**Medium composition and Amazonian oils for lipase production by *Candida viswanathii***

**Composição do meio de cultivo e óleos amazônico para a produção de lipase por *Candida viswanathii***

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**Key words:** carbon sources; enzyme production; fermentation parameters; triacylglycerol hydrolase

**Running title:** Amazonian plant oil for enzyme production

**ABSTRACT**

Lipases are successfully produced under submerged cultivation using olive oil as carbon sources and complex culture media. The aim of this work was to evaluate different media culture composition and to replace olive oil by plant oils from Amazonian region to produce lipase by *Candida viswanathii* under submerged cultivations. *C. viswanathii* was capable to produce high-level of biomass and lipase using little nutritional requirements, such as potassium, magnesium and yeast extract with olive oil as sole carbon source. Plant oils from Amazonian region were capable to support the cell growth, but pequi oil was found the best inducer for lipase production (22.5±1.3 U), which presented YP/S=56.15 U/g, YP/X=72.00 U/g, PL=15.60 U/h and qL=1.00 U/g/h. Organic nitrogen source induced the highest levels of lipase production compared to inorganic sources. Typical microbial growth curves and lipase production were also observed with high lipase production (35.23±2.3 U) after 60 h with peptone as nitrogen source, presenting YP/S=88.00 U/g, YX/S=70.43 U/g, PL=29.35 U/h and qL=1.17 U/g/h. Pequi oil is a potential carbon source for lipase production by *C. viswanathii* which, in turn, can be used as biocatalyst for oil modification in food and cosmetic industries.

Key words: carbon sources, enzyme production, fermentation parameters

**RESUMO**

As lipases são produzidas com sucesso sob cultivo submerso utilizando azeite como fontes de carbono e meios de cultura complexos. O objetivo deste trabalho foi avaliar a composição da cultura de diferentes meios e substituir o azeite por óleos vegetais da região amazônica para produzir lipase por *Candida viswanathii* sob cultivo submerso. *C. viswanathii* foi capaz de produzir alto nível de biomassa e lipase usando pequenas necessidades nutricionais, como potássio, magnésio e extrato de levedura com azeite como única fonte de carbono. Os óleos vegetais da região amazônica foram capazes de suportar o crescimento celular, mas o óleo de pequi foi considerado o melhor indutor para a produção de lipase (22,5±1,3 U), que apresentou YP/S=56,15 U/g, YP/X=72,00 U/g, PL=15,60 U/h e qL=1,00 U/g/h. A fonte de nitrogênio orgânico induziu os níveis mais altos de produção de lipase em comparação com fontes inorgânicas. As curvas típicas de crescimento microbiano ea produção de lipase também foram observadas com alta produção de lipases (35,23±2,3 U) após 60 h com peptona como fonte de nitrogênio, apresentando YP/S=88,00 U/g, YX/S=70,43 U/g, PL=29,35 U/h e qL=1,17 U/g/h. O óleo de pequi é uma fonte potencial de carbono para a produção de lipase por *C. viswanathii* que, por sua vez, pode ser usado como biocatalisador para modificação de óleo em indústrias de alimentos e cosméticos.

Palavras-chave: fonte de carbono, produção de enzimas, parâmetros fermentação

**INTRODUCTION**

Lipases (E.C. 3.1.1.3, triacylglycerol acyl hydrolase) are defined as enzymes which hydrolyze long-chain fatty acids esters linkage in triacylglycerol into diacylglycerol, monoacylglycerol, fatty acids and glycerol under aqueous conditions. Other enzymes, e.g. esterases and cutinases, may also break short-chain fatty acids ester bonds in triacylglycerols soluble in aqueous systems. On the other hand, which distinguishes lipases from these enzymes is their ability to identify an insoluble substrate and having its activity directly correlated with the total substrate area and not with the substrate concentration (Fojan, Jonson, Petersen & Petersen, 2000). Under certain conditions, they are also able to catalyze synthetic reactions such as acidolysis, alcoholysis, aminolysis, esterification and inter-esterification. Lipases are considered to have great potential as biocatalysts in numerous industrial processes, including synthesis of food ingredients, additives to detergents and to obtain enantiopure drugs and other refined products (Contesini, Lopes, Macedo, Nascimento & Carvalho, 2010).

