Journal of Bioscience and Bioengineering
VOL. 128 No. 1, 98–102, 2019

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Cost analysis of oil cake-to-biodiesel production in packed-bed micro-flow reactors with immobilized lipases

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Received 21 May 2018; accepted 11 January 2019
Available online 7 February 2019

Biodiesel production depends to a great extent on the use of cheap raw materials, since biodiesel itself is a mass product, not a high-value product. New processing methods, such as micro-flow continuous processing combined with enzymatic catalysis, open doors to the latter. As reported here, the window of opportunity in enzyme-catalyzed biodiesel production is the conversion of waste cooking oil. The main technological challenge for this is to obtain efficient immobilization of the lipase catalyst on beads. The beads can be filled into tubular reactors where designed packed-bed provide porous channels, forming micro-flow. It turns out, that in this way, the immobilization costs become the decisive economic factor. This paper reports a solution to that issue. The use of oil cake enables economic viability, which is not given by any of the commercial polymeric substrates used so far for enzyme immobilization. The costs of immobilization are mirrored in the earnings and cash flow of the new biotechnological process.

[**Key words:** Biodiesel; Cost-benefit; Immobilized lipase; Oil cake; Packed-bed microreactor]

With the increase of awareness to protect and preserve the environment, biodiesel production has rapidly moved to an industrial level all over the world. Due to its biodegradability, non-toxicity and low sulphur content, biodiesel is categorized as a green fuel even though the technological process of its production cannot be fully considered as green (1). Today's biodiesel industrial production is based on chemically catalyzed transesterification (2), which has a negative influence on the environment due to the generation of large amounts of wastewater and high energy consumption. Therefore, strong efforts are being made to make the current industrial biodiesel production ecologically friendlier.

The use of lipases as biocatalysts (3–6) and the application of the waste oil from the food industry or restaurants as feedstock (7–11) has been so far performed only at the laboratory scale. Special attention was given to the development of novel enzymatic bioreactors with lower energy consumption (12–16). With the appearance of micro-flow reactor systems (17–19), a new opportunity has emerged to overcome some of the limitations, such as pressure drop, glycerol deposition, mass transfer limitations (20,21).

The results derived from our previous works (13,22) highlight two basic obstacles for the economically viable industrial-scale biodiesel production in packed-bed micro-flow reactors: (i) the cost of the carrier for lipase immobilization, and (ii) the price of lipase. The next step is to find cheaper carriers and/or cheaper lipases. In this paper, the introduction of oil cake as a carrier for lipase immobilization and as a substrate for lipase production was suggested.

The annual amount of produced vegetable oil in the EU is 29 million tonnes according to FAOSTAT database (http://www.fao.org/faostat/en/#data/QD). Considering that during oil production 1/3 of oil and 2/3 of oil cake is generated, the annual amount of oil cake produced in the EU can be estimated to 58 million tonnes. The oil cake that lags behind the oil production is commonly marketed as an animal feed, but due to chemical composition, it can be suitable for the production of lipase by solid-state fermentation. In comparison to other agroindustrial waste, oil cakes contain higher amount of lipids that act as inducers for lipase production. Various microorganisms (fungi, bacteria, yeasts and actinomycetes) are known to be good lipase producers, such as *Candida*, *Pseudomonas*, *Mucor*, *Rhizopus* and *Aspergillus* species (24).

The possible utilization of oil cake in biotechnology includes the production of antibiotics, vitamins, antioxidants, amino acids (25) and enzymes (26,27) or the application as renewable carriers for enzyme immobilization (28,29). Oil cakes can be used as lipase carriers in immobilization process (28) but need to be defatted with respect to the amount of lipids remaining after oil production. One of the most successful immobilization method is multipoint covalent bonding in terms of the thermal and operational stability of the immobilized enzyme. Indirect immobilization by covalent attachment of the lipase to the carrier is achieved by binding via active group of enzymes. Glutaraldehyde is the most commonly used carrier activator for the enzyme immobilizing by covalent binding (30).

