Journal of Cleaner Production 59 (2013) 290–297

Contents lists available at SciVerse ScienceDirect

Journal of Cleaner Production

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

Utilization of oleochemical industry residues as substrates for lipase production for enzymatic sunflower oil hydrolysis

Wael Abdelmoez [a,*], N.A. Mostafa [b], Ahmad Mustafa [c]

[a] Chemical Engineering Department, Faculty of Engineering, Minia University, Minia, Egypt
[b] Faculty of Applied Medical Science, Taif University, Taif, Saudi Arabia
[c] Research and Development Sector, Oleo Misr for Oils and Soap Plant, Sadat City, Egypt

ARTICLE INFO

Article history:
Received 22 April 2013
Received in revised form
12 June 2013
Accepted 17 June 2013
Available online 27 June 2013

Keywords:
Lipase
Candida rugosa
Fermentation
Hydrolysis
Kinetic parameters

ABSTRACT

The present work deals with the production of lipase by *Candida rugosa ATCC 14830* and focuses on the effect of different substrates on lipase production by submerged fermentation. Two industrial wastes from oil refining and the oleochemical industry were evaluated as substitutes to olive oil for lipase production through aerobic fermentation. Lipase activity, protein yield and cell mass were studied using different substrates concentrations. First, olive oil (OO), fatty acid residues (FAR) and soapstock (SS) were tested individually as substrates. The activities of the produced lipases, utilizing these substrates, were found to be 12 U/ml, 7 U/ml and 7.4 U/ml, respectively. Second, a mixture of these substrates composed of fatty acid residues, olive oil, and soapstock (POS) were formulated and used for lipase production. The obtained results showed that the lipase activity achieved was 10 U/ml. The produced lipase has been used in sunflower oil hydrolysis. The results revealed that 39.5% of the original tested sunflower oil was hydrolyzed into free fatty acids after 24 h at 37 °C.

1. Introduction

Fatty acids currently are producing by very high capital and running cost processes. The currently used method of splitting of lipids to fatty acids and glycerol involves high temperature and pressure conditions for about 6 h to achieve the desired 96–99% conversion. When these extreme conditions are employed, polymerization of fats and by-products formation take place result in dark fatty acids and colored aqueous glycerol solution (Al-Zuhair et al., 2003). To remove the color and the by-products, further purification by distillation is required. Both hydrolysis and subsequent distillation of fatty acids and glycerol are energy intensive processes. A number of comparative environmental assessment studies have been conducted in the past 15 years to investigate whether using the enzymatic processes in oleochemical industry lead to environmental improvements and assess whether they could play a role in moving toward economic and cleaner industrial production. All agree that enzymatic processes are favorable to the environment compared with the traditional processes (Jegannathan and Nielsen, 2013). However the economic benefits of replacing the chemical methods with the enzymatic one in doubt until now. As the cost of enzymes are very high it became necessary to find an economical routes for enzyme production. The saving of energy and minimization of thermal degradation are probably the major attractions in replacing the current chemical technologies with biological ones.

Lipases (glycerol ester hydrolases EC 3.1.1.3) have versatile applications, importance and significance in oleochemical industry due to their wide variety of reactions (Ramani et al., 2010). The physiologic role of lipases is to hydrolyze triglycerides into di-glycerides, mono-glycerides, fatty acids, and glycerol. Also Lipases catalyze partial or complete hydrolysis of triacylglycerols, reaction of esterification, transesterification and interesterification of lipids (Colla et al., 2010).

The recent interest in the production of lipases is associated with their applications as additives in food (flavor modifications), fine chemicals (synthesis of esters), detergent (hydrolysis of fats), waste water treatment (decomposition and removal of oily substances), cosmetics (removal of lipids), pharmaceuticals (digestion of oil and fats in foods), leather processing (removal of lipids from animal skins) and biomedical assays (blood triglycerides) (Salihu et al., 2012). Additionally, lipases have an important application in the field of bioenergy, especially in biodiesel production, which is an expanding sector, as a result of the worldwide rising demand on the use of renewable energy (Colla et al., 2010).

* Corresponding author. Tel.: +20 1000859791.
E-mail addresses: drengwael2003@yahoo.com, ahmedm@oleomisr.com (W. Abdelmoez).

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http://dx.doi.org/10.1016/j.jclepro.2013.06.032

Lipases are produced by various microbes, such as bacteria, fungi, and yeast. They have also been reported in the sources of plants and animals. Lipases of microbial origin have greater industrial attraction because they are available in large quantities and can be produced with high yields (Das, 2005). In addition, the absence of seasonal fluctuation, and rapid growth of microorganisms on inexpensive media added more advantageous to the process (Govindarajan and Krastanov, 2008). Bioconversion of agricultural residues for lipase production as well as other value added products would hold a prominent position in future biotechnologies, mainly due to their low cost, accessibility and nutrient compositions containing carbon, nitrogen and minerals to (Salihu et al., 2012). In fatty acids fractionation plants, disposing of fatty acids residues (pitch) is a problem, in the same time it is not possible mixing it with the plant's waste water. Furthermore it accumulates in abundance during the production process, which in turn has a big role in filling the plant's storage tanks with worthless materials. Although the emergence of few pitch based applications such as production of low grade soap and some kinds of poultry feeds, however that didn't solve the problem. In this research fatty acids residues (pitch) were used as an effective nutrient for lipase production, which in turn could help in solving pollution problems which may be caused by waste disposal. Selection of an appropriate substrate is a key factor in submerged fermentation (Salihu et al., 2012). Recently, substrates like meal (Ul-Haq et al., 2002), Sugarcane bagasse (Babu and Rao, 2007), Bleaching oil cake (Balaji and Ebenezer, 2008), jatropha seed cake (Mahanta et al., 2008), and biopharmaceutical oil wastes (Mohanasrinivasan et al., 2009) have been used for the production of lipases.

