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Biomass production by *Yarrowia lipolytica* as a source of lipids: bench scale cultivation on raw glycerol-based medium

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Abstract

This paper analyzes the aeration and agitation effects in growth parameters and lipid content for *Yarrowia lipolytica* NRRL YB-423 growing in a raw glycerol-based medium. A central composite design (2^2 assays plus three central points) was made, with combinations of agitation (100 to 300 rpm) and aeration (0.5 to 1.5 vvm). The resulting K_La values ranged from 2.25 to 16.83 h^{-1}. The most favorable condition was 200 rpm and 1 vvm (K_La of 8.29), producing 19.14 g L^{-1} biomass with a lipid content of 7.87%. In relation to fatty acid profile, polyunsaturated fatty acids prevailed (62.55%), achieving 49.16% linoleic acid (C18:2). Therefore, this yeast biomass has the potential to constitute a sustainable and renewable alternative as a source of essential fatty acids in food formulations for animals and as a raw material for the development of glycerol-based biorefineries for fuels and chemicals.

Introduction

Petroleum is the main energy source used in the world, but its availability is limited and the search for new renewable energy sources is a major priority. Biofuels, such as ethanol and biodiesel, are among the most promising sources for the substitution of fossil fuels (Silva *et al.*, 2009). In relation to biodiesel, a rapid expansion in production capacity is taking place around the world (Almeida *et al.*, 2012).

Biodiesel is produced by the transesterification of triglycerides with a monovalent alcohol, such as methanol and ethanol, into fatty acid alkyl esters. Glycerol is formed as a byproduct, and corresponds to 10% (w w^{-1}). However, this value refers to pure glycerol. The raw glycerol obtained from the biodiesel synthesis is usually 55–90% pure, containing other constituents such as unconverted triglycerides, unconverted methanol or ethanol, biodiesel and soaps. Therefore, this crude glycerol contains too many contaminants for a useful application in chemistry or pharmacy and a purification treatment is required (Amaral *et al*., 2009).

The current annual amount of glycerol from biodiesel production exceeds the world market for pure glycerol with high quality for industrial applications (chemical and pharmaceutical). Consequently, glycerol has become an important feedstock and an abundant renewable carbon source for microbial cultivation (Silva *et al*., 2009; Amaral *et al*., 2009).

Therefore, glycerol from biodiesel production would be a good alternative as a competitive substrate for microbial cultivation since it is a byproduct and, consequently, its price is much lower than traditional carbon sources, such as glucose, sucrose and starch (Silva *et al*., 2009). Moreover, glycerol bioconversion by chemical or biochemical routes adds significant value to the production chain of the biodiesel industry, resulting in greater competitiveness (Vlysidis *et al*., 2011; Schultz *et al*., 2014).

Biomass production as a source of nutrients in human foods or animal feeds comprises the production of dried cells of different microorganisms (algae, bacteria, molds and yeasts) in large scale culture systems. The production of yeast biomass is advantageous because of its nontoxic nature, low level of nucleic acid content and high productivity. Moreover, yeast biomass is important as a raw material for the food, pharmaceutical, cosmetic and other industries, in addition to being an excellent source of nutrients, such as protein and lipids, vitamins of the B complex and essential minerals (Caballero-Córdoba and Sgarbieri, 2000).

Among yeasts, the unconventional yeast *Yarrowia lipolytica* has received much attention due to its biotechnological potential and ability to metabolize several important industrial and agro-industrial

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byproducts (Makri *et al.*, 2010). According to Zinjarde (2014), value-added products can be obtained from *Y. lipolytica*, such as single cell oil, single cell protein (as food and feed), aroma compounds, organic acids, polyalcohols, emulsifiers and surfactants. *Y. lipolytica* has been used extensively for the production of single-cell oil (Papanikolau *et al*., 2008; Sestric *et al*., 2014; Poli *et al*., 2014) from glycerol, resulting in an alternative source of triacylglycerols for biodiesel production.

