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Biodiesel Production from Dairy Waste Scum by Using a Efficient Nano-Biocatalyst

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In the present paper, magnetic nanoparticles (Fe_3O_4 NPs) covered with tartaric acid (TA) have been synthesized through an "eco-friendly approach" and subsequently used for direct physical immobilization of the lipase from *Thermomyces lanuginous* (TLL). The immobilized lipase was used for the biodiesel production from dairy waste scum oil in a solvent-free system. The maximum yield to biodiesel of the immobilized lipase is about 90%, at the alcohol/oil molar ratio of 6:1, in the same operating conditions free lipase exhibits, after 24 h, a maximum yield of 76% with a molar ratio of 3:1. The immobilized lipase showed excellent reusability. The biodiesel properties evidence the feasibility of the dairy waste scum oil as raw material for biodiesel production.

1. Introduction

Bio-fuel derived from vegetable oil and fat animal results in a crucial alternative to decreasing petroleum resources and increased pollution concerns.
Biodiesel has increased in popularity due to its environmental benefits and the need for a good substitute for conventional fuels. On the other hand, biodiesel is more expensive than fuels derived from petroleum because it is mostly made from expensive virgin vegetable oils (Al Hatrooshi et al., 2020). The cost of biodiesel is the major obstacle for its commercialization (Sivakumar et al., 2011). The cost of raw materials accounts for 75–85% of the production cost of bio-diesel (Demirbas, 2009). The high price of biodiesel made researchers look for newer ways to reduce the cost.
Therefore, in recent years, a hypothesis shift has resulted in the usage of non-edible oil such as microalgae, jatropha oil,waste grease, animal fats and waste cooking oil (Karmakar and Halder, 2020) as feedstock in biodiesel production. These feedstocks contain high water and free fatty acids (FFA) content, which require either a pre-treatment or additional esterification processes.
Dairy waste scum oil (DWSO) has produced by dairy industries that handle raw and milk products such as butter, yogurt, cheese, ice cream, etc. A large dairy, which processes 5 10^5 liters of milk per day, generates about 3 10^5 g of waste scum per day. A considerable amount, which makes it difficult for disposal (Kavitha et al., 2019). Dairy scum is a less dense, floating solid mass formed by a mixture of fats, lipids, proteins, etc., Most of the dairies dispose of this scum in solid waste disposal site or by incinerating (Kelessidis and Stasinakis, 2012). By doing so, it is economically wasteful and generates pollutants. Further, scum causes direct as well as indirect difficulties in handling and operations in effluent treatment plants. These dairy scums contain a large number of triglycerides (more than 80 % dry bases). Therefore, oil extracted from dairy scum can be an alternative feedstock for biodiesel production.
Another important aspect of the transesterification process is the proper choice of the catalyst, which determines the cost of production, in some cases leading to economic impediments. Most of these feedstocks utilize either sodium hydroxide (NaOH), potassium hydroxide (KOH) as their homogeneous base catalyst for the transesterification process due to their high catalytic activity under mild conditions. In spite of these advantages, these catalyst suffers severe limitations as they are corrosive to reactors and requires a large amount of water for purification of biodiesel which ultimately escalates the cost of production (Demirbas, 2008;

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Georgogianni et al., 2009). To overcome these difficulties, heterogeneous solid catalysts have gained a lot of importance due to high conversion efficiency and also for the reuse of the catalyst. However, costs and complicated procedures lead to the need for alternative methods for biodiesel production. Enzyme-catalyzed transesterification is an alternative method (Sarno and Iuliano 2018). In particular, lipases, due to their ability to catalyze transesterification of oils and fats, can be used for this purpose (Fernandez-Lafuente, 2010). Enzyme's poor stability towards pH, temperature and time, and their costs encourage the use of immobilizations to facilitate separation, recovery, and enhance activity. The immobilization of enzymes onto nanomaterials is a topic of great interest (Ansari and Husain, 2012; Sarno et al.,2017; Sarno and Iuliano 2019;).
In the present paper, for the first time, magnetic nanoparticles covered with tartaric acid (TA) have been synthesized through an "eco-friendly approach" and subsequently used for direct physical immobilization of the lipase from *Thermomyces lanuginous* (TLL). The activity of lipase was investigated at different conditions.

