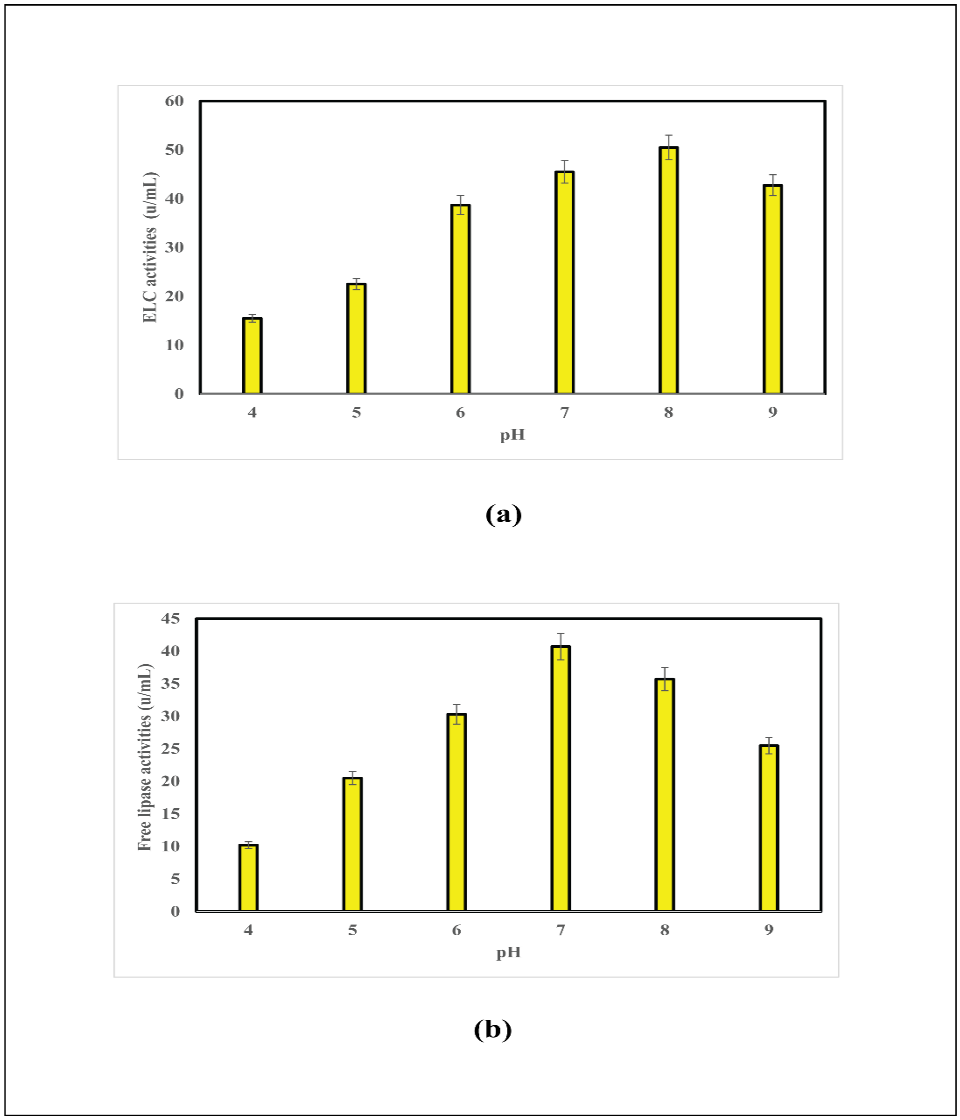
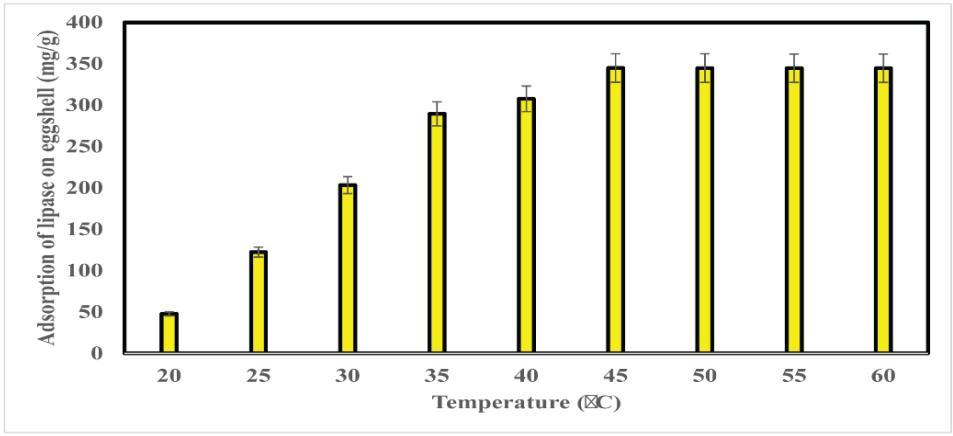
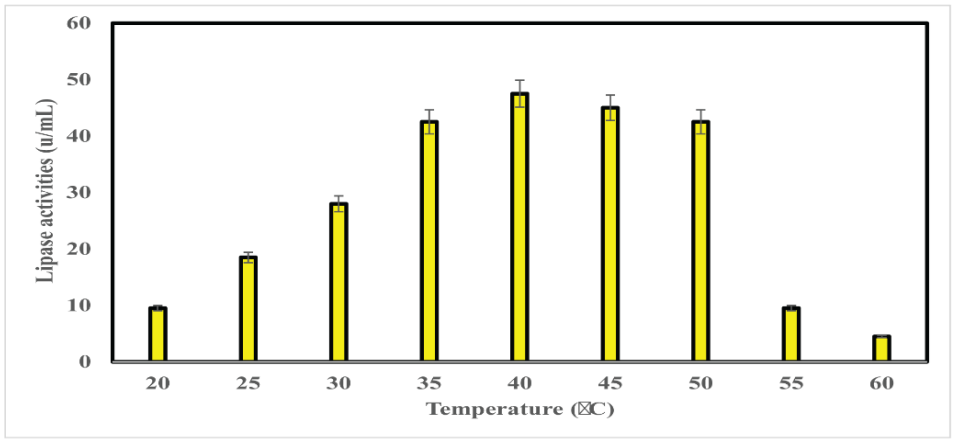


