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Glycerol bioconversion in unconventional magnetically assisted bioreactor seeking whole cell biocatalyst (intracellular lipase) production

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ABSTRACT

A novel technology that consisted of a magnetically assisted bioreactor to produce biomass and intracellular lipase (whole cell) from *Yarrowia lipolytica* (*Y. lipolytica*) has been studied. Fermentations were carried out at 1.5; 4.5, and 9.0 kA/m in different experimental setup: a) in conventional fermenter inside magnetic field; b) in conventional fermenter with cellular suspension recycled through spiral-shaped tube under external magnetic field; and c) in conventional fermenter with cellular suspension recycled through U-shaped tube system under external magnetic field. Control experiments were also carried out for comparative purpose. Thus, the yeast showed a little change in the cell growth under magnetic field respect to the control, reaching just an increase of 6% at 1.5 kA/m, using the U-shaped tube and transversal direction of field lines. However, around 66.8 units activity/g for lipase production were obtained using a transverse orientation of the field lines, at 9.0 kA/m and recirculation through the U-shaped tube system, resulting in 30% higher than control experiment. These effects may be attributed to magnetic field action at the vicinity of the cell-substrate interfaces, which are related to both, the intracellular metabolism and the mass transfer from the substrate to the cell walls, explained by micro-level dynamo concept (MLD).

1. Introduction

The production and use of renewable fuels is an alternative to reduce the generation of greenhouse gases. In this context, biodiesel constitutes an attractive proposal to mitigate the effects that contribute to global warming. However, the use of residual glycerol is an important issue, due to its huge accumulation as a sub-product of the biodiesel production since it represents about 10–15% of the total mass of produced biodiesel. Thus, researches on new applications of the residual glycerol are a hot topic with results that might affect both, the economic viability of biodiesel production and its environmental impact. Consequently, glycerol has been used as raw material to produce more value-added products by chemical or biochemical methods, such as 1,3-propanediol (Aquino de Souza et al., 2014), hydrogen or syngas (Mangayil et al., 2015; Sarma et al., 2012; Siew et al., 2015), biomass (Taccari et al., 2012); animal feed (Leoneti et al., 2012), bioethanol

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(Adnan et al., 2014; Aquino de Souza et al., 2014); biogas and biomethane (Athanasoulia et al., 2014; Robra et al., 2010); several organic acids (Leoneti et al., 2012) and biobutanol (Yadav et al., 2014); among others. Thus, the present work refers to improve cells growth and intracellular lipase production by the yeast *Yarrowia lipolytica* (*Y. lipolytica*) in a magnetically assisted bioreactor using glycerol as carbon source. Therefore, the results herein reported can be important to develop a novel biodiesel integrated process considering the glycerin reuse as carbon source during fermentation process to obtain a biocatalyst based in a whole cell (intracellular lipase). Thus, this strategy can allow redefine the conventional chemical process of biodiesel production by enzymatic route using this biocatalyst.

Evidences about the interactions between extremely low frequency electromagnetic field (ELF-EMF) and/or static magnetic field with living systems have been reported (Hunt et al., 2009; Polk and Postow, 1996; Santini et al., 2009; Stavroulakis, 2003). Particularly, bioprocesses assisted by electromagnetic fields constitute a relatively new area of great interest in the biotechnology (Hristov, 2010), including fermentation processes (Alvarez et al., 2006; Hristov and Perez, 2011; Perez et al., 2007). These results relate in some cases the stimulation of cellular growth (Moore, 1979; Rodriguez Justo et al., 2006) or inhibition (Fojt et al., 2004; Moore, 1979; Novák et al., 2007; Rodriguez Justo et al., 2006), and/or alterations in the metabolites production (Alvarez et al., 2006; da Motta et al., 2004; Justo et al., 2007; Perez et al., 2007; Santos et al., 2012). In this context, the Table 1 shows the performance of several biotechnology processes under magnetic field just for comparative purpose. These biological effects have been observed principally as a function of microorganism type, culture conditions, and several operational parameters from magnetic field generator, such as field strength, frequency and exposure time, field lines direction, system geometry, among others.

