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Lipase Production in Solid-State Fermentation Monitoring Biomass Growth of *Aspergillus niger* Using Digital Image Processing

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Abstract The aim of this study was to monitor the biomass growth of *Aspergillus niger* in solid-state fermentation (SSF) for lipase production using digital image processing technique. The strain *A. niger* 11T53A14 was cultivated in SSF using wheat bran as support, which was enriched with 0.91% (*m*/*v*) of ammonium sulfate. The addition of several vegetable oils (castor, soybean, olive, corn, and palm oils) was investigated to enhance lipase production. The maximum lipase activity was obtained using 2% (*m*/*m*) castor oil. In these conditions, the growth was evaluated each 24 h for 5 days by the glycosamine content analysis and digital image processing. Lipase activity was also determined. The results indicated that the digital image process technique can be used to monitor biomass growth in a SSF process and to correlate biomass growth and enzyme activity. In addition, the immobilized esterification lipase activity was determined for the butyl oleate synthesis, with and without 50% *v*/*v* hexane, resulting in 650 and 120 U/g, respectively. The enzyme was also used for transesterification of soybean oil and ethanol with maximum yield of 2.4%, after 30 min of reaction.

Keywords Lipase · Digital image processing · *Aspergillus niger* · Solid-state fermentation · Glycosamine · Vegetable oils · Biodiesel

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Introduction

Lipases (triacylglycerol hydrolases, EC 3.1.1.3) are enzymes that catalyze reactions such as hydrolysis, interesterification, esterification, alcoholysis, acidolysis, and aminolysis [1]. There is an increasing interest in the development of lipase applications to oleochemical transformations to obtain esters of long-chain fatty acids, as monoalkyl esters of fatty acids [2]. Utilization of lipase as a catalyst for the production of biodiesel, defined as a mixture of monoalkyl esters, is a clean technology due to its nontoxic and environmental friendly nature, requiring mild operating conditions compared with chemical method [3].

Lipases are produced by animals, plants, and microorganisms. Microbial lipases have a great potential for commercial applications due to their stability, selectivity, and broad substrate specificity. Among the high number of lipases described in the literature, only the enzymes belonging to a few species have been demonstrated to have adequate stability and biosynthetic capabilities to allow routine use in organic reactions. The most productive species belong to the genera *Geotrichum*, *Penicillium*, *Aspergillus*, and *Rhizomucor* [4]. Nevertheless, the commercial lipases currently available are very costly and unsuitable for biodiesel production.

Fungal species are easily cultured in solid-state fermentation (SSF). This culture mode offers many advantages over commonly submerged fermentation (SF) processes, such as, the recovery of more concentrated products, smaller residues generation, reduced water consumption, and the possibility of using by-products as substrate [5]. There are several methods proposed to monitor the SSF process, such as the biomass estimation techniques, which permit to establish a relationship among growth kinetics, biomass morphology, and the fermentation product, providing important parameters to improve the operational fermentation process. However, the monitoring of filamentous fungi biomass growth in SSF is laborious and slightly accurate, mainly because of the difficulties in biomass/fermentation medium separation [6].

Some methodologies have been proposed as indirect measurements of biomass quantification, for example the production of primary metabolites [7], the variation in the electrical conductivity between biomass and the solid substrate [8] and the changes in the fermentation medium color determined by reflected light [9]. In most cases, the proposed methodology was compared to the glycosamine method, which is considered the classic method for biomass quantification. Within this method, the quitine, a monomer present on the cellular wall of fungi and yeast, is measured [10]. Nevertheless, this method can present errors due to the variation in the glycosamine content at different growth stages and to the low specificity of glycosamine quantification.

The use of software for digital image processing can be an alternative to the traditional chemical method, as it enables the correlation among the morphological biomass features and other variables of the fermentation process, mainly in submerged fermentation [11, 12]. However, a few works are found reporting the use of this methodology for biomass monitoring in solid-state fermentation [6, 13–16]. Recently, Couri et al. [6] showed that there is a correlation between the fungi growth area determined by image processing technique and the polygalacturonase activity produced by SSF.

The objective of the present work was to improve lipase production of *Aspergillus niger* under SSF conditions by supplementary carbon source addition and to verify whether the digital image processing can be used to monitor the biomass growth of *A. niger* 11T53A14. The methodology proposed in this work is similar to that presented by Couri et al. [6] and was based on the measurement of the hyphae area, which demanded that a contrast difference in the acquired image be obtained to separate the gray level image pixels in 0

(black) and 1 (white) [17]. In addition, the produced lipase was investigated as a biocatalyst in the alcoholysis of soybean oil with ethanol.