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1389-1723/$ – see front matter
https://doi.org/10.1016/j.jbiosc.2019.01.004

Synthesis of biodiesel catalyzed by lipase produced by solid-state fermentation has been intensively investigated. For example, lipase produced during *Burkholderia cenocepacia* cultivation on bagasse/sunflower seed cakes was applied for biodiesel production. Oil conversion in fatty acid ethyl esters from 70% to 95% was obtained (31). Furthermore, Aguieiras et al. (27) performed biodiesel synthesis catalyzed by lipase produced during solid-state fermentation with *Rhizomucor miehei* cultivated on babassu cake. They reached fatty acid ethyl esters (FAEE) content of 90%. A wide range of oil conversion in fatty acid methyl esters (from 48% to 90%) was reported using lipase produced on a variety of agroindustrial wastes, such as tofu dregs, coconut dregs, corn bran and wheat straw (32,33). However, unpurified, crude lipase extracts, produced by solid-state fermentation by various microorganisms, possess low specific activities (1.15−5.31 U/mg) (34,35) compared with commercial lipase from *Thermomyces lanuginosus* (418 IU/mg) (36). Application of lipase of such low specific activities is insufficient to meet the amount of fatty acid methyl esters (FAME) in biodiesel prescribed by EN 14214 (37). Additional research was done on the immobilization of crude lipase extracts (38). Lipase produced by solid-state cultivation of *Aspergillus niger* on mangaba seeds was immobilized by indirect covalent bonding on glutaraldehyde activated sol-gel matrix, which resulted in an immobilization yield of 91.2%. On the other hand, various agroindustrial wastes were also tested as the enzyme immobilization carriers by adsorption or by covalent attachment, when pre-activated (39). Green coconut fibers are known to be used as carriers for immobilization of lipase and laccase by adsorption (40,41) or covalent binding (42,43). Other applied agroindustrial wastes used for enzyme immobilization (amylase, lipase and trypsin) are corn cob powder, corn stalks core, corn cob, rice hulls, banana stalk, Irvine leaves, and *Salacca wallichiana* stem (44−46). Optimization of the immobilization process of lipase from *T. lanuginosus* on olive pomace was investigated by Yücel (47). Covalent bonding using poly-glutaraldehyde as activation agent was performed. Immobilized lipase was used for biodiesel production from pomace oil and methanol. Maximal yield of biodiesel obtained after 24 h of process duration was 93%.

Therefore, the objective of this study was to determine the economic benefit of utilization of oil cake as a carrier in enzymatic biodiesel production in packed-bed micro-flow reactors. The analysis was done comparing the use of commercial lipase and lipase produced by solid-state fermentation on agroindustrial waste.

MATERIALS AND METHODS

The general idea proposed by this paper was to use the closed production cycle, from the waste (oil industry waste) to the product (biodiesel). Since it is very common that biodiesel plants own oil-producing facilities, the use of the waste/by-product (oil-cake) for biodiesel production could have benefits for the improvement of self-sustaining process.

Approaches and assumptions This paper is not a ready-made recipe but solid base in a search for a greener biodiesel option. Within this paper, the profitability of biodiesel production in micro-flow packed-bed reactors was analyzed using the waste cooking oil as a feedstock and methanol as acyl acceptor in molar ratio 1:3 in solvent free system. As a carrier for lipase immobilization by covalent attachment oil cake was suggested to be defatted with hexane and activated by glutaraldehyde. The ideal transesterification is assumed. The number of reactors is calculated based on the activity of immobilized lipase for the target annual production of 10,000 tones. Two cases were presented: case 1: application of oil cake as a carrier for immobilization by covalent binding of commercial lipase from *T. lanuginosus*; and case 2: application of oil cake as a carrier for immobilization by covalent binding of lipase produced by solid-state fermentation. The key parameters used for the calculation of cost analysis are presented in Table 1.

As previously highlighted (23), *T. lanuginosus* lipase was selected due to its high stability, tolerance to methanol and high productivity (47,48). Since the relevant literature lacks data relating to the immobilization of lipases produced by solid-state fermentation on agroindustrial wastes, for the calculation, data on lipase from *Schizophyllum commune* ISTL04 produced by solid-state fermentation on *Leucaena leucocephala* seeds (49) was used. This lipase was shown to have high specific activity (238.13 U/mg). It is assumed that this activity would be sufficient to reach the amount of fatty acid methyl esters in biodiesel prescribed by EN 14214 that is ≥96%.

In our previous work (23), in case 1 for the calculation to obtain a positive profit, the retention activity of 80 % was used. In this work, the retention activity of 50% was used in calculation, for both investigated cases. No values were found in the literature that would justify higher percentage. According to Chen et al. (50), immobilized lipase can be used for 7 days without significant loss of activity. Based on this work, the cost analysis for case 2 (lipase from *S. commune* ISTL04) was made. It is different from case 1 (commercial lipase from *T. lanuginosus*) where lipase could be reused for 10 days, based on Yucel's results (47). The cost analysis was carried out for 10,000 tonnes of target annual plant production capacity.