On the other hand, the study of operational conditions effects, such as temperature, medium composition, agitation and aeration, plays an important role in understanding microbial cultivation and can provide information for scale-up. Within this context, experimental design is an important tool for establishing optimal process conditions. This technique is advantageous compared to the conventional method, which handles a single parameter per assay. Various research workers have applied this technique, especially for the optimization of culture conditions (Manera *et al*., 2008; Rosa *et al*., 2010; Santos *et al*., 2012) and for the determination of optimal processing parameters (Burkert *et al.*, 2005; Alves *et al*., 2010). However, to the best of our knowledge, there are no data on the application of this tool simultaneously for agitation and aeration effects on the *Y. lipolytica* cultivation in the literature.

In a previous study (Santos *et al*,. 2012), a 2_v^{5-1} fractional design followed by a central composite design (CCD) was used in order to establish the medium composition for the yeast *Y. lipolytica* NRRL YB-423 growing on a raw glycerol-based medium in shaken flask cultivation in order to produce yeast biomass as a source of proteins. In the present work, a CCD (2^2 assays plus three central points) was proposed in order to evaluate the effects of aeration and agitation on growth parameters and lipid content in the cultivation of *Y. lipolytica* NRRL YB-423 in a 5 L batch stirred reactor.

Materials and Methods

Microorganism

Y. lipolytica NRRL YB-423 was provided by the Northern Regional Research Laboratory (Peoria, United States) and certified as GRAS (Generally Recognized As Safe). This strain was previously selected from a number of strains according to their growth capabilities on glycerol (Santos *et al*., 2013). The yeast was maintained on Yeast Malt (YM) Agar and stored at 4°C.

Raw glycerol

Raw glycerol was provided by BS Bios Indústria e Comércio de Biodiesel Sul Brasil S.A. (Passo Fundo, Brazil), derived from biodiesel production from soybean oil via methanolic route, with the following composition (% w v^{-1}): 83.08 glycerol; 6.37 ash; 10.31 humidity; 0.28 monoacylglycerols; pH 6.68.

Inoculum

Two tubes of microbial culture previously incubated at 25°C for 48 h were used. They were scraped with 10 mL of 0.1% (w v^{-1}) peptone diluent for each tube and transferred to 500 mL Erlenmeyer flask, totaling a 200 mL volume (180 mL medium and 20 mL microorganism suspension) with the following composition (g L^{-1}): 33.9 glycerol p.a; 0.6 yeast extract; 5.5 $(NH_4)_2HPO_4$; 1.5 peptone; 5.5 KH_2PO_4; 1.0 $(NH_4)_2SO_4$; 0.25 $MgSO_4.7H_2O$; 0.021 $CaCl_2.2H_2O$; and initial pH adjusted to 4.8. The suspension was incubated in a rotary shaker at 30°C and 180 rpm, and growth was monitored by counting in a Neubauer chamber.

5 L Bioreactor cultivation

The cultivations were done in a 5 L Biostat B bioreactor (B. Braun Biotech International, Germany), with a working volume of 4 L, containing optimized culture medium by Santos *et al.* (2012) with the following composition (g L^{-1}): 40.8 raw glycerol; 0.6 yeast extract; 5.5 $(NH_4)_2HPO_4$; 1.5 peptone; 5.5 KH_2PO_4; 1.0 $(NH_4)_2SO_4$; 0.25 $MgSO_4.7H_2O$; 0.021 $CaCl_2.2H_2O$ and initial pH adjusted to 4.8 before sterilization.

In all the bioreactor assays the inoculated yeast suspension volume was calculated so as to achieve a concentration of 10^7 cells mL^{-1} in culture medium to a final 4 L volume. The temperature was maintained at 30°C and pH at 4.8. Two Rushton type impellers were used for the agitation. The dissolved oxygen was monitored by a polarographic electrode (Mettler-Toledo, Brazil).

In order to evaluate the effects of agitation and aeration, a CCD was proposed (2^2 assays plus three central points). Samples were drawn at regular intervals of 6 h and centrifuged under refrigeration. Cells were recovered after washing twice with distilled water for the dry weight determination.

From the growth curves, the maximum biomass concentration (X_{max}) and volumetric productivity (Q) for each assay were calculated. Volumetric productivity was defined as the variation between the maximum and the initial biomass concentration and corresponding cultivation time required to achieve

maximum biomass (Juszczyk *et al*., 2013). At the end of the cultivation, the lipid content was determined. Under the best aeration and agitation conditions established by the experimental design, the fatty acids profile was also determined.