There is a considerable interest to optimize the carbon sources for lipase production and to try cheaper substrates alternatives (Walde and Schomärer, 1988). It was reported that it is difficult to get all required features from a single substrate; however it could be achieved by combination of different substrates (Edwinoliver et al., 2010). Accordingly, to enhance the growth and production of lipase from *Candida rugosa* ATCC 14830, a novel route has been tested. In the present work, a novel fermentation media using a combination of different substrates like fatty acids residues, soapstock, and olive oil were chosen for lipase production. In this way, a sustainable way of hydrolysis could be provided with keeping an economically feasible rout of production of fatty acids.

2. Materials and methods

2.1. Materials

Soapstock, fatty acid residues and refined bleached deodorized (RBD) sunflower oil were kindly provided by Oil Tec. Industries (Sadat city, Egypt). Soapstock is a by product of the caustic refining of oils and fats. Fatty acids residues are the spent viscous fatty acids that remained in the end of the fractionation process of fatty acids. The olive oil was purchased from local market. All reagents used were of analytical grade including, gum arabic, thymolphthalein indicator sodium hydroxide, ethanol (95%), Folin–Ciocalteu reagent, bovine serum albumin, sodium carbonate, peptone, yeast extract, glucose, copper sulfate, sodium tartrate, phenolphthalein and isopropyl alcohol were from Algomhorya for chemicals company (Cairo, Egypt).

Candida cylindracea ATCC 14830 strain was used throughout this study. The organism provided by microbiological resource center (MIRCEN) Faculty of Agriculture, Ain Shams University, Cairo, Egypt. The organism was maintained on peptone-glucose-yeast extract agar slant at 4 °C.

Table 1
Composition of soapstock obtained from soyabean oil refinery.

Test	SV[a]	FFA[b]	NO[c]	FM[d]	Moisture
	171 mg KOH/g	0	17.5%	23.5%	37%

[a] (SV) The saponification value was expressed as the number of milligrams of potassium hydroxide (KOH) required saponifying 1 g of the test sample.
[b] (FFA) Free fatty acids, due to the high concentration of sodium hydroxide in soapstock all the fatty acids are present as sodium salts.
[c] (NO) Neutral oil (%w/w, comprises acylglycerol).
[d] (FM) Fatty matter was expressed as the substances soluble in petroleum ether in that portion of the sample which is soluble in alcohol.

2.2. Methods

2.2.1. Microbial culture and inoculum preparation

Five milliliter of sterile production medium was dropped on the surface of the slant. Then, cells from the slants were scrapped off and suspended in 100 ml of media. The suspension was then shaken thoroughly to break up any aggregates. The used media consisted of 1 g of peptone and 2 g of glucose along with 1 g of yeast extract, the medium components were mixed together in 100 ml of water. The initial pH of the medium was adjusted to 6 prior to autoclaving at 120°°C for 20 min.

2.2.2. Fermentation medium

The oil-based medium contains different concentrations of oily materials. The fermentation media consisted of 1% (w/v) peptone, 0.5% (w/v) yeast extract and 0.25% (w/v) glucose, adjusting the pH to 6 in 1 L of water.

2.2.3. Lipase production in bench-top stirred tank bioreactor

A stirred tank bioreactor with a working volume of 1 L was used for the fermentation studies. Agitation was performed using two six bladed disc turbine impellers. Air flow rate was maintained using a blower. Sampling was taken at regular intervals followed by centrifugation at 4000 rpm for 20 min. The supernatants were used for the determination of lipase activity. The bioreactor and the agitator were designed according to the geometric proportions for a standard agitation system (Geankoplis, 1993). The cover on the top allows sampling and measurement of the temperature and pH.

2.2.4. Biomass estimation

For the determination of biomass content, the sample was centrifuged at 4000 rpm and the cell mass placed on a preweighted filter paper. The cells were washed with distilled water several times and finally dried in an oven at 105 °C to a constant weight.

2.2.5. Lipase assay

The activity of lipase was determined by the titrimetric method. In this procedure, native substrate (olive oil – gum arabic emulsion) was hydrolyzed to yield fatty acids at 37 °C in water bath under

Table 2
Composition of fatty acids residues resulted from mixed soybean and sunflower oil distillation.

Test	Result
Free fatty acids	33%
Soap[a]	5000 PPM
Moisture content	1.14%
Impurities	0.35%
Unsaponifiable matter[b]	18.6%
Esters, gums, waxes	47%

[a] Soap content was measured by the titrimetric method.
[b] Unsaponifiable matter includes higher aliphatic alcohols, sterols, pigments, and hydrocarbons.