Experimental Methodology

Microorganism, Maintenance, and Propagation

A. niger 11T53A14, a mutant from the Embrapa Food Technology collection, was maintained on dry sand at −18°C, activated in basic agar slant, and propagated for a corn cob medium [18, 19].

Chemicals

The reagents employed were commercial soybean oil (Sadia, Brazil), analytical grade ethanol, butanol, oleic acid (extra pure), and hexane (Merck, Darmstadt, Germany). Methyl heptadecanoate (a chromatographic standard) was acquired from Sigma (St. Louis, USA). Sodium hydroxide and acetone were purchased from Vetec (Brazil).

Fermentation Mediums and Processes

Oil Selection

Initially, tests were performed to verify the best supplementary carbon source for lipase production, using the conditions described by Penha et al. [19]. The experiments were carried out in Erlenmeyer flasks (250 ml), and the fermentation medium consisted of 100 g of powdered wheat bran (60% moisture adjusted adding a 0.91% (*m*/*v*) ammonium sulfate solution, pH=7.0) and 2% *m*/*m* of vegetable oil (castor, soybean, olive, corn, and palm oils). The medium was mixed and sterilized at 1.0 atm for 15 min, and afterwards, inoculated with 10^7 spores/g substrate. All flasks were closed and incubated in a biochemical oxygen demand (BOD) environment, keeping the moisture and ventilation conditions constant, at 32°C for 96 h. Then, the samples were analyzed to determine the glycosamine content and the lipase activity.

Effect of Castor Oil Concentration in Lipase Production

Experiments were carried out using a SSF column reactor to enhance lipase production using castor oil as the carbon source. The experiments were carried out in glass columns (210 mm height and 22 mm diameter), filled with approximately 16.0 g of wheat bran and variable castor oil concentration (0, 2, and 4% *m*/*m*). The medium moisture was adjusted to 60% using the same procedures described earlier. The columns were inoculated with a suspension of 10^7 spores/g of substrate, incubated in a thermostatic bath at 32°C, and aerated with saturated air at a rate of 4 l/h. The experiment lasted 96 h and was monitored every 24 h, for determining the glycosamine content and the lipase activity.

Biomass Growth Monitoring

For the monitoring of fungi growth using image processing techniques, biomass was grown in Petri plates containing 16.0 g of wheat bran (60% moisture) enriched with 2%

m/*m* of castor oil. Twenty of these plates were then inoculated with 10^7 spores/g, and five plates were reserved to be used as the blank test of the fermentation process. The plates were covered, and the experiments took place at 32°C in a BOD environment, for 96 h, with moisture and ventilation conditions kept constant. All the samples had their images acquired every 24 h for image processing procedures, and simultaneously, a group of five plates was removed for determination of the glycosamine content and the lipase activity.

Glycosamine Determination

The content of glycosamine was determined by the method revised by Penman et al. [20], in which a solution composed of 5 ml of the dried fermented medium previously digested in 70% *v*/*v* sulfuric acid for 24 h and sterilized for 1 h at 120°C and 1 atm, was neutralized and mixed with acetyl acetone, ethanol and *p*-dimethylaminobenzaldehide. After 45 min, the reaction was completed, and the absorbance data could be measured by using a Perkin-Elmer Lambda 10 spectrophotometer, at 530 nm.

Lipase Activity Assay

Samples were drawn periodically during the fermentation, and the extracellular lipase activity was analyzed using an olive emulsion method [21]. The fatty acids released were determined by titration with 0.05 M NaOH. One lipase activity unit (U_h) was defined as the enzyme required to release 1 μmol of fatty acid per minute at 35°C, pH=7.0.

