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# Valorization of Rice Bran Fatty Acid Distillate: A Green Approach to Ethanol-Based Biodiesel Production Using Eversa® Transform as a Recyclable Catalyst

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Rice bran fatty acid ethyl esters were produced using rice bran fatty acid distillate (FAD), a by-product of rice bran oil refining, and ethanol was obtained as a by-product from sugar factories. The reaction was done using *Aspergillus oryzae*-based liquid lipase (Eversa® Transform 2.0 FG). In the first set of experiments, the dosage of the lipase catalyst was set to 0.5% w/w FAD, and the separated emulsion layer was recycled over 4 experiments. In the second set, 15 experiments were performed by giving a 10% top-up dose of lipase every second experiment, and five experiments were conducted without top-up doses. The time of the reaction was set at 16 h, and FAD: ethanol molar ratio was set to 1:2. The moisture content of the reaction was maintained at 2%, pH between 6.5 and 7, and the reaction temperature between 40 ºC and 42 ºC. The results showed that the reaction ran up to 98.4% conversion. Furthermore, with ethanol being used as a solvent, the enzyme, with a 10% top-up dose, could be recycled for a minimum of 15 reactions with a minimum of 97.5% conversion. The manufactured ethyl ester also was observed to have excellent properties for use as biodiesel. The biodiesel produced with an extra polishing step met all the specification requirements for B-100 biodiesel as per ISO standards. The recycling of enzymes resulted in the unexpected production of a non-contaminated water layer as a by-product that can be easily reused in other applications. The research, thus, concludes that using ethanol instead of methanol in combination with Eversa® Transform 2.0 for making biodiesel lowers the processing cost, significantly reduces the number of enzymes, water, and subsidiary chemicals consumption, and simplifies the recycling of by-product water, thereby making the entire process much greener and cleaner. Therefore, it can be stated that a sustainable model for biodiesel production from the vegetable oil refining industry is well within the grasp.

**Keywords:** Rice bran fatty acid ethyl esters, Biodiesel production, *Aspergillus oryzae*-based liquid lipase, Enzyme recycling, Sustainable vegetable oil refining

## INTRODUCTION

In this age of transformation in the energy sector, renewable, carbon-neutral, and economic sources of fuel are being sought after with utmost priority. Low-cost feedstocks and cleaner and greener processes are the tools to achieve the goal of reducing carbon emissions and protecting the environment. By 2040, global fossil fuel demand is expected to surpass 100 million barrels per day, with diesel leading at over 5 million barrels per day [1-2]. Biodiesel, which was produced about 40 million tonnes in 2018, offers a sustainable solution to socioeconomic and fuel challenges by significantly reducing greenhouse gases and pollutants. Together with renewable sources, such as solar, wind, hydroelectric, and nuclear energy, it can decrease reliance on fossil fuels [3]. The major challenges associated with biodiesel are the added pressure on edible oils which are already in high demand, higher prices as compared to fossil fuel-based diesel, and the use of chemical catalysts that adds to environmental pollution.

Extensive research has been dedicated to enhancing the

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competitiveness of biodiesel through the development of sustainable production technologies, aiming to boost productivity [4]. Biodiesel primarily consists of fatty acid alkyl esters, with methanol or ethanol commonly used as the alkyl group. Biodiesel can be made from multiple routes using chemical catalysis *via* broadly acidic and alkaline catalysts. Recent research has also introduced a new method via an enzymatic process using lipase as a biocatalyst. A summary of different methods used for biodiesel production, along with their merits and demerits, is given in Table 1 [3]. The enzymatic process is suitable for low-quality feedstocks because it is generally insensitive to the presence of impurities in the feedstocks [2]. In addition, the enzymatic method, compared to chemical routes, consumes much lower utilities in terms of heating and cooling mediums. It also generates significantly lower amounts of effluent that can be easily treated. Hence, the enzymatic route is more sustainable compared to the chemical route.