Shaken flask cultivation

A comparison was made between shaken-flask cultivation in the previously described conditions (Santos *et al.*, 2012) and bench scale cultivation in the conditions established in this work. The flasks containing raw glycerol-based medium were inoculated with previously prepared yeast suspension in order to achieve 10^7 cells mL^{-1}. The flasks were maintained in a rotary shaker at 30°C and 180 rpm. Samples were taken at regular intervals and centrifuged at 1780 x g for 15 min, and cells were recovered after washing twice with distilled water in order to determine biomass concentration. Growth parameters were calculated as mentioned above.

Analytical methods

Biomass

For the bioreactor assays, the biomass was dried at 105ºC up to constant weight in order to calculate biomass concentration (g L^{-1}). For shaken flask assays, biomass was monitored by measuring the absorbance at 600 nm. A calibration curve between OD_{600} and the cell dry-weight concentration (g L^{-1}) was established first (Santos *et al*., 2013).

Lipid content

The methodology proposed by Manirakiza *et al.* (2001), based on the Bligh and Dyer method, was used for total lipid quantification through the extraction of polar and nonpolar lipids from the biomass using a mixture of methanol and chloroform.

Fatty acids profile

The lipid fraction was esterified in order to obtain fatty acid methyl esters, in accordance with Metcalfe *et al.* (1966). Samples were then analyzed by gas chromatography with a gas chromatograph (Varian 3400 CX, United States) equipped with a hydrogen flame ionization detector and a ZB-Wax column (30 m x 0.32 mm internal diameter) (Zebron, United States). Nitrogen was used as the carrier gas with a flow rate of 0.6 mL min^{-1}. Injector and detector temperatures were 250 and 300°C, respectively. The initial oven temperature was 100°C followed by an increase in 6°C min^{-1}, remaining 1 min at the respective temperature until reaching 160 ºC, where it remained for 5 min. This was followed by a further increase of 6°C min^{-1} until the temperature reached 230°C, at which it remained for 10 min, totaling 41 min. The amount of injected sample was 1 µL. The fatty acids were identified by comparing retention times with standard mixtures (Sigma Supelco, United States) and quantified using Varian Star 4.51 software (Varian, United States).

K_La measurement

The volumetric oxygen transfer coefficient (K_La) was measured in a cell-free reactor by the gassing method for each combination of aeration and agitation from the CCD. The method consisted of gassing nitrogen until the oxygen concentration drops to almost zero, at which point air started to be injected into the reactor. The dissolved oxygen concentration was then monitored by a polarographic electrode (Mettler-Toledo, Brazil) and the K_La determined in accordance with Eq. 1 (Galaction *et al*., 2004).

$$-K_L a * t = \ln\left(\frac{100 - DO}{100}\right) \qquad \text{(Eq. 1)}$$

Where DO is the dissolved oxygen and t is the time.

Statistical analysis

The statistical treatment of the data was carried out using Statistica 5.0 software (Stat Soft Inc., United States).

Results and Discussion

Growth parameters

The results obtained from CCD in terms of microbial growth parameters are shown in Table 1. Experiments 5, 6, and 7 (200 rpm agitation and 1.0 vvm aeration), corresponding to CCD central points, had the best values for maximum biomass concentration, producing an average 19.14 g L^{-1} biomass. The time taken to achieve maximum biomass concentration ranged from 36 h (assay 3) to 60 h (assay 6), corresponding to stationary phase for all the assays. Volumetric productivity ranged from 0.22 g L^{-1} h^{-1} (assay 2) to 0.39 g L^{-1} h^{-1} (assays 5 and 7). The highest values for this parameter were also observed in central point conditions.