Cost analysis In Table 2, the list of parameters with the corresponding prices used for making cost analysis is given. It is apparent that the price of solid-state fermentation produced lipase is significantly higher than the price of the commercial lipase. The crude lipase extract according to the literature (34,35), has low activity and it has to be purified. The most frequent purification process consists of enzyme precipitation with ammonium sulfate followed by separation using gel permeation column chromatography. It significantly increases the price of produced enzyme. The price of purified lipase thus reaches and even exceeds the price of the commercially available lipases (Table 2). According to Tufvesson et al. (51) guidelines for the cost analysis of the biocatalysis production, free enzyme price ranges from 297 to 1188 USD/kg, excluding the costs for research and development. Since in this paper a detailed cost analysis of production of lipase by solid-state fermentation was not given, but the production of biodiesel in packed-bed micro-flow reactors, the price of solid-state fermentation produced lipase was taken from the paper published by Tufvesson et al. (51).

TABLE 1. Description of parameters used for cost-benefit analysis for biodiesel production by application of oil cake as a carrier for lipase immobilization in packed bed micro-flow reactors.

Parameter	Description	
	Case 1	Case 2
Enzyme	Lipase from *Thermomyces lanuginosus* (TLL), commercially available	Lipase from *Schizophyllum commune* ISTL04, produced by solid-state fermentation on *Leucaena leucocephala* seeds (49)
Specific activity (U/mg)	418.00	238.13
Turn over number (TON) [g (substrate) min^{-1}/g (lipase)]	7.80	5.51
Number of reuses	10	7
Number of packed-bed reactors required	10	12
Carrier	Oil cake defatted with hexane and activated with glutaraldehyde	Oil cake defatted with hexane and activated with glutaraldehyde
Raw materials	Waste cooking oil and methanol	Waste cooking oil and methanol
Oil:methanol molar ratio	1:3	1:3
Activity retention (%)	50	50
Enzyme retention (%)	99	99
Target annual plant production capacity (tones)	10000	10000
Duration of production, year	1	1
Working days per year	300	300
Utilities	Water, electricity and natural gas	Water, electricity and natural gas

TABLE 2. Input and output parameters with corresponding prices used for cost-benefit analysis for biodiesel production by application of oil cake as a carrier for lipase immobilization in packed bed micro-flow reactors.

Parameters	Price
Waste cooking oil, USD/kg[a]	0.24
Methanol, USD/kg[a]	0.52
Lipase from *Thermomyces lanuginosus*, USD/L[b]	929
Lipase from *Schizophyllum commune* ISTL04, USD/L	1188.4 (51)
Defatted and activated oil cake, USD /kg[a]	95.78
Phosphate buffer, USD/L[a]	5.71
Labor, USD/year	224208
Capital costs: case 1, USD/t biodiesel	129.7 (23)
Capital costs: case 2, USD/t biodiesel	136.5 (23)
Electricity, USD/t biodiesel	2.84 (23)
Water, USD/t biodiesel	3.68 (23)
Natural gas, USD/t biodiesel	31.32 (23)
Biodiesel, USD/kg[a]	1.04
Glycerol, USD/kg[a]	0.8

[a] Retrieved March 17, 2018 from Alibaba Group, http://www.alibaba.com.
[b] Retrieved March 17, 2018 from Sigma–Aldrich Corporation, https://www.sigmaaldrich.com/european-export.html.

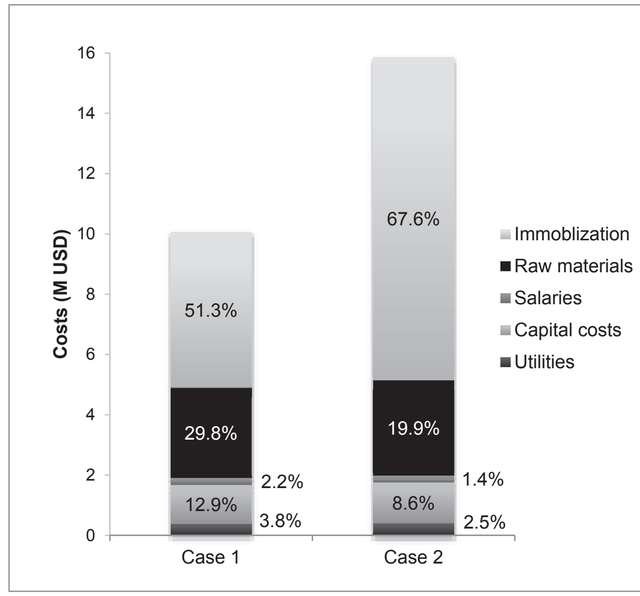

FIG. 1. Input costs and share of each component for both cases.