## Lipase and Biodiesel Production

Lipases are highly efficient biocatalysts used in diverse industries, including pharmaceuticals, food, and fine chemistry [5]. They play a key role in converting oil to biodiesel and can be sourced from animals, plants, fungi, yeasts, or bacteria. Free or immobilized lipases have been explored for biodiesel production, but their activity is affected by unfavourable conditions (pH, temperature, and solvent) [3]. Enzymatic catalysts yield biodiesel ranging from 70% to 99%, depending on the feedstock [4-5]. Eversa® Transform 2.0, a low-cost liquid lipase derived from genetically modified *Aspergillus oryzae*, has been introduced recently by Novozymes and generated an ester content of 97% using feedstocks containing ≥ 80% free fatty acid using a very low enzyme concentration (0.2 w%), a low methanol-to-oil molar ratio (4:1), and a low process temperature (40 ºC) [2]. Lipases can be used to catalyse biodiesel production in two main ways, by using free enzymes and by

**Table 1.** Advantages and Disadvantages of Different Biodiesel Conversion Methods [3]

| Method | Advantages | Disadvantages |
|---|---|---|
| Pyrolysis (Biofuel) | Co-production of value-added by-products such as syngas and biochar;<br>Satisfactory biofuel properties and no need for new infrastructure for up-take | High production cost with complex instruments;<br>More gasoline than diesel production |
| Microemulsion | Minimum by-products;<br>Higher liquidity and lower viscosity;<br>Lower NOx emissions | Inadequate combustion and heavy deposition of carbon residue;<br>Injector needle sticking |
| Catalyst-based esterification/transesterification | The most widely used methods;<br>Unreacted feedstock and by-products can be recovered by post-processing;<br>High biodiesel yield | High oil-to-alcohol ratio;<br>A non-eco-friendly production process ;<br>Sensitive catalysts to the impurities present in the feedstock;<br>Difficult by-product value addition |
| Enzyme-based esterification/transesterification | Environment-friendly and low energy consumption;<br>Reaction not affected by high FFA;<br>Recycling of immobilised enzymes for several times;<br>Easy downstream processing of biodiesel | Costly enzymes;<br>Longer reaction times;<br>Inhibition of gums, solvents, and by-products by enzyme activity;<br>Prevention of the recyclability of free enzymes by methanol |

immobilising the enzyme on a neutral substrate. Considerable research has been conducted using both of these methods. *Candida antarctica* lipase B (CALB) was magnetically immobilised on a hybrid sol-gel nanocomposite to produce biodiesel from waste cooking oil. The synthesized biocatalyst achieved a 96% yield at 40 °C, with a methanol to oil ratio of 4:1, 30 hours contact time, and 1% w/w catalyst dosage [6].

### Feedstock for Biodiesel Production

Biodiesel is chemically an ester made from an alkyl group and an alcohol group. The alkyl group is generally generated *via* the renewable oleochemical segment. Mohiddin *et al.* [7] suggested that the feedstocks could be classified into four generations. According to their classification, the first generation comprises edible oil sources, the second generation comprises non-edible oils, waste, and by-products of edible oil refining process and used cooking oils, the third generation is mainly classified as oils derived from algal sources, and the fourth generation is oil produced synthetically via micro-organisms. For the purpose of this study, rice bran fatty acid distillate (RBFAD; second generation), produced during the de-odorization of rice bran oil, was used as the feedstock. The most common alcohol groups used for biodiesel production are methanol and ethanol. Methanol has a pronounced effect of enzyme deactivation [8] added to its industrial production using steam reforming of natural gas [9], which is a fossil fuel-based process. Ethanol, on the other hand, has a lower impact on enzyme activity [8] and is made traditionally from biomass or related products *via* fermentation [10]. Ethanol was the solvent of choice.

Optimised reaction parameters for the enzymatic reaction, esterification, and transesterification have been studied in good detail. Generally, the reaction yields have been observed to be above 96%. The process parameters for using immobilised lipase have been varied greatly from an enzyme concentration of 2.5 wt% to 30 wt%, alcohol: feedstock molar ratio of 2:1 to 8:1, reaction temperature from 25 ºC to 50 ºC, reaction time from 8 to 90 h, in the presence or absence of co-solvents. This is primary because the support provides good robustness to the enzyme [11-12].