Approximately 90% of all industrial biocatalysts are produced by submerged cultivation using specifically optimized conditions and genetically manipulated microorganisms. Increasing lipase production during the fermentation process is also an important step in industrial application. Various methods were used to optimize the fermentation process to enhance production of lipase. However, few efforts have been made to improve the fermentation process by searching the variation of medium components and interactions among them during the cultivation process (Açikel, Ersana, Açikel, 2010). The medium composition is a critical factor in submerged conditions and studies have been undertaken to define the optimal culture and nutritional requirements for lipase production, such as type and concentrations of carbon and nitrogen sources, the culture pH, the growth temperature, and dissolved oxygen concentration (SHARMA et al., 2001).

Lipidic carbon sources seem to be generally essential for obtaining a high lipase yield. Oleic acid is regarded as one of the best inducers. Olive oil is composed by 80% of oleic acid in total fatty acid and is considered a standard carbon sources for lipase production (Sharma, Chisti, & Banerjee, 2001; Wei, Zhang, & Song., 2004). Alternative carbon sources for lipase production include palm oil, crambe oil, sesame oil, corn oil, beef tallow, and oil cakes from agroindustrial wastes. Oils from Amazonian region has high levels of monounsaturated fatty acids, tocopherol and carotenoids which have functional importance such as antioxidant, anti-inflammatory and antimicrobial activities and exhibited good digestibility (Silva , 2009; Che Man, & Marina, 2006; Parfene, Horincar, Tyagi, Malik & Bahrim, 2013). The use of whole-cell biocatalyst is attractive alternative to the modification of these plant oils with potential uses in pharmaceutical and cosmetic industries. In this study, lipase production by *Candida viswanathii* was evaluated in different media composition and with oils from Amazonian region as sole carbon sources, such as the nitrogen sources and fermentation parameters.

**MATERIALS AND METHODS**

***Microorganism and inoculum***

*C. viswanathii* strain is available in the laboratory of Biotechnology, Food Analysis and Products Purification, Habite – Biotechnology-based Company Incubator, Federal University of Tocantins, Gurupi, TO, Brazil. *C. viswanathii* was cultivated on malt extract agar (MEA) for 3 days, at 28oC, for inoculum preparation. Submerged cultivations were inoculated with 1.0 mL of cells suspension containing 1.0 × 107 cells/mL.

***Submerged cultivation and selection of medium culture***

Cultures were performed in Erlenmeyer flasks (125 mL) containing 20 mL of culture medium solution supplemented with olive oil (2%, w/w) as initial carbon/inducer source to lipase production and cell growth. Six media cultures formulations were used for *C. viswanathii* growth and lipase production, as following (g/L): Medium 1: NaH2PO4 12,0; KH2PO4 2,0; yeast extract 10, CaCl2•2H2O 0,03; ZnSO4.7H2O 0,03; FeSO4.7H2O 0,005 (Ülker, Özel, Çolak & Alpay Karaoğlu., 2011); Medium 2: Yeast extract 1.0, meat peptone 20.0, NaCl 5.0 (Freire, Teles, Bon, & Sant'anna Jr., 1997); Medium 3: K2HPO4 4.0, MgSO4.7H2O 1.0, yeast extract 5.0, Peptone 10.0 (Papagora, Roukas, & Kotzekidou, 2013); Medium 4: K2HPO4 5.5, KHPO4 15.0, MgSO4.7H2O 0.5, yeast extract 10.0 (Açikel et al., 2010); Medium 5: K2HPO4 5.5, KHPO4 15.0, MgSO4.7H2O 0.5, NaCl 0.1, CaCl2 0.1, (NH4)2SO4 5, yeast extract 10.0, micronutrient solution 1 ml\* (Dalmau, Montesinos, Lotti & Casas, 2000); Medium 6: Na2HPO4 6.0, KHPO4 15.0, MgSO4.7H2O 1.0, (NH4)2SO4 6.0, FeCl3.6H2O 0.010, urea 4, biotin 0.5 ml, micronutrient solution 1 ml\* (Wei, Zhang & Song, 2004).