Image Acquisition and Processing

At each fermentation time, one image was acquired from all the Petri plates. The first group of images obtained at 0 h fermentation was achieved by applying simple random sampling. After this fermentation time, all image samples were acquired from the same place to permit the further subtraction of 0 h data. All the images were attained using a stereomicroscope Carl Zeiss STEMI 2000-CS, coupled to a digital camera Sony Cyber Shot DSC-S75, at the same conditions described by Couri et al. [6] that are focused and with high illumination. Afterwards, all images were processed using the software KS400®, release 2.0 (Kontron Electronic GMB). Morphological data were obtained by applying math operations, binary mask, and filters (both linear and nonlinear ones) [17]. Figure 1 presents an example of the image sequence produced by using the procedures of the image processing for a sample at 96 h of fermentation. In the developed subroutine, Fig. 1a was the original acquired image, showing the hyphae and the wheat bran substrate mixed. The hyphae boundaries were then enhanced by using a high-pass filter (Fig. 1b). Then, a fixed threshold was applied to this image to separate the gray levels in black (the substrate) and white (the hyphae area). Finally, a pruning tool was applied to remove the debris and reach the final image to be measured. All the acquired images were submitted to the same subroutine.

Statistical Analysis

The biomass growth and the lipase activity results were statistically evaluated using the software Statistica (Windows release 6.0). As the data did not present normal distribution, nonparametric statistic was used. Therefore, the Kruskal–Wallis test was performed to

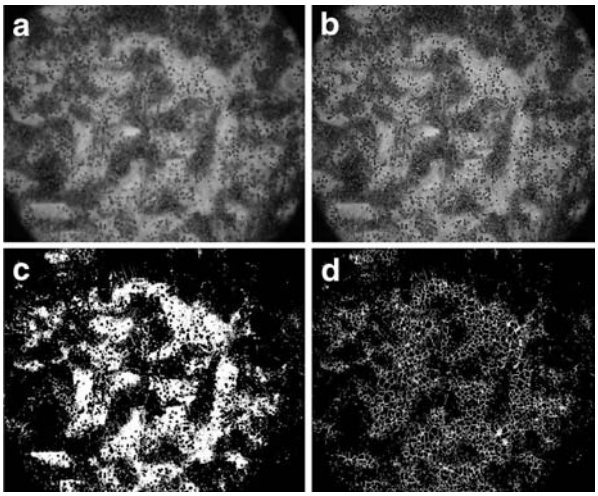

Fig. 1 Example of processing stages used to evaluate the images of biomass growth from solid-state fermentation (experimental condition: 24 h of fermentation): **a** acquired image; **b** a high-pass filter is applied; **c** a limited threshold is applied, and **d** debris are removed to reach the final image

evaluate all groups, while Mann–Whitney test was used to detect any differences between the groups. Parameters were considered statistically significant whenever the statistical analysis resulted in a p value less than 0.05. The experimental curves were fit using the software Microcal Origin® (Windows release 6.0).

Lipase Immobilization

The enzymatic extract produced was centrifuged, and the supernatant was dried by lyophilization. This dry product was solubilized in phosphate buffer (0.05 M, pH 7.0) and submitted to preferential immobilization by physical adsorption on hydrophobic support (Accurel® MP1000) according to Oliveira et al. [22].

Lipase Esterification Activity

The esterification activity of immobilized lipase was measured by the consumption of oleic acid at 45°C in the esterification reaction with butanol (equimolar ratio) with 3% *m*/*m* enzyme. One lipase activity unit (U_e) was defined as the amount of enzyme necessary to consume 1 μmol of oleic acid per minute under assay conditions. The enzyme activity was also evaluated in a reaction medium containing 50% *v*/*v* of hexane.

Lipase Catalyzed Transesterification (Biodiesel Synthesis)

The transesterification reactions between soybean oil and ethanol using 7% *m*/*m* of immobilized lipase at 40°C were conducted in closed 15 ml batch reactors, with constant mechanical stirring, coupled to condensers to avoid alcohol loss by volatilization. The water circulating in the condenser was cooled by a thermostatic bath. The reaction temperature was kept constant by circulating ethylene glycol from a thermostatic bath (Haake DC10) into the reactor's jacket. Reaction progress was monitored by taking duplicate samples (50 μl), which were diluted in hexane and mixed with methyl heptadecanoate as an internal standard before analysis by gas chromatography.

Chromatography Analysis

The samples were injected into a Varian gas chromatograph (CP-3380 model), equipped with a flame ionization detector (FID) and a CP WAX 52 CB capillary column 30 m×0.25 mm×0.25 μm, and a split injection system with a 1:20 ratio. Injector and detector temperatures were kept at 250°C. The oven was initially maintained at 200°C for 4.5 min, then heated up to 210°C, and kept constant at this temperature for 0.5 min. After that, it was heated to 220°C for 0.5 min. The oven was heated again to 250°C and maintained at this temperature for 1.5 min. Hydrogen was used as the carrier gas at a 1.8 ml/min flow rate; column pressure was set at 12 psi. A computer loaded with the Star Workstation 6.2 software was connected to the GC by a Star 800 Module Interface to automatically integrate the peaks obtained. Methyl heptadecanoate was the internal standard used.