When working with liquid lipases or soluble lipases, these parameters were changed in the following manner: enzyme concentration from 0.3 wt% to 10 wt%, alcohol:feedstock molar ratio from 2:1 to 7:1, reaction temperature from 35 ºC to 45 ºC, reaction time from 8 to 24 h, and water concentration from 2 wt% to 10 wt%. Generally, this process produces yields between 70% and 99%, depending on the feedstock type [13-17]. Integrating the technology backward, Cesarini *et al.* showed that combining liquid lipase with phospholipase can provide a single tank º umming and transesterification of crude oil to biodiesel, with process parameters falling within the above ranges. The process parameters used in this study were decided based on optimal parameters for similar raw materials from prior literature and from guidelines given by Novozymes India.

Taking THE sustainability and economic feasibility of liquid lipases into account, some researchers have reported a whole-cell catalyst based on *Pichia pastoris* with intracellular Tll (*Thermomyces lanuginosus lipase*) having a 78% activity after 3 reaction cycles in used cooking oil and methanol system. Remonatto *et al.* used Eversa transform 2.0, immobilised them on hydrophobic (Sepabeads C18) support, and reported 75% activity after 4 reaction cycles using hexane as a solvent. They also observed that the lipases were much more stable in ethanol compared to methanol [17]. Several researchers have reported the reusability of liquid lipase by separating the middle emulsion phase between FAAE and glycerol-water using the gravity of centrifugal separation and the middle emulsion layer with 90%-95% of the enzyme activity [13-15]. Kua *et al.* used crude Jatropha seed oil and reported yields of 94.9%, 81.1%, and 53.0% in the first, second, and third cycles using recombinant CRL2 and CRL4 isozymes recycled by gravity separation [17]. Moreover, Wancura *et al.* reported biodiesel yields of 85.08%, 76.32%, 50.19%, and 50.00% for four subsequent reuse cycles from beef tallow as feedstock using Eversa Transform, without any added treatments, and recovering the lipase using centrifugation [13].

## MATERIALS AND METHODS

### MATERIALS

Rice bran fatty acid distillate was procured from M/s. Vishal Oleochem. Mumbai Ethanol (trade name Manosol 1A) used for the reactions was procured from M/s. Coatings

and Coatings India Pvt. Ltd., Maharashtra. The ethanol had 5% moisture, 99.9% GC purity, and 0.1% acetone. Eversa Transform 2.0 FG (*Aspergillus oryzae)* was procured from Brenntag Ingredients Pvt. Ltd., India (manufactured by Novozymes, Denmark). Sodium Hydroxide was procured from M/s. Meghmani Industries Pvt. Ltd., Vapi, Gujarat. Water used for all purposes was distilled from MIDC RO Water at M/s. Kedia Organic Chemicals Pvt. Ltd., Ambernath, Maharashtra. All reactions and analyses were carried out at their laboratory. Characterizations were carried out at the Institute of Chemical Technology, Mumbai, Kedia Organic Chemicals Pvt. Ltd. (R&D Centre) and Trans Thane Creek WMA laboratory, Mahape. All the chemicals and solvents used in analyses were procured from laboratory suppliers (manufactured by Merck India and of LR/AR grade).

Reactions were carried out in a 4-necked round bottom flask with 1 l capacity. The round bottom flask was set up in a water bath with an attached thermostat made by Shanti Scientific Industries. An additional thermometer with a range of -10 to 100 ºC was set up in a glass thermowell in one of the necks of the flask. The central neck was fitted with a stirrer made by Galaxy Scientific Equipments (India) with an RPM ranging from 0 to 300. The third neck was fitted with a water-cooled jacketed glass condenser, and the final neck was used for ethanol charging. The neck was fitted with a glass stopper when not in use. The glass stirrer rod was 8 mm thick, and stirrer blades were made from PTFE. All the glassware was made from borosilicate glass and bought via standard suppliers. Digital pH meter model EQ 610 was procured from Equiptronics. Karl Fischer auto-titrator was from Veego Scientific. All other materials and equipment used were procured from standard suppliers and calibrated as per standard laboratory practices.

## METHODS

### Neutralisation of Excess Acidity

RBFAD was preheated in a water bath to 50 ºC and added to a separating funnel. An equal amount of water, preheated to 80 ºC, was added to the funnel and the stopper was fixed. The contents were mixed for 10 semi-circular rounds and allowed to settle for 15 min. The aqueous extract was separated from the bottom, and its pH was checked using a pH meter. The amount of sodium hydroxide to be added was calculated using the relation between the pH and the dosage of sodium hydroxide, as mentioned in Table 2.