Micronutrient solution: C6H8O7 5.0g, ZnSO4.7H2O 5.0g, Fe(NH4)2(SO4)2.6H2O 1.0g, CuSO4.5H2O 0.25g, MnSO4.H2O 0.005g, H3BO3 0.005g, NaMo4.2H2O 0.005g, distilled water 100 ml.

Flasks containing liquid cultures were autoclaved at 121 oC, for 20 min. Cultures were carried out at 28 oC, pH 6.0, and 200 rpm for 72 h.

***Selection of vegetable oil***

Five vegetable oils, such as andiroba (*Carapa guianensis*), buriti (*Mauritia flexuosa*), castor (*Ricinus communis*), pequi (*Caryocar brasiliense*) or tucum (*Bactris setosa*) were evaluated for lipase production in substitution to olive oil. The cultures were prepared in Erlenmeyer flasks (125 ml) containing 20 mL medium previously selected, supplemented with 2.0% (w/v) vegetable oil, and pH adjusted to 6.0. The media were kept at 200 rpm, 28 °C for 72 h. The cultures were performed in triplicate.

***Selection of nitrogen source***

Nitrogen sources [meat peptone, yeast extract, soy protein, casein, urea, NH4Cl, or (NH4)2SO4] were added to the culture media at 1.0 % (w/v). The cultures were carried out in Erlenmeyer flasks (125 ml) containing 20 mL medium previously selected, supplemented with 2.0% (w/v) pequi oil, and pH adjusted to 6.0. The media were kept at 200 rpm, 28 °C for 72 hours. The cultures were performed in triplicate.

***Separation of biomass and protein extract***

Biomass was separated from the fermentation broth by filtration (membrane cellulose acetate 0.45 μm cut-off) and dried at 105 °C until constant weight. Cell free broth was used for lipase activity assays.

***Determination of residual olive oil***

The culture supernatant was transferred to Erlenmeyer flasks (125 mL) and acidified to pH 1.0 with concentrated sulfuric acid. Then, 10 mL of hexane were added to the samples, which were vigorously agitated and maintained in a separating funnel until the separation of organic and aqueous phases. The organic phase was oven-dried at 40 °C and the mass was measured in analytical balance.

***Lipase Activity Assay***

Lipase activity was assayed with p-nitrophenyl-palmitate (p-NPP) as substrate (Almeida, Tauk-Tornisielo & Carmona, 2013). p-NPP was first dissolved in 0.5 mL of dimethyl sulfoxide and then diluted to 0.5 mM with McIlvaine buffer pH 4.0 containing 0.5% Triton X-100. The hydrolysis of p-NPP was determined discontinuously at 40 oC by releasing p-nitrophenolate (p-NP). After 5 min of preincubation of 0.9 mL of this substrate solution in water bath, the reaction was started by addition of 0.1 mL of appropriately diluted sample. The reaction was stopped at different intervals by heat shock (90 oC, 1 min), followed by addition of 1 mL of saturated sodium tetraborate solution. The absorbance was measured at 410 nm and the activity was determined according to the standard curve carried out with p-nitrophenol (p-NP molar extinction coefficient: 1.72×104 M/cm-1). Controls were prepared without enzyme. One unit of enzyme activity was defined as the amount of enzyme that releases 1 𝜇mol of p-NP per mL per min.