Results and Discussion

Carbon Source Evaluation

Lipidic carbon sources (as vegetable oils) seem to be essential for obtaining high lipase yield in fermentation process due to their potential inducing ability [23–25]. Dalmau et al. [24] related that the mechanisms regulating biosynthesis vary widely in different microorganisms. Results obtained by these authors with *Aspergillus* showed that lipase production seems to be constitutive and independent of the addition of lipidic substrates to the culture medium, although their presence enhanced the level of lipase activity produced.

To study the ability of different supplementary carbon sources to enhance the lipase production, several kinds of vegetable oils (castor, soybean, olive, corn, and palm oils) were fed at 2% *m*/*m* to the fermentation process (Fig. 2). In the experimental conditions used, castor oil presented the best results, reaching lipase activity of 23.7 U/ml.

Rodriguez et al. [5] studied the effect of different triglycerides (olive, sunflower, corn, peanut walnut, and grape seed oils) on the production of lipases by culturing *Rhizopus homothallicus* in SSF. They observed that this fungal strain was able to produce similar high lipase activities with all studied oils. This is quite important as it proved that the

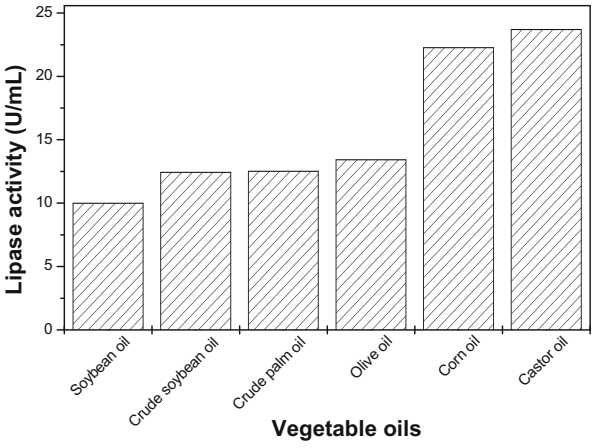

Fig. 2 Effect of feeding different vegetable oils as supplementary carbon sources (2% *m*/*m*) on lipase production by culturing *Aspergillus niger* 11T53A14 in SSF

cheapest and/or available oil could be used as a convenient carbon source for industrial scale lipase production.

Castor oil is largely produced in Brazil. Moreover, according to Fig. 2, the highest lipase activity was obtained with this vegetable oil. Therefore, different levels of this supplementary carbon source (0, 2, and 4% *m*/*m*) were added to respective batches of fermentation medium. As shown in Fig. 3, maximum lipase activity was obtained with 2% *m*/*m* of castor oil. This result is in agreement with that of Gombert et al. [26], who also studied the effects of different supplementary carbon sources concentration on lipase activity. They have shown that the best condition was obtained when 2% *m*/*m* of olive oil was added.

On the other hand, at the highest castor oil concentration studied (4% *m*/*m*), a significant drop in lipase activity was observed. Probably, the lipase production was repressed by the continuous release of fatty acid due to the castor oil hydrolysis in the fermentation broth. Li et al. [23] observed the inducing effect of oleic acid in lipase production and showed that when oleic acid concentration was higher than 0.4% *m*/*m*, it repressed lipase production.

Monitoring Biomass Growth in SSF

Design and operation of a fermentation process could be improved by using methodologies for biomass estimation, which permit to investigate the relationship among growth kinetic, biomass morphology, and the fermentation product. Biomass growth in SSF can be usually measured by indirect methods, as the variation in glycosamine content [19] or the production of a metabolite [7], in our case, the production of lipase.