The values obtained for the maximum biomass concentration of *Y. lipolytica* NRRL YB-423 growing on raw glycerol-based medium were significant when compared to those quoted in the literature. Papanikolaou *et al*. (2008) achieved 7.1 g L^{-1} at 92 h of cultivation using *Y. lipolytica* ACA-DC 50109 growing on a medium containing raw glycerol (20.5 g L^{-1}) from biodiesel synthesis. Musial *et al.* (2011) observed a maximum biomass concentration of 19 g L^{-1}

Table 1. CCD matrix with encoded (real) values and responses obtained for each assay

Assay	Agitation (rpm)	Aeration (vvm)	K_La (h^{-1})	X_{max} (g L^{-1})*	Time (h)	Q (g L^{-1} h^{-1})	L (%)*
1	-1 (100)	-1 (0.5)	2.25	14.49 ± 0.04	54	0.23	5.48 ± 0.01
2	+1 (300)	-1 (0.5)	13.71	13.03 ± 0.02	42	0.22	5.68 ± 0.03
3	-1 (100)	+1 (1.5)	9.01	13.26 ± 0.02	36	0.33	5.96 ± 0.01
4	+1 (300)	+1 (1.5)	16.83	15.80 ± 0.10	48	0.30	5.28 ± 0.02
5	0 (200)	0 (1)	8.34	19.20 ± 0.01	48	0.39	7.88 ± 0.01
6	0 (200)	0 (1)	8.25	19.15 ± 0.01	60	0.31	7.86 ± 0.01
7	0 (200)	0 (1)	8.29	19.07 ± 0.03	48	0.39	7.87 ± 0.02

X_{max}: maximum biomass concentration; Time: time to reach X_{max} (stationary phase); Q: volumetric productivity: K_La: volumetric oxygen transfer coefficient; L: lipid content.
* Mean value ± standard error of two determinations.

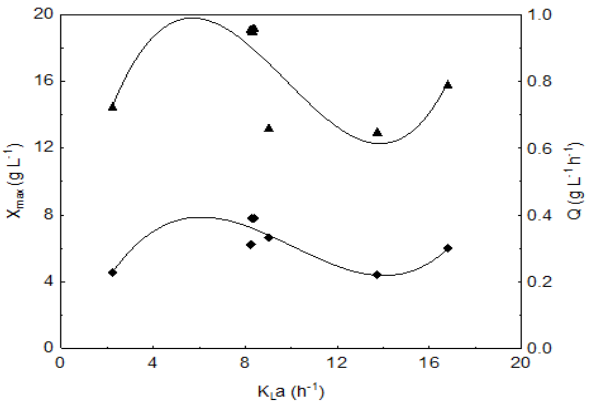

Figure 1. Growth parameters as a function of the K_La in the 5 L batch stirred reactor. (▲) Xmax; (♦) Q. For shaken flasks cultivation: X_{max} = 15.74 g L^{-1}; Q = 0.11 g L^{-1} h^{-1})

for *Y. lipolytica* A-101, but using a medium containing 20 g L^{-1} of crude rapeseed oil as the main carbon source. In the work of Juszczyk *et al*. (2013), biomass production on media containing raw glycerol reached 11.6 g L^{-1} with *Y. lipolytica* S10 cultivated in a 5 L bioreactor with a working volume of 1.5 L, aeration rate of 1 vvm and agitation of 550 rpm.

Regarding the volumetric productivity, the values obtained in this work were lower compared with those presented by Juszczyk *et al.* (2013), 1.06 g L^{-1} h^{-1} for *Y. lipolytica* S10 cultivated in the same conditions as described above. The K_La is related to agitation, aeration, impeller type, culture medium viscosity, and bioreactor geometry. This study had a K_La value ranging from 2.25 h^{-1} (assay 1) to 16.83 h^{-1} (assay 4), corresponding to the mildest aeration and agitation conditions (100 rpm and 0.5 vvm) and the most severe (300 rpm and 1.5 vvm).

According to Figure 1, the oxygen supply, represented by the K_La values, proved to be an important factor affecting growth parameters. It is likely that the high agitation rate (300 rpm), consequently a high K_La value, promoted excessive shear stress resulting in a decrease in biomass concentration and volumetric productivity. On the other hand, the lower K_La value, corresponding to mild aeration (0.5 vvm) and agitation (100 rpm) conditions, is likely to have caused a deficient oxygen supply, resulting in a decrease in growth parameters. Therefore, an intermediate level of K_La was preferred, maintaining agitation and aeration rates at approximately 200 rpm and 1 vvm, respectively. Similar behavior was observed by Burkert *et al*. (2005) in the cultivation of *Geotrichum candidum* in stirred and air-lift reactors for lipase production.