Fig 3. Effect of pH on enzyme activity

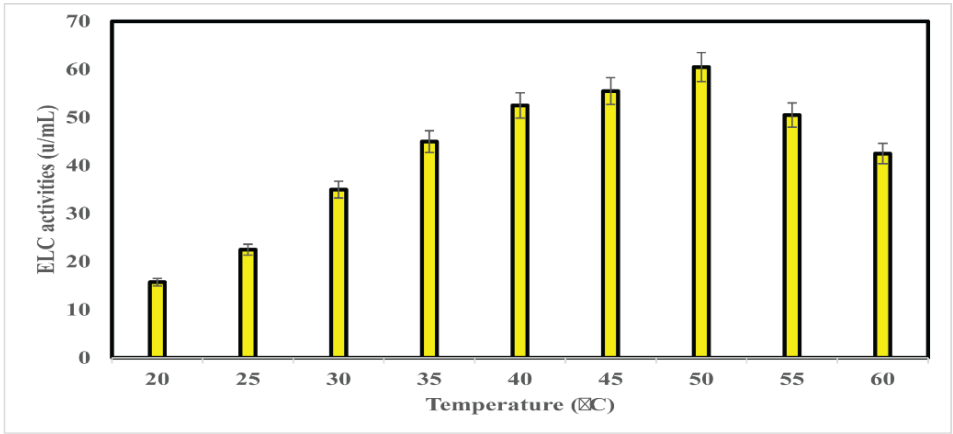
The ELC activities was gradually varying with pH of the Eggshell-lipase immobilised catalyst solution, As it is shown in the Fig.3(a), Lowest at pH-4 its activities was 15 u/ml and at highest pH-8 , it was 50 u/ml, when pH increases from 4-8 ,the activities were gradually increases from 15 to 50 u/ml,it is due to adsorption of the lipase on the eggshell and further increase in the pH from 8 to 9,the activities were started decreasing from 50u/ml to 40 u/ml,it is due to saturation of the adsorption of the lipase on the eggshell. All the unpaired electrons of lipase get bounded with eggshell and remaining unbounded lipase will be freely available, which can be removed by washing with sodium buffer solution and finally by DM water[32]. As result maximum activities was obtained at pH-8, which will give maximum yield of the biodiesel. Free lipase activity of the lipase –eggshell catalyst was gradually increases with increase in pH value. When pH increases from 4 to 7, then the corresponding free activity increases from 10 u/ml to 40 u/ml and further increase in the pH, the free activity decreases from 40 u/ml to 20 u/ml. The Maximum free activity was 40 u/ml at pH of 7.The free activity of the lipase-eggshell is neither acidic nor basic in nature[33], the free activity is the activity of loose lipase, which has not form bound with eggshell, it is not useful for production of biodiesel, besides it get washed with DM water[34] and only activity of lipase-eggshell catalyst at pH- 8, shows maximum activity of 40 u/ml, this pH confirmed the lipase-eggshell catalyst was basic in nature.