The methodology proposed in this work was based on the area of the hyphae determined by image processing technique. For the sampling and image process procedures, it has been considered that the fungi grew as an isotropic interconnected system of tubules or fibers, surrounded by the fermentation medium. As the practical sections were very thick compared to the tubular diameter, the magnitude of the bias due to incorrect handling of any edge effect can be probably considered unimportant [27]. The results are shown in Fig. 4a. The proposed methodology was able to detect a significant growth trend during the SSF experiment ($p<0.003$) and a significant growth ($p<0.05$) between the samples at 24 and 48 h of fermentation. However, no significant difference could be noted among the other analyzed groups. Similar methodology used by Couri et al. [6], also detected a

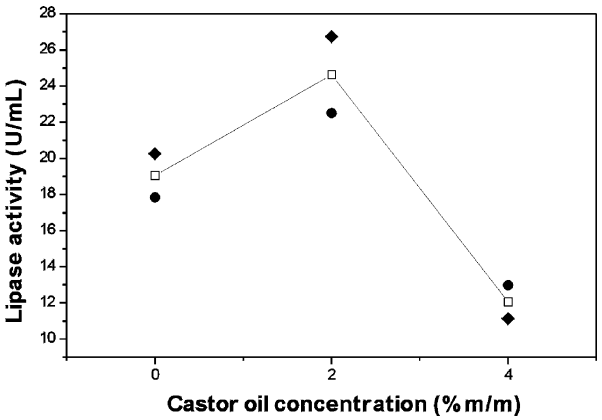

Fig. 3 Effect of castor oil concentration (% *m*/*m*) on lipase production by culturing *Aspergillus niger* 11T53A14 in SSF. *Filled circles*—experiment 1; *filled diamonds*—experiment 2; *empty squares*—average result

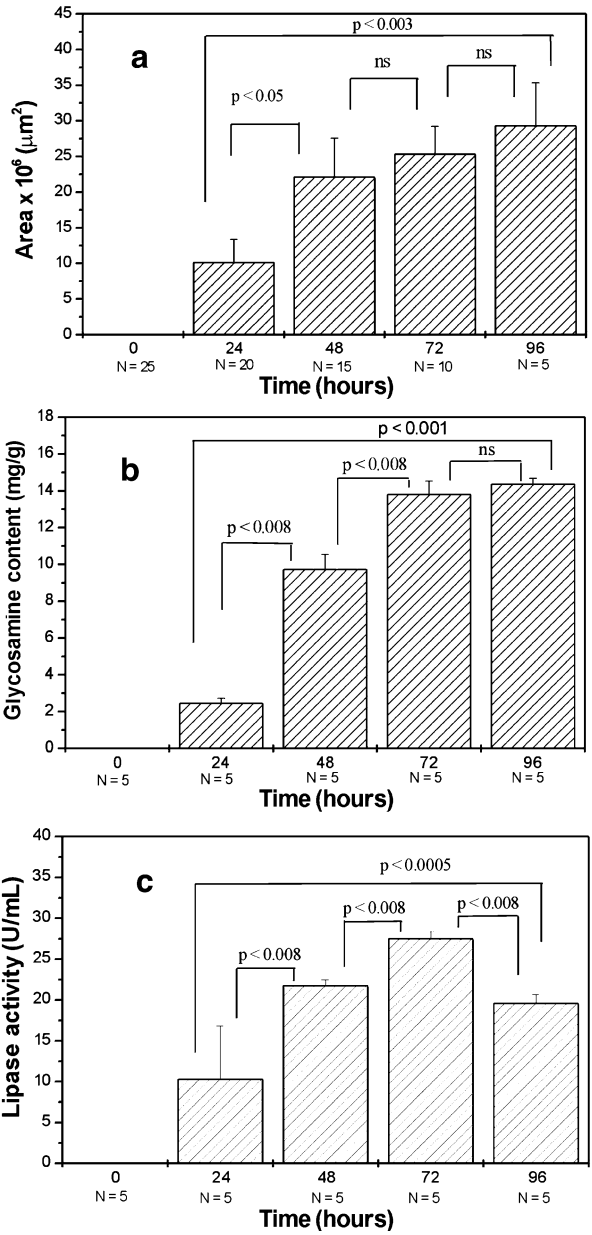

Fig. 4 Statistical results of the biomass growth and the enzyme production in solid-state fermentation: **a** Biomass growth measured by the digital image processing; **b** Biomass growth measured by the glycosamine method; **c** Lipase activity

