Fuel Processing Technology 165 (2017) 1–8

Contents lists available at ScienceDirect

Fuel Processing Technology

journal homepage: www.elsevier.com/locate/fuproc

Research article

Optimization of lipase production from organic solid waste by anaerobic digestion and its application in biodiesel production

Selvakumar P., Sivashanmugam P. *

Department of Chemical Engineering, National Institute of Technology, Tiruchirappalli-620 015, Tamil Nadu, India.

ARTICLE INFO

Article history:
Received 14 February 2017
Received in revised form 29 April 2017
Accepted 29 April 2017
Available online xxxx

Keywords:
Garbage lipase
Organic solid waste
Response surface methodology
Artificial neural network
Biocatalytic activity
Biodiesel production

ABSTRACT

Lipases are particularly important due to the fact that they specifically hydrolyze the acylglycerols into free fatty acids and glycerol, which is of greater interest for biodiesel production. In this study, garbage lipase was produced from organic waste (Pomegranate (P), Orange (O) and Pineapple (PA) peels) by optimization the organic waste composition, ultrasonic pre-treatment time and process parameters. Optimization of process parameters using RSM and ANN modelling was performed with the composition of 35(P): 20(O): 35(PA) and 15 min (pre-treatment). The maximum lipase activity of 57.43 U/mL was obtained at pH (6), temperature (33 °C), agitation (210 rpm) and time (4 days). The effects of pH, temperature, organic solvents on the stability of partially purified lipase (PPL) and enzyme kinetic parameters were also investigated. PPL yielded 88.63% conversion of palm oil into biodiesel. Hence, garbage lipase would be a potential biocatalyst for biodiesel conversion and other industrial catalytic processes.

1. Introduction

Recently, the transesterification reaction of different oils and alcohols with suitable catalyst has gained much attention towards biodiesel production in industries. In this reaction, oils/fats (mono-, di-, triglycerides, non-lipid esters etc.) react with short chain alcohols like ethanol or methanol in the presence of either acid or base catalyst and most often with an alkaline catalyst to produce mono-alkyl ester and glycerol. However, alkaline catalyzed transesterification has its own disadvantages due to its high sensitivity towards free fatty acids, difficulties in the recovery of the catalyst and requirement of plenty of water for purification makes the process a little less viable [1]. In addition to this, when the substrate contains >0.5 wt% of free fatty acids, it has a negative impact on the yield due to the formation of soap [2]. The acid catalyst can also be used but the rate of conversion is unsubstantial when compared to alkaline catalyst [3]. Therefore, there is a need for profuse search for a new catalyst to overcome these drawbacks.

Enzymatic catalyst i.e., lipase (E.C.3.1.1.3) has become a potential option because they are non-toxic, organic solvent tolerant, reusable and insensitive to free fatty acids. Therefore, the oil does not require a pre-treatment, and both the esterification and transesterification can take place at the same time to produce mono-alkyl esters without any hindrance. But, enzymes are less stable, expensive, and have poor recyclability and this causes difficulty in wide acceptance for enzyme catalyzed transesterification process. Enzyme immobilization technology helps to overcome some of the disadvantages by increasing the enzyme stability, decreasing inhibition rate and facilitating enzyme reuse [4,5].

Response Surface Methodology (RSM) is a commonly used statistical method for optimization of process parameters. For lipase production from various sources, Central Composite Design (CCD) was used [6], while only a few studies were done using Face centered CCD model. Artificial Neural Network (ANN) is an efficient prediction and modelling tool. Comparing the predicted results from RSM with the ANN results would help to determine which tool among them gives more accurate data regarding optimization of parameters [7].