### Synthesis of Fatty Acid Ethyl Ester

First, 660 g of RBFAD was added to the 4-necked round-bottom flask using a liquid funnel. The thermostat of the water bath was set to 42 ºC to ensure 40 ºC temperature inside the reactor. The reactor temperature was separately monitored using a glass thermometer. The stirrer was set to 80 RPM. The total water to be added to the system was calculated based on 2% of RBFAD weight. Water was added via ethanol (5% water wt/wt), trace moisture content in RBFAD (0.01% wt/wt), and water to make NaOH solution of 5% strength. Then, 0.12 g of NaOH (calculated as per Table 2) and 2.40 g of water were mixed to make a dilute alkali solution; this solution was added to the reaction mass. The sample was taken out to check the final pH of the mixture. The contents were then stirred for 15 min to achieve uniform mixing and a constant temperature of 40 ºC. Once the pH, temperature, and stirring conditions were stabilised, 3.3 g of Eversa Transform 2.0, stored at 20 ºC, was added to the flask. The contents were allowed to mix for another 15 min until a uniform distribution was obtained. Ethanol dosing was started at this time, taken as 0 h. The dosing recipe was set as mentioned in Table 3. Samples were taken out every two hours to monitor the progress of the reaction using the acid value.

One hour of maintenance was provided after the final dosage, and the stirring was stopped. The contents were transferred to a separating funnel and allowed to settle for 4 h. The top layer of fatty acid ethyl ester was taken for solvent and water recovery under reduced pressure. The mid layer of enzyme and emulsion was transferred to a secondary smaller separating funnel and kept under additional settling for 24 h to obtain a concentrated enzyme layer. The bottom aqueous layer was collected as effluent.

### Recycling of Enzyme Layer

The enzyme layer, which was transferred to the secondary separating funnel, displayed further separation by gravity. The bottom aqueous layer was added to the effluent collection while the top enzyme layer was recycled in its current state to the subsequent reactions. If any ethyl ester

**Table 2.** The Relationship between the pH and the Quantity of Sodium Hydroxide

| pH of Aqueous extract | Sodium hydroxide* % (wt/wt of RBFA ) |
|---|---|
| 3 | 0.08 |
| 3.5 | 0.06 |
| 4 | 0.04 |
| 4.5 | 0.03 |
| 5 | 0.02 |
| 5.3 | 0.01 |

**Table 3.** Ethanol Dosage Recipe

| Hour | Quantity (g) | Hour | Quantity (g) | Hour | Quantity (g) |
|---|---|---|---|---|---|
| 0 | 26.87 | 6 | 8.95 | 12 | 8.95 |
| 1 | 26.87 | 7 | 8.95 | 13 | 8.95 |
| 2 | 26.87 | 8 | 8.95 | 14 | 8.95 |
| 3 | 26.87 | 9 | 8.95 | 15 | 8.95 |
| 4 | 8.95 | 10 | 8.95 | | |
| 5 | 8.95 | 11 | 8.95 | | |

layer was observed, it was separated, weighed, and added to the ethyl ester layer prior to recovery.

### Obtaining Pure Ethyl Ester of Rice Bran Fatty Acid

The fatty acid ethyl ester layer was subjected to heat up to 85 ºC under reduced pressure of 150 mm Hg generated by a water jet venturi system. Solvent and water were distilled off to obtain the ethyl ester of rice bran fatty acid. The material was weighed and then analysed for acid value, moisture, viscosity, cloud point, pour point, free glycerine content, total glycerine content, combined glycerine content, soap content, density, calorific value, and gas chromatography. The ethyl ester from all the batches was homogenised and treated with sodium hydroxide and isopropanol to further reduce its acid value to meet the ISO/ASTM standards.

## EXPERIMENTAL SECTION

### Experiments 1 to 5

An initial set of 5 experiments was carried out to understand the performance of the enzyme on 100% recycling. The experimentation procedure was carried out as described above in Section 2.2. Table 4 details the quantities of the raw materials charged in the reaction from experiments 1 to 5.