significant growth trend ($p<0.000007$) and a significant difference ($p<0.003$) between zero and 24 h of fermentation, during the SSF production of polygalacturonase. Conversely, in the present work, there was no area measured in the initial point of fermentation. As reported by Couri et al. [6], a contrast difference is demanded in the digital processing technique, to separate the image pixels in 0 (black) and 1 (white), during the binary operation. As the wheat bran is a nonhomogeneous surface, a contrast difference between the grains in the bottom and at the surface of the Petri plate exists, causing the measurement

of some "data", even though no real hyphae is present. Therefore, an amount of area is always measured by the subroutine at 0 h fermentation, even though no growth occurred. This problem was avoided in the present work by acquiring the images from the same region of the Petri plate, so the blank value (0 h) could be discounted. Alternatively, the mathematical algorithm used for image processing was improved for 0 h fermentation, during the stage where a limited threshold is applied.

The variation of the glycosamine content within the fermentation time is shown in Fig. 4b. As it is widely known that the colorimetric methodology used to quantify the glycosamine in the fungi cell wall can also determinate other glycolized substances in the vegetal tissue, such as those present in the wheat bran [6, 19], in this analysis, the data obtained from the non-fermented medium (blank) have been previously discounted to avoid any interference in the results presented in this figure. The observed profile shows an intense growth up to 72 h, followed by a stationary stage after this fermentation time. There is a significant growing trend ($p<0.001$) in the glycosamine content during the SSF process and between the results obtained for 24 and 48 h ($p<0.008$) and 48 and 72 h ($p<0.008$) of fermentation. It suggests that at 72 h, the nutrient source was probably exhausted. Furthermore, knowing that the solid-state medium is a stress fermentation condition after the high enzyme production range, the microorganism can either keep its mass constant or overcome lyses processes.

The lipase activity varied significantly from 24 to 96 h of fermentation ($p<0.0005$), as shown in Fig. 4c. Moreover, there were also significant differences between 24 and 48 h ($p<0.008$), 48 and 72 h ($p<0.008$), and 72 and 96 h ($p<0.008$) of fermentation. It can be observed that the maximum lipase activity occurred at 72 h, followed by a decrease at 96 h of fermentation. Couri et al. [28] have shown similar effect for SSF production of polygalacturonase using *A. niger* 3T5B8. The authors related this effect to the possibility of protease production after the maximum point of enzyme activity, which could probably cause hydrolysis of the enzyme and decrease its activity.

It is interesting to note that this maximum was not observed for the results of glycosamine analysis or digital image processing (Fig. 4a and b). In the present work, images observed from 48 h on also presented spores on the surface, which were detected and also measured by the applied subroutine. The amount of these spores increased quickly from 72 to 96 h, probably by the substrate exhaustion, resulting in the area increase noted in the end of the experiment. Therefore, an enhancement of the method is needed to avoid the spore counting during the image processing.

Comparison Among the Biomass Quantification Methods

The determination of glycosamine content is the methodology usually used for comparison when a new biomass quantifying method is proposed. To verify if the digital image processing methodology could be applied for quantifying the microbial growth, its response to this parameter was then compared with the one produced by the glycosamine method. The results from both image and glycosamine experiments were plotted against each other to verify if any correlation between the methods would exist (Fig. 5). The correlation coefficient was higher than 0.97. Using a methodology similar to the one proposed in this study, Couri et al. [6] obtained a lower correlation coefficient ($R\approx0.86$) for comparison between the image processing and glycosamine results. The authors suggested that a high number of experiments was necessary to increase this correlation. In this work, the image process subroutine proposed also counted the spores' areas as hyphae, which could result in a higher difference between the analyses. However, it seems that this effect did not

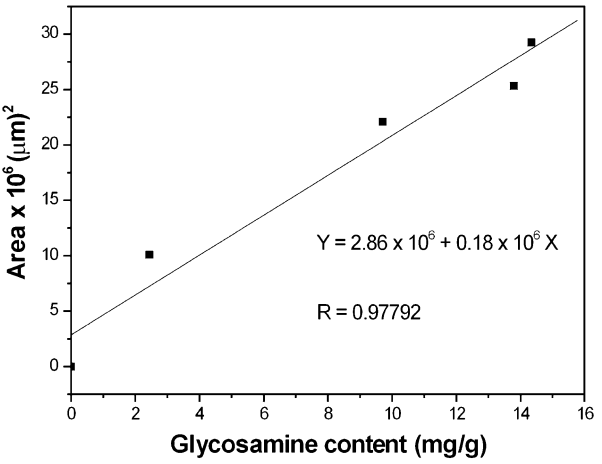

Fig. 5 Correlation between the digital processing analysis and the glycosamine methodologies

influence the correlation between the methods, as a high correlation coefficient value was obtained. The results suggest that the subtraction of the zero fermentation data may have enhanced the quality of the data.