For the purposes of calculations within this paper, the price for lipase produced by solid-state fermentation (case 2) of 1188 USD/kg was taken as the maximum load. Thus, the maximum price is the largest load that is more realistic than the minimum or average price for the cost analysis performed here.

Before its use in lipase immobilization, oil cake needs to be defatted and activated. Defatting should be carried out with non-polar solvents. In this paper, due to the low costs, hexane was considered to be used for the purpose of de-fatting. Activation of the cake for indirect immobilization by enzyme covalent binding is usually carried out with glutaraldehyde, which was also used here in the calculation. The final price of the carrier is expressed as the price of defatted and activated oil cake (Table 1).

Based on the paper by Peters et al. (52) the cash flow analysis by present value method was made.

RESULTS AND DISCUSSION

From the obtained results of cost analysis, for both cases, it is evident that the immobilization costs have the greatest impact on the total costs (Fig. 1). Total immobilization costs (which includes the raw material for immobilization and associated operating costs) are the most affected by the high price of lipase.

In the majority of industrial plants as well as in the real industrial biodiesel plant in Croatia (23), the share of costs for materials-capital-other follows a distribution of 70-10-20%. When using oil cake as a carrier, the results are quite different (Fig. 1).

The percentage share of raw materials in the total costs is recorded to be 30%, while the capital costs contributes with 13%. The biggest share in the total costs is immobilization (51%) due to the high price of commercial lipase (case 1). For case 2, because of the higher price of free solid-state fermentation produced lipase, the total costs of immobilization is 67.6%. Due to the higher immobilization costs, the raw materials contribute with nearly 20% and capital costs with 9%. Nor does case 1 nor case 2, here agree to the previously mentioned most common distribution of the costs of real industrial biodiesel production (70–10–20% break up of material–capital–other costs). However, results in Fig. 2 show that in the first year of the production in a packed-bed micro-flow biodiesel plant with commercially available lipase immobilized on the oil cake (case 1), it is possible to achieve a positive profit. For case 2 it is not possible, yet. Why is that so? Because the production of cheap enzyme/lipase by the solid-state fermentation procedure is still catching up. This is still a relatively new field with regard to economic viability for commercial use and it is quite difficult to estimate the real price of biocatalyst (51).

Based on these results, again, the price of the lipase appears as the main issue and obstacle to achieve an economically viable and environmentally unburdened biodiesel production process.

The total sales represents the price of the produced biodiesel for the plant targeted production of 10,000 tons per year (Fig. 2). The case 1 requires 10 packed-bed micro-flow reactors (according to the detail calculation presented in Budžaki et al. (23)) for annual production of 10,145.91 tones. With the market price of biodiesel at 1.04 USD/kg it is annually worth 11.4 million USD. For case 2, according to the calculations, the minimal number of reactors needed is 12 (10, 670.16 tonnes per year) and the total sales worth is 12 million USD per year. The difference in the amount of annually produced biodiesel is affected by specific lipase activity packed into a single packed-bed micro-flow reactor.

As previously pointed out, it is assumed that the process of biodiesel production is ideal, and that the technological problems of biodiesel production (pressure drop, glycerol deposition, and mass transfer limitations), that usually affect conversion of oil into fatty acid methyl esters, are excluded. Accordingly, the cost-benefit analysis shows the total positive profit (Fig. 2) with respect to lower cost of the carrier for immobilization, resulting in significant reduction in operating costs.

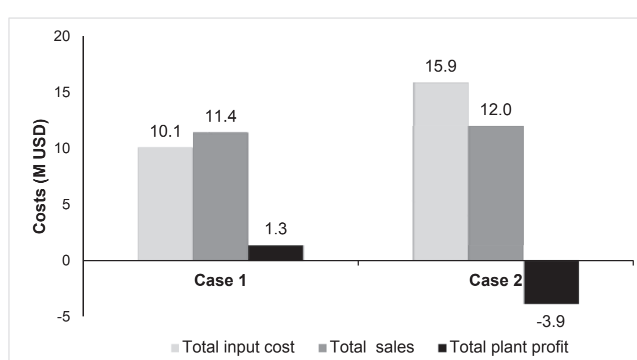

FIG. 2. Plant profit for first year of production.