In addition, *Y. lipolytica* it is a well-known yeast, considered by many authors as an "unconventional fungus," being a strictly aerobic microorganism from the kingdom Fungi (Amaral et al., 2006; Barth and Gaillardin, 1997). This yeast produces metabolites of great interest, as for example, lipases (Triacylglycerol hydrolases - EC 3.1.1.3) that are capable to hydrolyze triacylglycerol to form fatty acids and glycerol, and can also be used as a biocatalyst (whole cells) in biodiesel production and other industrial applications (Amaral et al., 2006; Fickers et al., 2005). In addition, this yeast has showed a great potential to use low cost substrates as carbon sources, such as n-alkanes, oils, fats, fatty acids, and glycerol in several applications (Darvishi Harzevili, 2014).

2. Materials and methods

2.1. Materials

The lyophilized strain of *Y. lipolytica* NRRL-1095 was obtained from ARS Culture Collection, USA. All chemicals and culture media used in this work were analytical grade and purchased from Sigma-Aldrich and Merck, respectively.

2.2. Fermentation procedure

2.2.1. Inoculum preparation

The culture of *Y. lipolytica* was carried out in a culture medium containing (g/L): yeast extract (3), malt extract (3), glucose (10), and peptone (5). The pH was adjusted to 7.0 with NaOH or HCl 2 mol/L. Then, 100 mL of culture medium were added to Erlenmeyer flasks (250 mL), autoclaved at 121 °C during 15 min. Further, culture media were inoculated by yeasts (10% v/v) and incubated on rotational shaker (Marconi Equipment for Laboratory Ltda., Brazil) at 28 °C and 200 rpm during 24 h. The cells number were determined in the Neubauer chamber using a Nikon microscope, model Eclipse E200 (Nikon Corporation), coupled to digital camera Moticam 1000 (Motic, Asia).

2.2.2. Fermentation

The culture medium for fermentation was the same used for pre-culture preparation. The fermentations were carried out in a VirTis Omni-Culture Fermenter (USA) with a working volume of 1 L and inoculated with 10% (v/v) of the pre-culture cells at 28 °C, 200 rpm, and 1 vvm. In the fermentation process glycerol (10 g/L) was used as carbon sources in despite of glucose. Olive oil (20 g/L) was also used as an inducer for the lipase production.

2.2.3. Experimental setup for fermentation assisted by electromagnetic field

The homogeneous magnetic fields assisting the fermentation were generated by square Helmholtz coils (Fig. 1) allowing various orientations of the field direction with respect to the vertical axis of the fermenter (VirTis Omni-Culture Fermenter, USA) i.e., from axial to transversal (Hristov, 1996). The coils are supported by a nonmagnetic (wooden) frame with a pendulum swing supporting the fermenter or other vessels where the cells suspension can be subjected to the magnetic field action. This system allows easily change the field lines direction by simple rotation of the coils since that the pendulum swing always stays vertical. The coils were energized by DC current supplied by a Greatz rectifier and controlled by a digital Ampermeter.

Several situations (see Fig. 1) were considered for electromagnetic treatment of the cellular suspensions during fermentation. As describe previously fermentation experiments were carried out in the fermenter (VirTis Omni-Culture Fermenter, USA) assisted by electromagnetic field, using a working volume of 1 L, inoculated with 10% (v/v) of the pre-culture cells at 28 °C, 200 rpm and under 1 vvm. Thus, the experimental setup considering different arrangements were: a) recycling the cellular suspensions through a spiral-shaped tube, external to the fermenter with a magnetic field parallel to the coil axis; b) recycling the cellular suspensions through a spiral-shaped tube with a transverse field to its axis; c) recycling the cellular suspensions through a U-shaped tube system with a magnetic field parallel to its axis of symmetry; d) recycling the cellular suspensions through a U-shaped tube system, but with a transverse magnetic field; e) fermentation carried out in a fermenter entirely encircled by the magnetic field, i.e., the vessel was placed inside the Helmholtz coils without external recirculation of the cellular suspensions.

Moreover, the magnetic field intensity and its distribution inside the Helmholtz coils were monitored by GM08 Gauss-meter (Hirst Magnetic Instruments Ltd., UK). Control experiments were carried out to evaluate the effect the external recirculation setup and also including, both, bioreactor and conventional shaker systems, on fermentation performance in absence of the electromagnetic field for comparative purpose.