***Fermentation parameters***

Biomass and lipase yield (*Y*):

$Y\_{X/S}=\frac{X\_{1}- X\_{0}}{S\_{0}- S\_{1}}$ Equation 1 $Y\_{P/S}=\frac{P\_{1}- P\_{0}}{S\_{0}- S\_{1}}$ Equation 2 $Y\_{P/X}=\frac{P\_{1}- P\_{0}}{X\_{1}- X\_{0}}$ Equation 3

where, *X* biomass (g/L); *L* lipase (U); *YX/S* biomass yield on consumed substrate (g/g); *YP/X* lipase yield on biomass produced ((U/g)

Productivity and specific rate

$P\_{X }=\frac{X\_{1}-X\_{0}}{t}$ Equation 4 $P\_{L }=\frac{P\_{1}-P\_{0}}{t}$ Equation 5 $q\_{L }=\frac{P\_{X/S}}{t}= \left(\frac{P\_{f}- P\_{0}}{X\_{f}-X\_{0}}\right)\* \frac{1}{t}$ Equation 6

*PX* biomass productivity (g/h); PL lipase productivity (U/h); qL specific rate of lipase production (U/g biomass/h)

***Statistical analysis***

The results were expressed as mean ± standard error of the mean and subjected to variance analysis, followed by Tukey test.

**RESULTS AND DISCUSSION**

Initially, the nutrient media compositions were evaluated for *C. viswanathii* growth and lipase production supplemented with 2.0% olive oil (Figure 1).



Figure 1. Effect of different medium composition on the lipase production (▬) and cell growth (■) of *C. viswanathii* in submerged cultivation. Culture conditions: cultivations were carried out using 2% olive oil, pH 6.0, 200 rpm, 72 h at 28 oC.

The highest lipase production and growth was observed with medium 4 (31.50 U and 38.83 g/L, respectively). The medium 4 was composed by K2HPO4 5.5 g/L, KHPO4 15.0 g/L, MgSO4.7H2O 0.5 g/L, yeast extract 10.0 g/L. This medium composition was used for lipase production by *Rhizopus delemar* using sunflower oil and Tween 80 as carbon sources.3 Intermediary value was observed with the medium 5 which presented the second highest lipase production and biomass (27.5 U and 35.7 g/L, respectively), followed by medium 6 that presented lipase production of 22.0 U and biomass of 30.9 g/L. The medium 5 was composed by K2HPO4 5.5 g/L, KHPO4 15.0 g/L, MgSO4.7H2O 0.5 g/L, NaCl 0.1 g/L, CaCl2 0.1 g/L, (NH4)2SO4 5 g/L, yeast extract 10.0 g/L, 1 ml micronutrient solution; since the medium 6 was composed by Na2HPO4 6.0 g/L, KHPO4 15.0 g/L, MgSO4.7H2O 1.0 g/L, (NH4)2SO4 6.0 g/L, FeCl3.6H2O 0.010 g/L, urea 4 g/L, biotin 0.5 ml, micronutrient solution 1 mL (Wei, Zhang, & Song., 2004) Low values of enzyme activity and growth were observed with the media 1, 2 and 3.

The parameters biomass yield on substrate consumed, biomass productivity, lipase yield on substrate consumed, lipase productivity, and specific rate of lipase production were calculated for all media compositions (Table 1). Medium 4 indicates that this nutrient composition is suitable for lipase production and biomass, showing *YP/S* = 81.35 U/g, *YX/S* = 2.01 g/g, *YP/X* = 40.52 U/g biomass, *PL* = 21.86 U/h, *PX* = 0.54 g/h and *qL* = 0.56 U/g.h. However, the composition of the medium 5 also presented fermentation parameters such as lipase yield on biomass produced (*YP/X* = 38.56), lipase productivity (*PL* = 19.11 U/h) and specific rate of lipase production (*qL* = 0.53 U/g.h).