(a)



(b)



(c)

Fig 4. Effect of temperature on enzyme activity

The temperature on enzyme activity was also investigated, demonstrating that the enzyme activity was increased at 50°C[35]. However, the free enzyme activity was decreased, which implies that the thermal stability of ELC was found to be higher than free lipase.

Adsorption of lipase is depends on the temperature[36], at low temperature eggshell atoms are in packed condition, which is resisting to adsorb the lipase .In Fig. 4(a), the adsorption of lipase was observed, at 20 ℃

was 50 mg/g and when temperature gradually increases, the adsorption of lipase also increases, it was increased to 50 mg/g at 40 ℃ , further if the temperature increased its adsorption decrease gradually to 10 mg/g at 55 ℃ . It is due to destruction of the position of the atoms[37]. So the atoms of eggshell cannot hold the lipase as result lack of adsorption at highest temperature. Maximum adsorption was observed at 40℃ . Apart from that the lipase get degraded at the highest temperature[38], so it is not in position to get adsorbed at highest temperature[38]. The Activity of lipase was monitored at different temperature, it was found that the activity of lipase at 20℃ was 10 u/ml and it was gradually increases to 45 u/ml when temperature increase to 40℃, it is due to more contraction of lipase atoms at lower temperature, when temperature gradually increases, the atoms get excited its position and hence activity was more[39-44]. When the temperature further increases from 40℃ to 60℃ the activity of lipase was gradually decreased to 30 u/ml, it is due to degradation of lipase atoms and hence it is loses the functionality of the lipase at highest temperature as shown in Fig.4(b),similar result was obtained by Hyun Tae Hwang et.al. (2014)[45.] When these lipase immobilized with eggshell and form EIC (eggshell immobilized catalyst), which activity was monitored at different temperature as shown in Fig.4(c). At 20 ℃ its activity was monitored as 15 u/ml and temperature increases the activity gradually increases to 60 u/ml at 50 ℃ and further increases the temperature, slowly activity decreases and finally reached to 30u/ml at 60℃. It is due to the atom of eggshell at low temperature is not excited form and less formation of bond of lipase with eggshell, when temperature increases gradually eggshell atoms become opened and form more porous hole in the eggshell atoms and hence more lipase get adsorbed and immobilized[46], As resultthe activity of the EIC was increased,when temperature was too high, the atoms of eggshell get penetrated and loses the adsorbing characterizations and hence less amount of lipase was adsorbed at the highest temperature. As result the maximum optimized temperature for high activity of EIC was 50℃, similar result was obtained by Onyinyechukwu. J et.al. (2018)[46].

The enzyme activity was also investigated for the effect of immobilization time in detail. In the case of ELC, immobilization time of 4 min recorded 99 % of enzyme activity. Effect of temperature on adsorption of lipase was conducted. Maximum adsorption of lipase on eggshells (344.6 mg/g) was recorded at 55°C and 60°C. Re-usability of the ELC was done by measuring enzyme activity at different cycles ranging from 1to 10. A gradual decrease in enzyme activity was recorded with increased number of cycles.

Adsorption concentration of lipase on the eggshell depends on the time of adsorption, it was observed that the adsorption of lipase on eggshell from 1 hr to 10 hrs in evacuator. It was 50 , 120 , 200 , 350, 349, 345 mg/g for a time intervals of 1, 2,3, 4, 8 and 10 hrs respectively in the evacuator. The maximum adsorption was observes as 350 mg/g at 4hr. of duration. Initially the adsorption was increased with time up to 4hr and from 4hr. to 10 hrs, the adsorption gradually decreasing to 345 mg/g. It is due to formation of bond between lipase and eggshell from 1hr to 4hr and all the molecules of lipase get attached with eggshell, once all the eggshell molecules are being attached and no more molecules were remains for attachment then it almost maintained the concentration of 345 mg/g at 8 h duration, further increase in duration time of adsorption, some of the weakened bond of lipase get detached from the eggshell and hold as free molecules, hence the concentration was decreased to 345 mg/g at 10 h, these free molecules of lipase were removed with washing with DM water to achieved desired concentration of lipase with eggshell in the immobilization process. The similar result was observed by Muktesh Mohan et.al.(2020)[47.]