### Experiments 6 to 25

The second set of experiments was undertaken to establish the optimum recyclability of the enzyme. To this end, 10% (weight by weight) of the initial enzyme dosage was added as a top-up dose to the reaction system for every alternate batch, followed by 100% recycling from experiments 21 to 25. Table 5 lists the quantities of raw material used in this experiment series.

## RESULTS AND DISCUSSIONS

The designing of the reaction in this research was done by keeping in mind the objective of immediate scalability to ton-scale batches. Factors such as initial capital investment in enzymes, generation of easily re-usable effluent, low energy

**Table 4.** Experiments 1 to 5, 100% Catalyst Recycling

| Input component / Sr. Nr. | Rice bran fatty acid distillate (g) | Ethanol (g) | Eversa transform 2.0 (g) | Enzyme recycle layer (g) | Sodium hydroxide (g) | External water (g) |
|---|---|---|---|---|---|---|
| Experiment 1 | 660 | 215 | 3.3 | 0 | 0.12 | 2.4 |
| Experiment 2 | 660 | 215 | 0 | 5.4 | 0.12 | 2.4 |
| Experiment 3 | 660 | 215 | 0 | 5.4 | 0.12 | 2.4 |
| Experiment 4 | 660 | 215 | 0 | 5.3 | 0.12 | 2.4 |
| Experiment 5 | 660 | 215 | 0 | 5.5 | 0.12 | 2.4 |

**Table 5.** Experiments 6 to 25, Optimisation of Recycling Quantity

| Input component / Sr. Nr. | Rice bran fatty acid distillate (g) | Ethanol (g) | Eversa Transform 2.0 (g) | Enzyme recycle layer (g) | Sodium hydroxide (g) | External water (g) |
|---|---|---|---|---|---|---|
| Experiment 6 | 660 | 215 | 3.30 | 0.00 | 0.12 | 2.4 |
| Experiment 7 | 660 | 215 | 0.33 | 5.60 | 0.12 | 2.4 |
| Experiment 8 | 660 | 215 | 0.00 | 5.70 | 0.12 | 2.4 |
| Experiment 9 | 660 | 215 | 0.33 | 5.60 | 0.12 | 2.4 |
| Experiment 10 | 660 | 215 | 0.00 | 5.80 | 0.12 | 2.4 |
| Experiment 11 | 660 | 215 | 0.33 | 5.65 | 0.12 | 2.4 |
| Experiment 12 | 660 | 215 | 0.00 | 5.70 | 0.12 | 2.4 |
| Experiment 13 | 660 | 215 | 0.33 | 5.60 | 0.12 | 2.4 |
| Experiment 14 | 660 | 215 | 0.00 | 5.80 | 0.12 | 2.4 |
| Experiment 15 | 660 | 215 | 0.33 | 5.60 | 0.12 | 2.4 |
| Experiment 16 | 660 | 215 | 0.00 | 6.00 | 0.12 | 2.4 |
| Experiment 17 | 660 | 215 | 0.33 | 5.70 | 0.12 | 2.4 |
| Experiment 18 | 660 | 215 | 0.00 | 5.80 | 0.12 | 2.4 |
| Experiment 19 | 660 | 215 | 0.33 | 5.80 | 0.12 | 2.4 |
| Experiment 20 | 660 | 215 | 0.00 | 6.00 | 0.12 | 2.4 |
| Experiment 21 | 660 | 215 | 0.00 | 6.00 | 0.12 | 2.4 |
| Experiment 22 | 660 | 215 | 0.00 | 5.80 | 0.12 | 2.4 |
| Experiment 23 | 660 | 215 | 0.00 | 5.90 | 0.12 | 2.4 |
| Experiment 24 | 660 | 215 | 0.00 | 5.70 | 0.12 | 2.4 |
| Experiment 25 | 660 | 215 | 0.00 | 5.60 | 0.12 | 2.4 |

involved in processing, and fewer unit operations were given high importance in deciding the parameters of the experimentation.

The raw material was analysed for the saponification value and acid value, which yielded a difference of 2 units, signaling a 1% presence of triglyceride in the raw material. The amount of unsaponifiable matter was 0.4%. The GC method employed for the analysis of the fatty acid ethyl ester did not detect these unsaponifiable compounds.