Table 1. Fermentation parameters of biomass and lipase production by *C. viswanathii* using different medium composition.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Medium composition | *Y X/S* | *Y P/S* | *Y P/X* | *PL* | *PX* | *qL* |
| 1 | 1.47 | 23.93 | 16.32 | 6.38 | 0.39 | 0.23 |
| 2 | 1.56 | 12.52 | 8.04 | 3.33 | 0.41 | 0.11 |
| 3 | 1.66 | 37.23 | 21.34 | 9.84 | 0.43 | 0.29 |
| 4 | 2.01 | 81.35 | 40.52 | 21.86 | 0.54 | 0.56 |
| 5 | 1.81 | 69.72 | 38.56 | 19.11 | 0.49 | 0.53 |
| 6 | 1.60 | 56.85 | 35.51 | 15.27 | 0.43 | 0.49 |

Culture conditions: cultivations were performed with 2 % (w/v) olive oil, pH 6.0, 72 h, 200 rpm at 28 °C.

According to these results, *C. viswanathii* it showed capable to produce high-level of biomass and lipase activity using little nutritional requirements, such as potassium (K+), magnesium (Mg2+) and yeast extract. In the medium 5, the presence of sodium (Na+), calcium (Ca2+), ammonium (NH4)2+ and the addition of micronutrient solution promote a decrease of the lipase production and microbial growth. Mg2+ has been well related in the literature as important macronutrient for microbial growth and lipase stabilization (Sharma et al., 2001; Tan, Zhang, Xu, & Zhang, 2004). The addition of Ca2+ and Fe2+ inhibited lipase production from *Penicillium camembertii*, since these ions were reported to form complex with ionized fatty acid and then changed their solubility and behviour at the oil-water interfaces (Tan et al., 2004). Lipase production by *Pseudomonas gessardi* was slightly inhibited in the presence of Na+, Fe2+ and Mg2+, excepted for Ca2+ that presented stimulatory effect on lipase productivity (Ramani, Kennedy, Ramakrishnana, & Sekarana, 2010). In this sense, lipase production by *C. viswanathii* using minimal basal medium is an advantageous step for scale-up process of submerged fermentation, reducing the costs of enzyme production and facilitate the downstream processes used for isolation and characterization of the enzyme produced.

Pequi oil is composed by 65.5% of oleic acid and 34.5% by palmitic acid of the total fatty acids profile; while buriti oil is composed by 84.4% of oleic acid and 16.6% of palmitic acid (Ferreira et al., 2011). Oleic acid is considered the most important fatty acid present in triacylglycerol that induces the lipase production by several micro-organisms, including yeasts, bacteria and filamentous fungi (Sharma et al., 2001, Treichel, Oliveira, Mazutti, Luccio & Oliveira, 2010). The fatty acid composition and nutrition value present in pequi oil could provide the highest lipase production compared to buriti oil. Andiroba and tucum oils induced the intermediary levels of lipase production (~6.0 U), while castor oil induced the lowest lipase production of 2.5 U and biomass (13.2 g/L).



Figure 2. Effect of Amazonian oils on lipase production (▬) and growth (■) of *C. viswanathii*. Culture conditions: cultivations were carried out using 2% of each oil, pH 6.0, 200 rpm, 72 h at 28 oC.

The result found for lipase production with castor oil could be related to the anti-nutritional effect of the ricinoleic acid presenting 88.3% of the total fatty acids, an unsaturated omega-9 fatty acid called 12-hydroxy-9-cis-octadecenoic acid. The inhibition effect of unusual hydroxyl group linked to fatty acid in lipase production and microbial growth was also observed for *Candida rugosa* in submerged cultivation, using coconut oil that present the hydroxyl substituted C-14 fatty acid (Lakshmi, Kangueane, Abraham, & Pennathur, 1999). Fermentation parameters demonstrate that pequi oil is efficient in lipase production since the lipase yield on substrate consumed (*YP/S* = 62.84 U), lipase yield on biomass (*YP/X* = 82.62 U/g), lipase productivity (*PL* = 17.45 U/h) and specific rate of lipase production (*qL* = 0.83 U/g biomass.h) (Table 2).