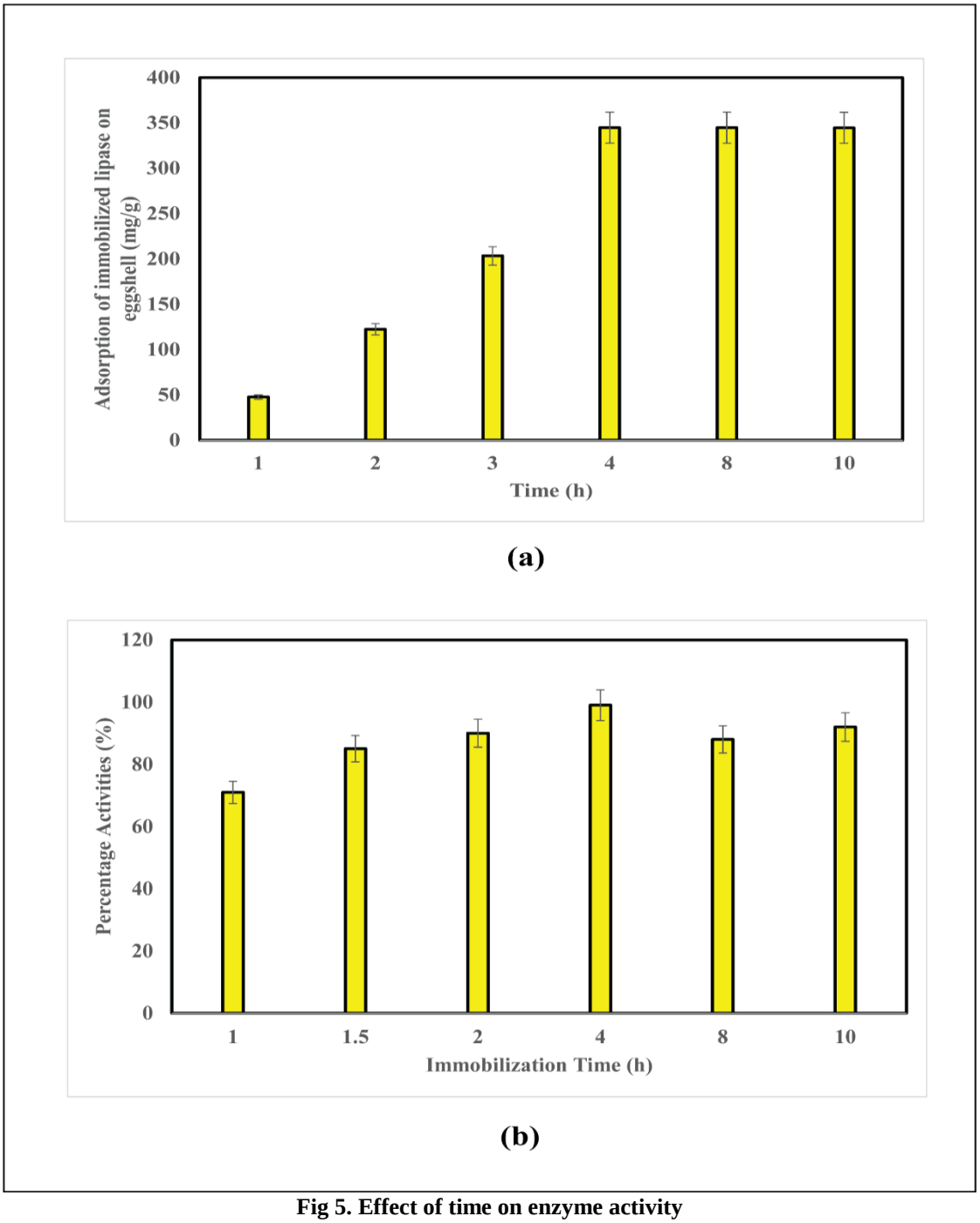


Fig 5. Effect of time on enzyme activity

CONCLUSION:

Biodiesel production using low-cost, cheap, and readily available resources has gained more attention due to its high efficacy and eco-friendly. In this study, eggshell immobilized lipase was prepared for the transesterification of waste cooking oil into high strength biodiesel.Optimizing parameters like pH, temperature, initial concentration reveals highly stable enzyme preparation towards alkaline pH and elevated temperature. Adsorption efficiency was also found to be increased. Further studies using the pilot-scale level will be useful to utilize biodiesel from waste cooking oil as the cheap resource for biodiesel production in large quantities.

CONFLICT OF INTEREST/PLAGIARISM REPORT:

I, On the behalf of the Authors declared that I have no known conflict financial interest and no personal relational ship to the influence of this work reported in this paper. We have checked the plagiarism of this paper and no such significant plagiarism has found.

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