2. Material & Method

Dairy Waste Scum was chosen as the feedstock for the present study and was collected from a local dairy (Giffoni, South of Italy). Ferric chloride hexahydrate ($FeCl_3$•6H2O, 98%), urea (100.5%), ethylene glycol (EG), tartaric acid (99.9%), ethanol, lipase from *Thermomyces lanuginous* (TLL) (solution ≥100,000 U/g), bovine serum albumin (BSA), polyvinyl alcohol (PVA), potassium hydroxide (KOH), olive oil (highly refined-low acidity), heptane, methyl heptadecanoate of known purity (99 %) (C17:0), boron trifluoride-methanol solution (BF3-Methanol) and methanol were acquired from Aldrich Chemical Co. All chemicals were of analytical grade.

2.1 Synthesis Fe_3O_4@TA nanoparticles

Synthesis procedure, $FeCl_3 \cdot 6\ H_2O$ (3 mmol), Urea (30 mmol) and tartaric acid (0.5 mmol) were dispersed in 30 ml of ethylene glycol. The mixture was ultra-sonicated for 5 min. Subsequently, the solution was transferred into a Teflon-lined stainless steel autoclave and then heated at 200°C for 3 h. After cooling down to room temperature the black material was washed with ethanol for numerous time and then dried at 60°C for 24 h to obtain Fe_3O_4@TA NPs.

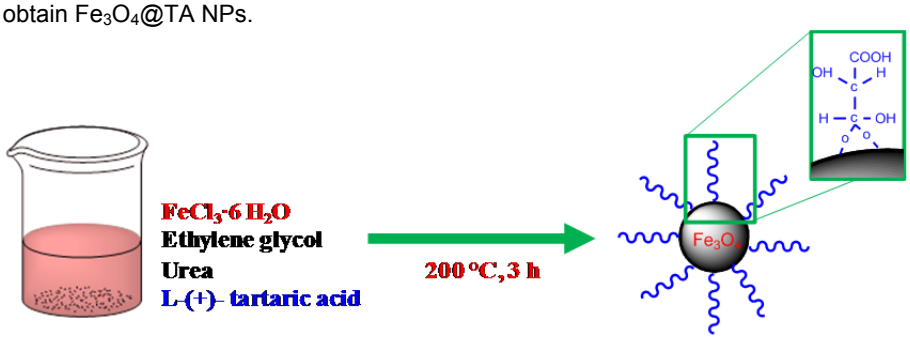

Figure 1: Synthesis Fe_3O_4@TA nanoparticles

2.2 Enzyme immobilization

Fe_3O_4@TA NPs (50 mg) and 1, 2, 4 mg of lipase from *Thermomyces lanuginous* (TLL) in 10 ml of phosphate buffer pH=3 were mixed at 4°C for ~ 3 h. Finally, immobilized enzymes were separated by an external magnetic field. The nanoparticles with anchored TLL were gathered and rinsed three times with buffer phosphate (pH 3.0) to definitely remove free enzymes. In order to evaluate the amount of enzyme loading, the residual enzyme in the collected supernatant was measured using UV/Visible spectroscopy. The immobilized enzyme was dispersed in the buffer and stored at 4°C for further measurements.

2.3 Enzyme loading determination

The amount of immobilized enzyme on Fe_3O_4@TA was determined by subtracting the initial amount of enzyme from the amount of enzyme remaining in the supernatant. The concentration of unbound enzymes that were in the supernatant was determined with a calibration curve and then the amount of enzyme immobilized on Fe_3O_4@TA nanoparticles was obtained. In particular, the enzyme attachment percentage was calculated by the Bradford method (Bradford, 1976).