Table 2. Fermentation parameters of biomass and lipase production by *C. viswanathii* using Amazonian oils.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Vegetable oil | Predominant Fatty acids (%) | Y X/S | Y P/S | Y P/X | PL | PX | qL |
| Andiroba | 16:1 (28.3); 18:1(49.8)  | 0.78 | 15.61 | 20.13 | 4.34 | 0.21 | 0.28 |
| Buriti | 16:1 (17.3); 18:1(73,3) | 0.83 | 42.19 | 50.74 | 11.72 | 0.23 | 0.70 |
| Pequi | 16:1 (38.8); 18:1 (56.0) | 0.78 | 62.84 | 80.62 | 17.45 | 0.21 | 0.83 |
| Tucum | 16:1 (22.9); 18:1 (67.6) | 0.66 | 15.10 | 22.92 | 4.20 | 0.18 | 0.32 |
| Castor | 18:1-ωOH (88.3) | 0.72 | 6.25 | 8.63 | 1.74 | 0.20 | 0.12 |

Culture conditions: cultivations were performed in medium 4 with 2 % (w/v) vegetable oils, pH 6.0, 72 h, 200 rpm at 28 °C.

Biomass production using the different vegetable oil did not present significant statistical difference (p<0.05), indicating that *C. viswanathii* could use these vegetable oil to grow, but these oils may present some inhibitor for lipase synthesis. In this sense, it is important highlight that this study related first-time the use of pequi oil for lipase production in submerged cultivation. The edible oil and fruits of pequi are used in cooking as well as in traditional medicine for the treatment of colds, coughs, bronchitis, edema and burns; and buriti oil has found applications in the cosmetic industry due to its emollient properties and can be used as adjuvant in sun protection (Ferreira et al., 2011). The use of these oils as carbon sources for lipase production and growth can indicate an important use of *C. viswanathii* lipase as effective biocatalyst when pequi or buriti oils is used as substrate.

Organic and inorganic nitrogen sources play an important role in the synthesis of enzymes, since inorganic nitrogen sources can be used quickly and organic sources can supply many cell growth factors and amino acids needed for cell metabolism and enzyme production .13 Different nitrogen sources are used in lipase fermentation. In this study, organic and inorganic nitrogen sources were evaluated at concentration of 1% (w/v) being them: yeast extract, meat peptone, soy protein, casein, urea, NH4Cl and (NH4)2SO4 (Figure 3). The culture media were supplemented with 2% (w/w) of pequi oil.

Among organic nitrogen sources, peptone and yeast extract supported the highest cell growth (16.8 g/L and 15.5 g/L, respectively) and the highest lipase productions (25.2 U and 28.4 U, respectively). Intermediary cell growths were observed with casein and soy protein (13.7 g/L and 12.6 g/L), however a negative effect on lipase production was observed. Urea provided the lowest growth (9.8 g/L) and lipase production (2.36 U). For other parameters analyzed using peptone, *C. viswanathii* presented high biomass production (*YX/S* = 1.31 g/g), lipase yield on substrate consumed (*YP/S* = 83.74 U/g), lipase productivity (*PL* = 19.80 U/h), biomass productivity (*PX* = 0.31 g/h) and specific rate of lipase production (0.89 U/g biomass.h) (Table 3). Inorganic nitrogen sources, such as NH4Cl and (NH4)2SO4, could support the *C. viswanathii* growth, but the lipase production using these sources also decreased.



Figure 3. Effect of nitrogen sources on lipase production (▬) and growth (■) of *C. viswanathii*. Culture conditions: cultivations were carried out using 2% of pequi oil, pH 6.0, 200 rpm, 72 h at 28 oC.

Organic nitrogen sources, such as meat peptone, soy peptone, casein, yeast extract, were related as suitable sources for lipase production with filamentous fungi and yeast (Tan et al. 2004). On the other hand, inorganic nitrogen source has been controversially related in the literature for lipase production. The production of lipase from an indigenously isolated marine *Aspergillus sydowii* was maximum using ammonium chloride at 3.5% (Bindiya, & Ramana, 2012) Tan, Zhang, Wang, Ying, & Deng, (2003) related that the highest lipase production by *Candida lipolytica* 90-17, isolated from soil from fish processing factory, was observed with ammonium sulfate. The medium supplementation with ammonium sulfate provide the highest lipase production by *Penicillium aurantiogriseum*; however, the lipase production with potassium nitrate was relatively low (Lima et al., 2003).