In recent years, the developing food processing industries, vegetable markets, restaurants, fruit markets, etc., are producing large quantities of organic solid waste such as vegetable dregs and fruit peels. Their disposal without causing any harm to the environment or personal health is a major issue all over the globe [8]. Organic wastes are rich in nutrients, easily degradable and have high moisture content. These properties make them useful for the production of electricity, fuels and high value biochemical such as garbage enzymes, organic acids, etc. [9–12]. Therefore, usage of such wastes for production of garbage enzyme reduces the production cost and also decreases the pollution load to the environment. The garbage enzyme is composed of different types of enzymes and their function can be of four categories: catalyze, decompose, compose and transform [13].

Lipase production from fungal species (*Candida*, *Geotrichum*, *Mucor*, and *Rhizopus*) and bacterial species (*Bacillus*, *Pseudomonas*,

* Corresponding author.
E-mail address: psiva@nitt.edu (P. Sivashanmugam).

http://dx.doi.org/10.1016/j.fuproc.2017.04.020
0378-3820/

Staphylococcus, and *Streptomyces*) was widely reported [14–16]. But, only a few studies were done on lipase production using organic solid waste. Apart from biodiesel conversion, lipase enzymes are used in biotechnological industries such as food, dairy, cosmetic, pharmaceutical, detergent, leather industries, used as a biosensor, bioremediation and peptide synthesis, etc. due to their distinctive huge catalytic potential. So, lipases are considered as one of the most crucial industrial enzymes [17,18].

Till now, no one has made an attempt to make use of garbage lipase from organic wastes for biodiesel production. Hence, the present study was focused on the production of garbage lipase from organic solid waste (Pomegranate, Orange and Pineapple peels) through anaerobic fermentation and analysis of the ability of produced enzyme towards biodiesel production. To improve the lipase activity of the produced enzyme, different organic waste ratios were taken and pre-treatment was performed using sonicator. The process parameters of enzyme production were optimized using RSM and ANN to maximize lipase activity. The lipase obtained was used for producing biodiesel using palm oil which was analyzed by Thin Layer Chromatography (TLC) and ^{1}H NMR spectroscopy.

2. Materials and methods

2.1. Determination of lipase activity of garbage enzyme

The lipase activity of garbage enzyme was assessed spectrophotometrically using *p*-nitrophenyl palmitate (p-NPP) as a substrate [19]. The reaction mixture containing 0.1 mL of enzyme fraction, 0.3 mL of 0.05 M phosphate buffer (pH 8) and 0.1 mL 0.8 mM p-NPP was kept at 37 °C for 10 min and then the reaction was terminated by the addition of 1 mL ethanol. Concurrently, control was prepared without the addition of enzyme fraction. The reaction resulted in the formation of yellowish color which was measured at 410 nm in a spectrophotometer. One unit of garbage lipase activity was referred as the amount of enzyme liberating 1 μmol of p-nitrophenol per min from p-NPP under optimal assay conditions.

2.2. Effect of organic solid waste composition on lipase activity of garbage enzyme

Pomegranate (P), Orange (O) and Pineapple (PA) peels were taken to make different compositions in order to assess the lipase activity of garbage enzyme. 10 g of pomegranate, 40 g of orange and 40 g of pineapple peels were mixed to obtain a ratio of 10(P): 40(O): 40(PA) which was denoted as composition C1. 3 parts of this mixture were taken in an airtight container, 1 part of industrial molasses and 10 parts of water were added. The container was incubated for 6 days in a dry, cool and well-ventilated space for fermentation. After 6 days, the fermentation broth was filtered and subsequently centrifuged at 4000 rpm for 30 min. The obtained supernatant (garbage enzyme) was used for further exploration. The same methodology was followed to assess the lipase activity of garbage enzyme produced from various ratios of organic wastes as mentioned in Table 1.

Table 1
Composition of different organic wastes with corresponding lipase activity.

Ratio of organic wastes (fruit peel)	Pomegranate (g)	Orange (g)	Pineapple (g)	Lipase activity (U/mL)
C1	10	40	40	6.8 ± 0.12
C2	20	35	35	8.1 ± 0.17
C3	30	30	30	8.4 ± 0.14
C4	40	10	40	8.6 ± 0.23
C5	35	20	35	9.7 ± 0.20
C6	40	40	10	6.5 ± 0.13
C7	35	35	20	7.3 ± 0.10

The values presented in the table are average values of three repetitions.