The production of a primary metabolite, such as the lipase enzyme (whose production occurred during the same fermentation time of the biomass growth) can be related to the microbial growth [7]. Therefore, in the SSF experiments used in this work, the activity of lipase was also used as a reference to verify how efficient the applied methodologies to quantify the biomass growth during the process were. Therefore, a correlation between the enzyme activity and the results obtained for both image processing and glycosamine analysis is presented in Fig. 6. It can be observed that both curves present a correlation coefficient above 0.9, showing a direct and linear relationship between the two methodologies used to indirectly determine the biomass content and the enzyme activity.

Lipase Esterification Activity

One of the restrictions associated to the enzymatic route to biodiesel production is the cost of enzyme. So it is important to produce a lipase with lower cost and that it works out well

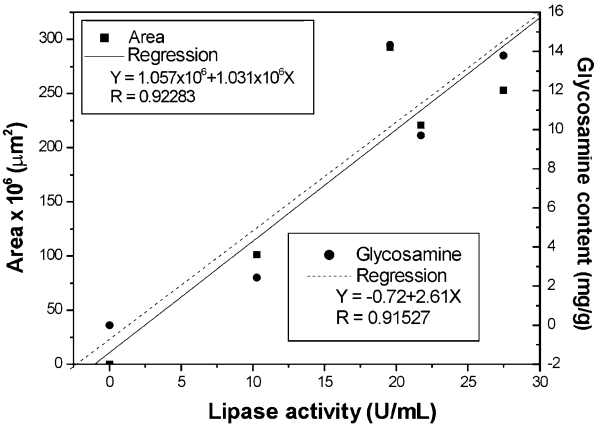

Fig. 6 Correlation between the lipase activity and the biomass contents indirectly measured by the glycosamine content and the digital image processing

for catalyzing the transesterification of vegetable oils with alcohols. The immobilized enzymes are generally known to give better catalytic performance in nonaqueous media. Therefore, in this work, the immobilization of the enzymatic extract obtained in SSF was performed. The carrier with enzyme was assayed for lipase esterification activity as described previously. The enzyme activity was determined by the initial reaction velocity in butyl oleate synthesis in a solvent-free system and in a reaction medium containing 50% *v*/*v* hexane, the values obtained were 120 and 650 U/g, respectively. As demonstrated by these results, the immobilized lipase produced by *A. niger* 11T53A14 presents considerable esterification activity. The commercial immobilized lipase Lipozyme RM-IM (Novozyme A/S) has an esterification activity of 2,250 U/g approximately, under the same conditions.

The time course curves of oleic acid consumption with immobilized lipase are shown in Fig. 7, and it can be seen that the fatty acid conversion was higher in the presence of hexane 50% *v*/*v*. At 45 min of reaction, oleic acid conversion in a solvent-free system was negligible; otherwise in a medium containing hexane 50% (*v*/*v*), the maximum conversion (approximately 30%) was observed.

The use of organic solvents in synthesis catalyzed by lipase has been reported. The nature of the solvent influences the activity and stability of the enzyme to a large extent. The polarity of the solvent plays a key role [29]. Log *P* has often been used to characterize solvents. Log *P* is defined as the logarithm of the partition coefficient of a substrate in the standard 1-octanol–water two-phase system. Normally, solvents with high log *P* values (log $P>4$, hydrophobic solvents) cause less inactivation of biocatalysts than more hydrophilic solvents [29]. The lipases have higher stability in a hydrophobic organic medium, such as hexane (log $P=3.5$).

According to the results shown in Fig. 7, in a solvent-free system, the enzyme drastically loses its activity after 30 min of reaction, indicating that the presence of hexane enhanced lipase stability.

Lipase Catalyzed Transesterification

As both methyl and ethyl alkyl esters are considered biodiesel, the enzymatic transesterification of soybean oil with ethanol was studied in this work. In Brazil, the production of ethyl esters is a sustainable technology, as ethanol can be easily produced from fermentable sugar (biomass).