## Recyclability of Liquid Lipase Catalyst (Eversa Transform 2.0 FG)

The recyclability of the enzyme was studied in 3 sets of experiments to ascertain the recyclability performance of the lipase catalyst. The conversion of the reaction was defined by the conversion of RBFA to its ethyl ester and was calculated based on the acid value. The yield of the reaction was based on gas chromatography results and was calculated as the percentage of ethyl ester present in the chromatograph divided by the theoretical maximum. Finally, the correction for unsaponifiable content was factored into the results.

## Direct Recycling at 0.5% Dosage

In the first set, from experiments 1 to 5, 0.5 wt% dosage of the lipase was employed in the first reaction (experiment 1), and the results of 100% recycling of this enzyme were observed over the next 4 reactions (experiments 2 to 5). A total of 5 reactions were carried out. As can be seen in Fig. 1, the conversion dropped gradually over the batches from 98% to 75%. The trend of the results showed that the conversion kept dipping further. A notable result obtained from the gas chromatograph of the samples was that even though the conversion dropped, there was not a significant change in the selectivity over the batches, confirming that the lipase catalyst was highly selective in the esterification process despite losing some activity.

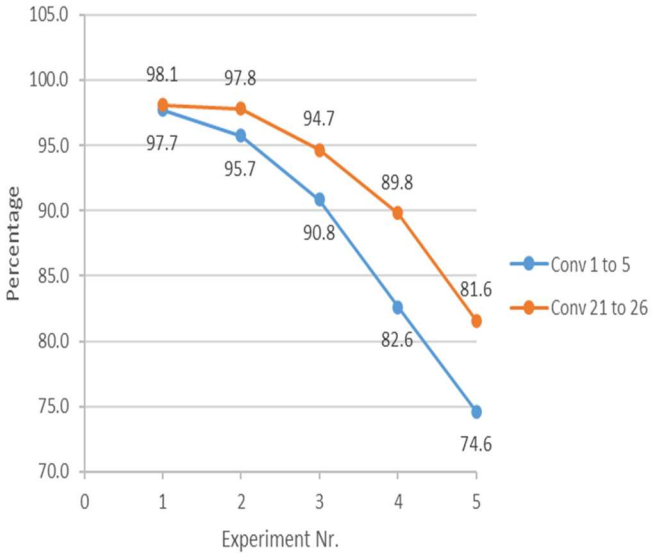



**Fig. 1.** Conversion (%) v/s experiment number and yield (%) v/s experiment number for experiments 1 to 5.

## Top-up Recycling Method

Taking the feedback from the first set of experiments, in the second set, from experiments 6 to 20, a top-up recycling method was used. In experiment 6, 0.5% dosage of the lipase was given; then, in experiment 7, 10% of the initial dosage of the lipase was added as a top-up dose. In experiment 8, 100% recycling was done. In this fashion, a 10% top-up dose of lipase was given to every alternate batch. In total, 15 experiments were carried out using this system, and the results showed more than 97.5% conversion in all the batches with yields of more than 98.5%. Figure2 shows the conversion and yield data from experiments 6 to 20. The high yields indicate that the lipase had successfully transesterified the triglyceride present in the raw material. The quantity of lipase catalyst recycling is depicted in Fig. 3, which shows a gradual increase in the quantity of lipase recycled over the batches. The minimum quantity of lipase recycled in any batch was 90%. It can be inferred from the data that as the number of batches increased, the cost saving also kept increasing as the recycling percentage increased over the batches. The effective enzyme dosage over the set of 15 experiments reached 0.056%. As the optimal lipase dosage was 0.5%, 88.5% less lipase was used for converting the raw material to the ethyl ester. Figure 4 is the plot of net savings in lipase consumption against the batch number. Extrapolating the results at 100 reactions, the effective enzyme dosage per batch was obtained as 0.03% and the net reduction in the lipase use was 94%. It is noteworthy that the percentage of the saved lipase went beyond 75% in the 5th experiment and beyond 85% in the 10th experiment.

## Direct Recycling at Higher Concentrations

At the end of the 15th experiment of the set (experiment 20), the absolute lipase concentration was estimated to be 0.85%. Another set of 5 experiments from experiments 21 to 25 were carried out at full recycling of the lipase with no additional top-up doses. The results indicated a much slower decline in the conversion as compared to experiments 1 to 5, for which a similar method at a lower concentration had been employed. Comparative results are illustrated in Fig. 3. This suggests the possibility of better recycling potential at higher doses. The results are also in accordance with the recyclability of immobilised lipase which has been extensively explored using various strategies.