2.3. Effect of pre-treatment on lipase activity in garbage enzyme

The organic waste mixture C5 (35(P): 20(O): 35(PA)) and phosphate buffer solution (pH 7) were taken in a glass beaker and was exposed to ultrasonic pre-treatment using a sonicator (Model: Lark Classic Model, frequency: 20 kHz, power: 45%, temperature: 30 °C) at different time intervals such as 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 min. The control sample was not exposed to pre-treatment. The industrial molasses and water were added to each pre-treated organic waste mixture including control sample and incubated for 6 days in a dry, cool and well-ventilated space for fermentation. After 6 days, the fermentation broth was filtered and subsequently centrifuged at 8000 rpm for 30 min. The obtained supernatant (crude enzyme) was used for the determination of lipase activity [20].

2.4. Optimization of lipase enzyme production

2.4.1. RSM

For this study, process input variables which impact on lipase yield were examined through RSM using organic solid waste composition C5 after pre-treatment for 15 min. The levels taken for the four independent variables such as pH, temperature, agitation speed and fermentation time were presented in Table 2. These ranges were selected based on preliminary experiments (Fig. A1–A4). To avoid redundancy, i.e. avoiding unnecessary repetition of experiments, a fractional factorial Central Composite Design (CCD) (Design Expert 10.0.3, Stat-Ease Inc. 2020 east Hennipin Ave., suite 480 Minneapolis, MN 55413) was applied to produce 30 experimental conditions (Table A.1). Six center points were used to appraise the lack of fit and pure error of the recommended model [21]. Numerous deteriorations were applied to fit the coefficient of the quadratic polynomial regression model of the response. The excellence of the fitted quadratic polynomial response model was evaluated by significance test and analysis of variance (ANOVA) and the fitted model is represented in Eq. (1).

$$
\begin{aligned}
Y_i = {} & \beta_0 + \beta_1 * X_1 + \beta_2 * X_2 + \beta_3 * X_3 + \beta_4 * X_4 + \beta_{12} * X_1X_2 + \beta_{13} \\
& * X_1X_3 + \beta_{14} * X_1X_4 + \beta_{23} * X_2X_3 + \beta_{24} * X_2X_4 + \beta_{34} * X_3X_4 \\
& + \beta_{11} * X_1{}^2 + \beta_{22} * X_2{}^2 + \beta_{33} * X_3{}^2 + \beta_{44} * X_4{}^2 \qquad (1)
\end{aligned}
$$

where, Y_i is the dependent variable (lipase), β_0 is the intercept value, β_1, β_2, β_3 and β_4 are the first order coefficients, β_{12}, β_{13}, β_{14}, β_{23}, β_{24} and β_{34} are the interaction coefficients, β_{11}, β_{22}, β_{33} and β_{44} are representing the quadratic coefficients and X_1, X_2, X_3 and X_4 denote the independent variables.

2.4.2. ANN modelling

The neurons in the ANN modelling are controlled by transfer and summing function. The most predominantly used transfer functions are Linear Transfer Function (purelin), Log-Sigmoid Transfer Function (logsig) and Tan-Sigmoid Transfer Function (tansig) [22]. In this study, MATLAB (R2014 a) mathematical software, Neural Network (NN) tool box was used for prediction and validation of optimal conditions for the production enrichment of garbage lipase from the organic waste composition C5 (Supplementary data).

Table 2
Experimental ranges and coded levels of the independent variables for RSM modelling.

Variables	Code	Levels		
		−1	0	+1
pH	X_1	3	6	9
Temperature (°C)	X_2	33	37	41
Agitation speed (rpm)	X_3	180	210	240
Time (days)	X_4	2	4	6