Furthermore, the cash flow analysis for case 1 after the first year of production shows net present value (NPV) as negative (−0.84 million USD) (Fig. 3). After the second year of production NPV gains a positive value, which is, according to Sotoft et al. (53), necessary payback period for the viability of the process. The cash flow analysis for case 2 shows a negative economic balance during 10 years of production.

Despite the evolution, and constant balance between the needs and constraints of sustainability, the essence of the idea remains unchanged (54). Shrivastava and Hart (1) defined green production: Green production focuses upon three fundamental goals: (i) to minimize emissions, effluents, and accidents; (ii) to minimize the use of virgin materials and non-renewable forms of energy; and (iii) to minimize the life-cycle cost (cradle to grave) of products or services. Introducing waste/by-products from the oil industry as energy and nutritionally high-value material (25,55) into the technological process of biodiesel production, makes it greener. This implies an environmentally acceptable production that leaves less of an environmental impact than the existing one and makes biodiesel obtained in such a way, the preferred product.

The most prominent use of food processing residues is its use for animal feeding. A few decades ago, a certain imbalance between the generation of food bio-waste and the number of livestock was perceived. With the growth of food industry, accumulation of food industry waste occurs, but the demand for feed decreases due to the decline in livestock farms (55). According to the data of FAOSTAT (http://www.fao.org/faostat/en/#data/QA/visualize, accessed September 24, 2018. The data about number of live animals were collected according to selected parameters as follows: cattle, goats, horses, mules, pigs, and sheep for Europe + (Total) from 2007 to 2017), the trend continues until today. Awareness about the growth of environmental loads with wastes or by-products originating from the food industry, increases tendency for the search of the new methods of its exploitation. Here, the two possibilities are presented.

In conclusion, given the fact that the lipase immobilization process as well as the biodiesel production process for this cost-benefit analysis are assumed to be ideal, this cost-benefit analysis can be considered as the basis for the optimization of the process of biodiesel production in packed-bed micro-flow reactors with special emphasis on immobilization techniques. The intention was to demonstrate two possible ways of utilization of oil cake in biodiesel production: as a carrier for lipase immobilization, and as a substrate for lipase production. Here the authors put the focus on immobilization techniques because better immobilization efficiency (higher lipase activity retention) can furthermore reduce total input costs and consequently increased profit. The cash flow analysis conducted via NPV method shows that at the given process conditions, biodiesel production is economically viable, since second year of production NPV was positive (+0.44 million USD).

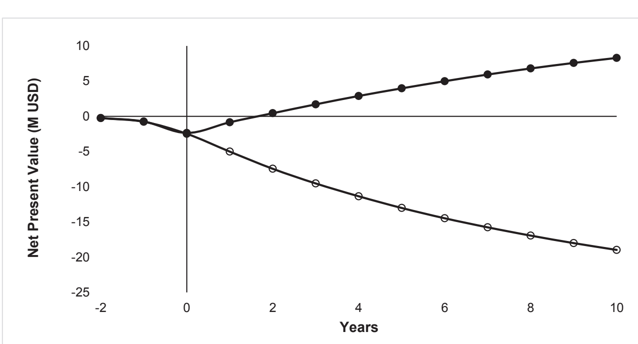

FIG. 3. Cash flow analysis for a 10 years production period.

It was expected that the cost analysis would show positive profit in view of significant immobilized lipase price reduction, but this is only an incentive for further research in terms of optimizing lipase production and immobilizing it on waste from the oil industry, as well as approaching the implementation of packed-bed micro-flow reactors with immobilized lipase at an industrial scale. The realized cost-benefit analysis shows the possibility of implementing waste from the oil industry in the production of biodiesel, which presents another way of using the waste, thereby it generates added value and minimizes such waste.

Biodiesel production has significantly evolved up to date: from homogeneous via to heterogeneous chemical catalysts to biocatalysts. With this work, we have made a step forward in the direction of greener biodiesel production that not only does not burden the environment but relieves it by using the waste/by-product (oil cake) from food industry as a carrier for the immobilization of lipase and/or as a substrate for the production of a cheaper biocatalyst. Furthermore, the introduction of a packed-bed micro-flow reactor, which are proven process intensifiers, in combination with an oil cake and rounded with cost analysis, represents a pointer to biodiesel commercialization.

ACKNOWLEDGMENT

This work was supported by the Croatian Science Foundation (grant number IP-2016-06-7993).

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