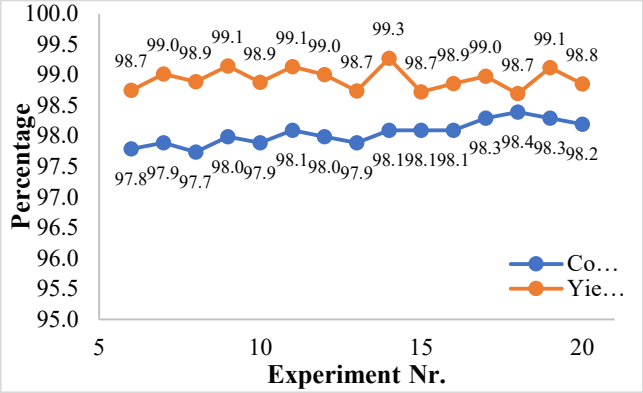



**Fig. 2.** Conversion (%) v/s experiment number and yield (%) v/s experiment number for experiments 6 to 20.

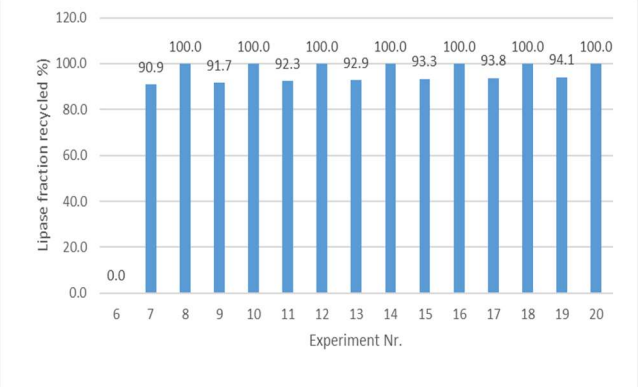
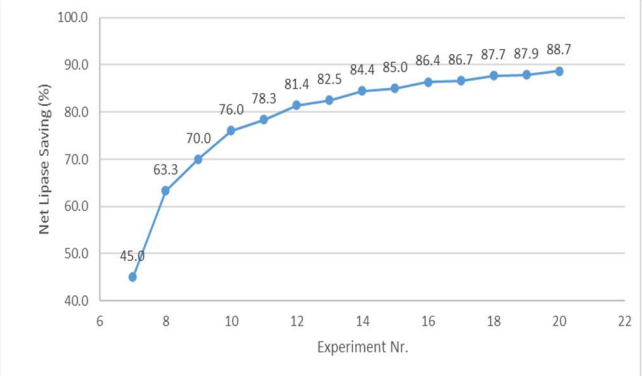



**Fig. 3.** Lipase recycling quantity (%) v/s experiment number for experiments 6 to 20 (calculated as the $[enzyme]_{n-1}/[enzyme]_n$ and expressed in %, where 'n' is the experiment number).




264 et savings for lipase calculated using the following formula: total lipase consumed as per original experiment - the total lipase consumed up to the experiment number/total lipase that consumed as per original experiment.

## Use as Biodiesel

The properties of the ethyl ester produced were checked and compared with that of the ISO Standard B-100 biodiesel. As can be seen in Table 6, only the free fatty acid was above the specified limits. An extra polishing step was done to reduce the acid value, and the test results of that sample are also shown in Table 6.

The higher AV ethyl ester was tried in industrial combustion applications at the steam baby boilers in M/s. Kedia Organic Chemicals Pvt Ltd along with three other companies in replacement for HSD and the performance was at par. The use of the RBFA ethyl ester produced as biodiesel for industrial applications was thus established. More long-term studies are needed to investigate the effects on boiler parts to establish the relationship between the present unreacted fatty acids and the corrosion of metal and rubber parts of the boiler assembly.

A comparison between the properties of commercial high-speed diesel and the RBFA ethyl ester is presented in Table 7. The calorific value of the RBFA ethyl ester is equivalent to that of HSD when compared in the Kilo Calories/litre. It is important to note that the fuel is sold in litres and not kilograms, so if the user buys RBFA ethyl ester at the same price as HSD, the energy value obtained will be similar.