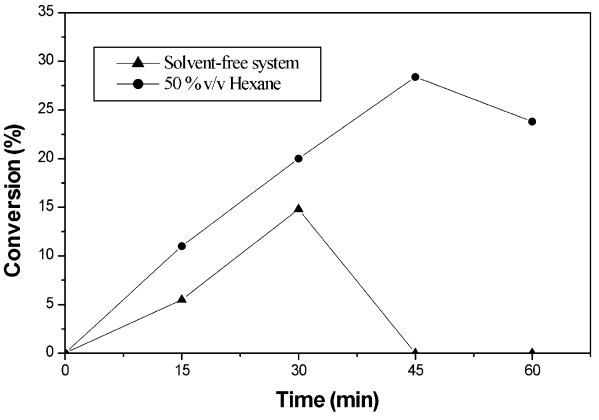

Fig. 7 Effect of hexane addition on oleic acid enzymatic conversion. Reaction conditions: oleic acid/butanol molar ratio of 1, with 3% (*m*/*m*) immobilized lipase from *A. niger* 11T53A14, at 45°C

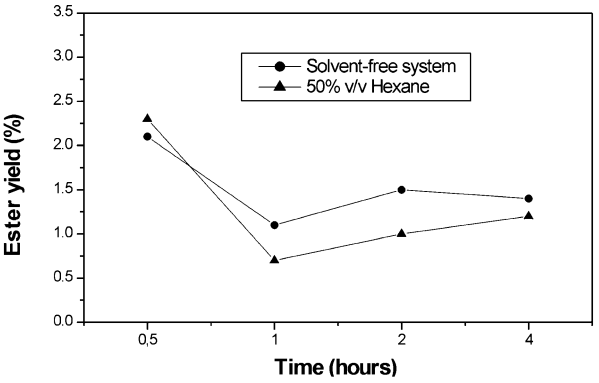

Fig. 8 Effect of hexane addition on the transesterification of soybean oil using an ethanol/soybean oil molar ration of 3 (stepwise ethanol addition: 1/3 at 0 h, 1/3 after 0.5 h, and 1/3 after 1 h), 7% *m*/*m* immobilized lipase from *A. niger* 11T53A14, at 40°C

The use of organic solvents is not indicated for biodiesel production due to the high risk of explosion and to the need of an additional step for solvent removal [30]. On the other hand, immobilized lipases show high conversion rates in nonpolar organic solvents. Therefore, there are several studies of triglyceride enzymatic alcoholysis in organic solvents [31]. Then, the effect of the addition of 50% *v*/*v* hexane was evaluated on the transesterification reaction of soybean oil with ethanol, at 40°C, with 7% *m*/*m* immobilized lipase and with the reactants' stoichiometric ratio (ethanol/soybean oil molar ratio of 3). The stepwise addition of ethanol (three consecutive steps) was used to avoid the lipase deactivation by a high initial alcohol concentration. However, the results (Fig. 8) show that even in these conditions, the yield of biodiesel obtained was only 2.4% after 30 min of reaction. This result is not in agreement with Bernardes et al. [32], who obtained yields of biodiesel higher than 50% using 7% *m*/*m* of commercial immobilized lipase (Lipozyme RM IM, Novozymes A/S) under similar reaction conditions (temperature of 50°C, with the stepwise addition of ethanol in three steps and using ethanol/soybean oil molar ratio of 3). These authors also evaluated the effect of the addition of 50% *v*/*v* hexane on the transesterification reaction of soybean oil with ethanol and verified that the use of hexane enhanced the biodiesel production. Therefore, the Fig. 8 results indicated that immobilized lipase produced by *A. niger* 11T53A14 is not suitable for transesterification reactions.

Conclusions

Lipase was produced by *A. niger* 11T53A14 using SSF process, using wheat bran as support. Several vegetable oils were tested as supplementary carbon sources, and the maximum lipase activity was achieved using castor oil. The best condition for producing the enzyme was evaluated in a column reactor, and it was obtained using 2% *m*/*m* of castor oil.

The results show that digital image process may be a promising tool to indirectly estimate of the biomass produced in SSF, by measuring the hyphae area in the acquired image. The correlation between the proposed methodology and the glycosamine determination was high and can be considered satisfactory, concerning the measurement of biological data. Moreover, the correlation between the proposed image processing methodology and the lipase activity was higher than 0.9, which agrees that the measured parameter (hyphae areas) might be applied to monitor the fungi growth at solid-state medium.

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