Table 3. Fermentation parameters of biomass and lipase production by *C. viswanathii* using nitrogen sources.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Nitrogen sources | YX/S | YP/S | YP/X | PL | PX | qL |
| Yeast extract | 0.78 | 62.84 | 80.62 | 17.45 | 0.21 | 0.83 |
| Peptone | 1.31 | 83.74 | 63.90 | 19.80 | 0.31 | 0.89 |
| Soy protein | 1.19 | 8.10 | 6.78 | 2.07 | 0.30 | 0.09 |
| Casein | 1.30 | 17.94 | 13.75 | 4.15 | 0.30 | 0.19 |
| Urea | 0.79 | 7.63 | 9.65 | 1.64 | 0.17 | 0.13 |
| NH4Cl | 0.69 | 13.20 | 19.15 | 2.83 | 0.15 | 0.27 |
| (NH4)2SO4 | 0.77 | 14.80 | 19.24 | 3.39 | 0.17 | 0.27 |

Culture conditions: cultivations were performed in medium 4 with 2 % (w/v) pequi oil, pH 6.0, 72 h, 200 rpm at 28 °C.

Mineral nitrogen sources showed no significant effect on both cell growth of *Yarrowia lipolytica* LgX64.81 and lipase production; by contrast, marked increase in lipase productivity has been observed upon addition of some organic nitrogen sources, e.g. tryptone (Fickers, Nicaud, Gaillardin, Destain, & Thonart, 2004). Among various inorganic nitrogen sources tested for lipase production by *Aspergillus terreus* none increased enzyme production significantly (Gulati, Saxena, & Gupta., 2000), and the concentration of sodium nitrate was further standardized and it was possible to decrease its concentration from 0.6 to 0.2% (w/v) without affecting enzyme production.

The time-course of lipase production and microbial growth were assayed for 96 h in medium with 2.0% (w/v) pequi oil and 1.0 % (w/v) peptone. The highest enzyme production was observed in 60 h cultures (35.23 U) (Figure 4). Fermentations parameters under this conditions were *YP/S* = 88.00 U/g, *YP/X* = 70.43 U/g, *PL* = 29.35 U/h, *YX/S* = 1.25 g/g, *PX* = 0.41g/h and *qL* = 1.173 U/g biomass.h. Typical microbial growth curves and lipase production were also observed. Lipase production was cell growth associated, and lipase production decreased in stationary phase.



Figure 4. Time-course of lipase production (■) and growth (●) of *C. viswanathii*. Culture conditions: medium 4, 2.0 % (w/v) pequi oil and, pH 6.0, 200 rpm at 28 ºC.

*C. viswanathii* cultivation showed that the medium composition influenced more the lipase production than the microbial growth. Potassium and magnesium showed be important nutrients for lipase production, while the presence of sodium, calcium, ammonium may act as inhibitor. Olive oil was replaced for oils from Amazonian region, among of them pequi oil presented the highest levels of lipase production and fermentation parameters after 60 h, when the fermentations were carried out with meat peptone as sole nitrogen source. These results suggest that pequi oil can be used as potential carbon source for lipase production and the *C. viswanathii* strain can be used as biocatalyst for oil modification in the food industry by use of the lipase produced under submerged conditions.

**CONCLUDING REMARKS**

*C. viswanathii* cultivation showed that the medium composition influenced mainly the lipase production than the microbial growth. The presence of potassium and magnesium showed an important nutrient for lipase production, while the presence of sodium, calcium, ammonium may act as inhibitor. Pequi oil was used as carbons source for growth and induced the highest levels of lipase production afterr 60 h when the cultures were carried out with meat peptone as sole nitrogen source. These results suggest that *C. viswanathii* strain can be used as biocatalyst for oil modification by use of the lipase produced under submerged conditions.

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