2.4 Scum oil: preparation and characterization

100 g of the dairy waste scum Figure 2a were heated up to 110 °C, until the water was completely removed (Figure 2b). After filtering through a stainless steel mesh to remove any suspended impurities, the dairy waste scum oil was obtained, Figure 2c.
The fatty acid (FA) composition of dairy waste scum oil (DWSO) was analyzed using GC-MS, Thermo-Fischer gas chromatography equipment. A capillary column, Trace-GOLD TG-POLAR GC Columns 0.25 µm×0.25 mm×60 m. The start temperature of the column was 150°C and it was gradually raised at the rate of 15°C/min to temperature of 190°C, after it was progressively raised at the rate of 4°C/min to final temperature of 230°C, while the injector and detector were maintained at 250°.

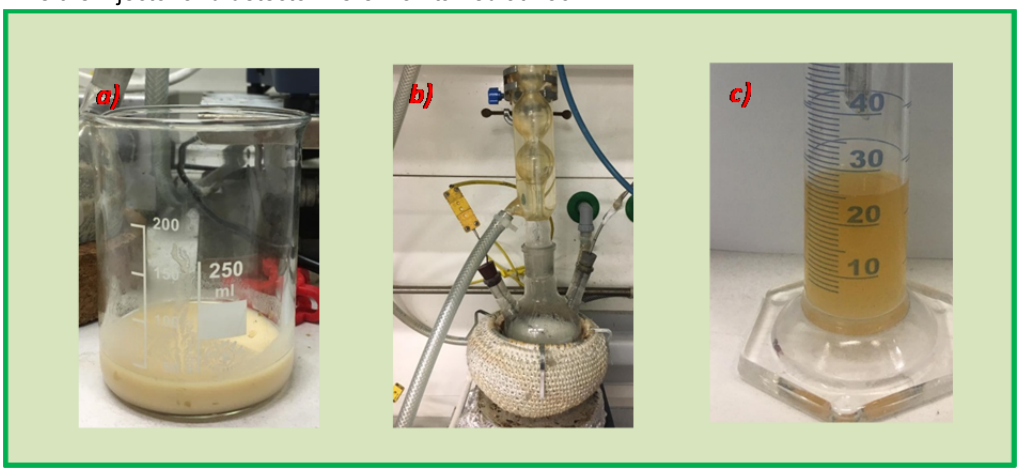

Figure 2: Scum oil preparation: (a) dairy waste scum; (b) dairy waste scum heated to 110°C and (c) dairy waste scum oil.

2.5 Transesterification

Transesterification reactions were carried out to 45 °C for 24 h at 220 rpm in a 50 mL-conical flask in a solvent-free system. Immobilized lipases 10 % (g enzyme /g oil) was added at reaction blend of 10 g of DWSO and methanol (with alcohol/oil molar ratio of 3:1 M, 6:1 M and 9:1). After the reaction time, the enzyme was recovered by magnetic separation, and the product purified at 60 °C under vacuum in a rotary evaporator to remove residual methanol, after washed with hot water and finally dried. The yield of DWSO to methyl esters was determined by the following equation:

$$yield\ (\%) = \frac{m_e}{m_{DWSO}} * 100\% \qquad (1)$$

where: m_e = weight (g) of the product after drying, m_{DWSO} = weight (g) of DWSO.

The quality of the biodiesel obtained has been evaluated in compliance with EN14214; in particular total ester content was determined as described in EN14103 in the presence and absence of methyl heptadecanoate (margaric acid methyl ester) as an internal standard.

3. Result & Discussion

3.1 Transmission electron microscopy analysis

The morphological and structural characteristics of the NPs were determined by transmission electron microscopy (TEM) analysis (FEI Tecnai electron microscope operating at 200 KV) equipped with an EDX probe). Highly uniform size nanoparticles were formed that, once deposited over a TEM grid, tend to self-organize in a hexagonal layer. From a statistical analysis of about 400 nanoparticles, the particle size distribution was obtained, indicating that the average diameter of the inorganic core is d = 6.7 nm with σ = 1.5 nm.

3.2 Oil property

The fatty acid composition of the scum oil was described in Table 1. The fatty acids were identified in dairy waste scum oil. It includes of 52.61% saturated fatty acids and 47.63% unsaturated fatty acids. The main fatty

acid results in palmitic acid, which is 43.17% and oleic acid 34.51%. The high proportions of saturated and mono-saturated fatty acids are a strong advantage in the formation of a fuel which polymerization during combustion will be substantially less than what occurs with a polyunsaturated fatty acid-derived fuel (Sivakumar et al., 2011).