## Truly Biological Biodiesel

The synergetic relationship between lipase catalyst recycling and ethanol presents an additional hidden benefit. In most commercial applications today, methanol is used as a solvent of choice due to its abundant availability, reasonable cost, low-cost volatility, and non-azeotropic combination with water. Methanol is produced commercially by the catalytic hydrogenation of synthetic gas obtained from the steam reforming of natural gas, essentially making it a fossil fuel-derived alcohol. Ethanol was primarily made by the fermentation of sugars present in sugarcane bagasse. Recently, the developments in technology regarding the production of ethanol have made it possible to use a variety of bio-based feedstocks ranging from starch-based crops to cellulosic feedstocks, such as grass, wood, and crop residues. Hence, the use of bio-catalyst, bio-ethanol, and renewable fatty acid feedstocks make the rice bran fatty acid ethyl ester a truly biological biodiesel.

**Table 6.** Properties of Rice Bran Fatty Acid Ethyl Ester, As Such and Polished, for Application as Biodiesel

| Sr. Nr. | Characteristic | Reference test method | ISO Standard | RBFA ethyl ester (as such) | RBFA ethyl ester after polishing |
|---|---|---|---|---|---|
| 1 | Density at 15 ºC, kg $m^{-3}$ | IS 42-1979 P:42 | 860 to 900 | 882 | 880 |
| 2 | Kinematic Viscosity at 40 ºC, cSt | IS 1448 P:25 | 2.5 to 6.0 | 5.17 | 5.1 |
| 3 | Acid Value, mg KOH/g, Maximum | IS 1448 P:1 | 0.5 | 3.2 | 0.2 |
| 4 | Moisture, mg/kg, Maximum | IS 326:2001 | 500 | 400 | 400 |
| 5 | Cloud Point, ºC, Maximum | ASTM D2500-17a | 12 | 4 | 4 |
| 6 | Pour Point, ºC, Maximum | ASTM D 2500 | 6 | -1 | -1 |
| 7 | Free Glycerine Content, % by Mass, Maximum | ASTM D 6584 | 0.02 | 0.017 | 0.014 |
| 8 | Total Glycerine Content, % by Mass, Maximum | ASTM D 6584 | 0.25 | 0.20 | 0.2 |
| 9 | Combined Glycerine Content, % by Mass, Maximum | | 0.23 | 0.18 | 0.19 |
| 10 | Calorific Value, kcal $kg^{-1}$ | IS 1448 P:61 | To report | 9980 | 10050 |
| 11 | Soap Content, mg/kg | | To report | 0 | 0 |
| 12 | Ester Content, % by mass, Minimum | EN 14103 | 96.5 | 98.2 | 99.6 |

**Table 7.** Fuel Property Comparison, Rice Bran Fatty Acid Ethyl Ester v/s High-Speed Diesel

| Sr. Nr. | Characteristics | RBFA ethyl ester | HSD (BS IV) |
|---|---|---|---|
| 1 | Colour | Yellow to Orange | Light yellow |
| 2 | Density at 15 deg C, kg $m^{-3}$ | 0.882 | 0.82 to 0.845 |
| 3 | Flashpoint, Abel, Deg C, Minimum | 150 | 35 |
| 4 | Kinematic Viscosity at 40 deg C, cSt | 5.17 | 2 to 4.5 |
| 5 | Calorific Value, kcal $kg^{-1}$ | 9980 | 10800 |
| 6 | Calorific Value, kcal $l^{-1}$ | 8802.36 | 8850 |
| 7 | Sulphur, mg/kg, Maximum | 10 | 50 |
| 8 | Moisture, mg/kg, Maximum | 400 | 200 |

## CONCLUSIONS

It is common knowledge that lipase catalyst can provide very good catalytic activity, but its commercial applications have been limited due to the cost factor. With the novel alternate dosage plan explored in the present research, the savings were found to be sufficiently substantial to overshadow acid catalysts. Furthermore, the newly developed method introduced in this study is environment-friendly, non-hazardous, safe to handle, and consumes much less power. In the words of a wise man, true innovation does not require a premium from the users, it pays for itself.

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