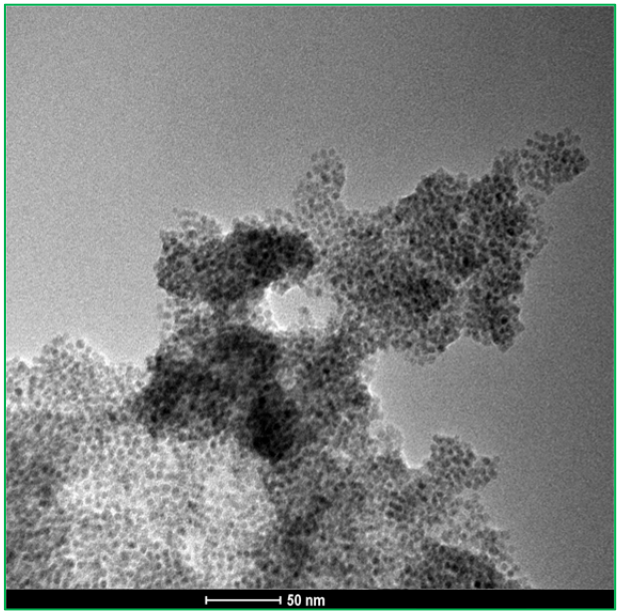

Figure 3: TEM image of Fe_3O_4@TA.

Table 1: Chemical composition of dairy waste scum oil

Fatty acid	Carbons	Value (wt.%)
Capric acid	10:0	0.19±0.02
Lauric acid	12:0	0.60±0.03
Myristic acid	14:0	1.00±0.06
Palmitic acid	16:0	43.17±0.04
Stearic acid	18:0	6.09±0.05
Oleic acid	18:1	34.51±0.04
Linoleic acid	18:2	12.21±0.06
Linolenic acid	18:3	0.71±0.03
Behenic acid	20:0	1.52±0.12

3.3 Effect of alcohol: oil molar ratio on trans-esterification reaction

The molar ratio of alcohol to oil is one of the important factors that affect the yield efficiency as well as the production cost of biodiesel. The molar ratio is the ratio of a number of moles of alcohol to the number of moles of glycerides in the oil. Theoretically, the transesterification reaction requires for each mole of oil three moles of alcohol. However, in reality, the molar ratio (alcohol/oil) should be higher than that of the stoichiometric ratio in order to drive the reaction towards completion. The effect of molar ratio on yield to biodiesel for immobilized lipase and free lipase is shown in Figure 4. The maximum yield achieved with the immobilized lipase is about 90% at the molar ratio of 6:1, soluble lipase shows a maximum yield of 76% at a molar ratio of 3:1, which decreases under molar ratio increase (see Figure 4).

The yield reduction observed at molar higher than 6:1 and 3:1 for immobilized and free lipase, respectively, is probably due to methanol inducing catalyst deactivation, that may occur in the presence of excess methanol (Yucel, 2011; Lu et al., 2007) and can also depend on how methanol was added (Yucel, 2011).

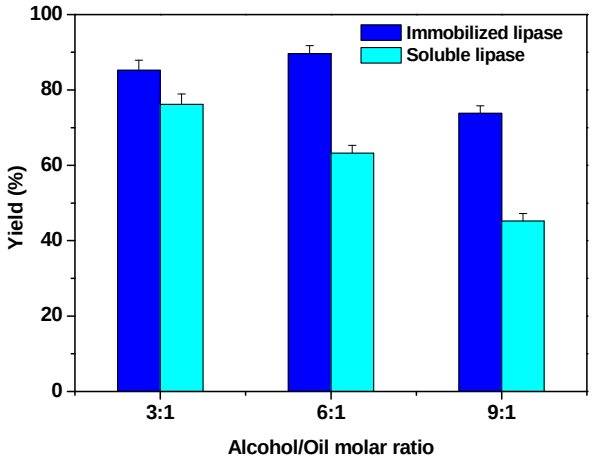

Figure 4: Effect of alcohol to oil molar ration on free and immobilized lipase for biodiesel production. Immobilization condition: coupling temperature 4°C; coupling time 3 h; coupling pH 3; lipase amount 1 mg/ml. Reaction conditions: reaction time 24 h; reaction temperature 45°C; lipase concentration 10%.

3.4 Reusability of nano-bio-catalyst

The key aspect of the enzyme recycling and reuse was investigated under cycles of 24 h without interruption, and the results are shown in Figure 5. The yield to biodiesel of the immobilized lipase was measured five times over a period of 5 days, for the methanol/oil molar ratio 6:1. Before each cycle, the immobilized lipase was washed with sodium phosphate buffer solution (0.1 M, pH 7) after reactions easily separated from the product by a magnet, and next reused in a new experiment. The yield to biodiesel after the first 4 cycles results of 84 %, showing excellent reusability at the molar ratio methanol/oil of 6:1.

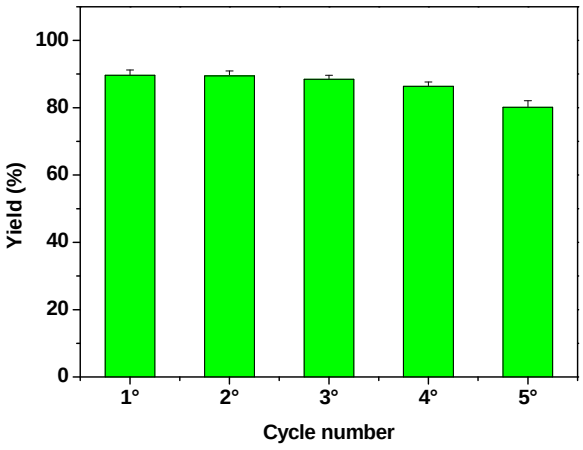

Figure 5: Effect of cycles use on immobilized lipase for biodiesel production. Immobilization condition: coupling temperature 4°C; coupling time 3 h; coupling pH 3; lipase amount 1 mg/ml. Reaction conditions: reaction time 24 h; reaction temperature 45°C; lipase concentration 10%; alcohol/oil molar ratio 6:1.

3.5 Properties of biodiesel

Biodiesel from DSWO, obtained by using our immobilized lipase with alcohol/oil molar ratio 6:1, presents linolenic methyl-ester amount equal to about 0.62%±0.07, that is in agreement with the EN14214. Ester content was calculated according to the method reported in EN14214. Fatty acid methyl Ester (FAME) content results equal to 96.6%±0.02, in agreement with the EN14214. The acid value calculated according to EN14214 results equal to 0.25 mg KOH/g in accord with the European standard. In particular, in Table 2 the results of biodiesel characterization are reported, demonstrating the feasibility of DWSO biodiesel as fuel.

Table 2: Property of Biodiesel

Property	Biodiesel
FAME content (%m/m)	96.6
Linolenic methyl ester (%m/m)	0.62
Viscosity at 40°C (mm^2/s)	3.85
Flashpoint (°C)	>150
Moisture content (ppm)	Trace
Acid value (mg KOH/g)	0.25
Polyunsaturated (≥4 double) methyl esters (% m/m)	0.0
Methanol content (% m/m)	0.1
Density at 15 °C (Kg/m3)	890

4. Conclusions

Magnetic Fe_3O_4@TA NPs were synthesized using a solvothermal method. Lipase was immobilized with success on the Fe_3O_4@TA NPs, via interaction between functional support groups and the enzyme. The immobilized TLL was used as an enzymatic catalyst for biodiesel production from dairy waste scum oil. The nanocatalyst, due to the nanosize of the support and the efficient immobilization, results highly active in the biodiesel production. Biodiesel DWSO presents linolenic methyl ester amount equal to 0.62%±0.07, and the total FAMEs amount of about 96.6%%±0.02 in agreement with the EN14214. The results of biodiesel characterization demonstrate the feasibility of DSWO biodiesel as a fuel.

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