The statistical analysis for aeration and agitation effects on the maximum biomass of *Y. lipolytica* NRRL YB-423 is shown in Table 2. An estimate of the main effects was obtained by evaluating the differences in process performance caused by a change from low (−1) to high (+1) levels of the corresponding variable (Rodrigues and Iemma, 2012). At a confidence level of 95% ($p>0.05$), the variables did not have a significant effect on the maximum biomass concentration and volumetric productivity, when aeration and agitation were changed from 0.5 to 1.5 vvm and from 100 to 300 rpm, respectively. However, as mentioned above, the maximum values for these parameters were observed in the conditions corresponding to the central point (200 rpm and 1 vvm), allowing the establishment of conditions for aeration and agitation with the best responses in the range studied.

Table 3 shows a comparison between microbial cultivation in a 5 L bioreactor, corresponding to the central point condition (200 rpm and 1 vvm) and the culture in shaken flasks using a culture medium with the same composition and the same incubation temperature (30ºC). This pointed to a significant

Table 2. Effects of agitation and aeration in maximum biomass concentration (g L^{-1}), volumetric productivity (g L^{-1} h^{-1}) and lipid content (%)

Factor	Effect			Stardard Error			t(3)			p		
	X_{max}	Q	L	X_{max}	Q	L	X_{max}	Q	L	X_{max}	Q	L
Mean	16.29	0.31	6.57	1.43	0.03	0.65	11.41	10.25	10.13	0.001	0.002	0.002
Agitation	0.54	-0.02	-0.24	3.78	0.08	1.72	0.14	-0.25	-0.14	0.895	0.819	0.898
Aeration	0.77	0.09	0.04	3.78	0.08	1.72	0.20	1.13	0.02	0.851	0.342	0.983
Agitation x Aeration	2.00	-0.01	-0.44	3.78	0.08	1.72	0.53	-0.13	-0.26	0.633	0.908	0.814

X_{max}: maximum biomass concentration; Q: volumetric productivity; L: lipid content.
*Mean value ± standard deviation of three assays. Different letters represent significant differences in the line ($p<0.05$)

Table 3. Comparison between *Y. lipolytica* NRRL YB-423 cultures performed in bench scale bioreactor and shaken flasks

Growth Parameter	Cultivation*	
	5 L Bench Scale Bioreactor	Shaken Flasks
X_{max} (g L^{-1})	19.14 ± 0.06[a]	15.74 ± 0.29[b]
Q (g L^{-1} h^{-1})	0.36 ± 0.05[a]	0.11 ± 0.01[b]

X_{max}: maximum biomass concentration; Q: volumetric productivity.
*Mean value ± standard deviation of three assays. Different letters represent significant differences in the line ($p<0.05$)

difference between microbial cultures in shaken flasks and in a bioreactor, regarding the evaluated cell growth parameters (X_{max} and Q), demonstrating that the established aeration and agitation conditions had a positive impact on cell growth, providing the microorganism with a more efficient supply of oxygen.

Lipid content

Table 1 shows the lipid content found in the biomass of *Y. lipolytica* NRRL YB-423 obtained as the cultures ended. *Y. lipolytica* NRRL YB-423 had quite high lipid contents in comparison, for instance, with *Saccharomyces cerevisiae* (0.5%, according to Yamada and Sgarbieri, 2005). Therefore, it can be an important source of lipids in animal feed, in comparison to the broadly used yeast *S. cerevisiae.*

Papanikolaou *et al.* (2008) obtained 6-14% of lipids during the stationary phase of *Y. lipolytica* ACA-DC 50109 in a medium containing raw glycerol (45.9 g L^{-1}). In turn, Papanikolaou *et al*. (2002) produced biomass at the concentration of 6-7.5 g L^{-1} containing 5-10% of lipids in shaken flask culture with *Y. lipolytica* LGAM SC711.

According to Table 2, agitation and aeration did not have a significant effect ($p>0.05$) on the lipid content, when aeration and agitation were changed from level -1 (0.5 vvm and 100 rpm, respectively) to level +1 (1.5 vvm and 300 rpm, respectively). However, similar to the behavior observed for growth parameters, it is possible to establish that the best condition for lipid content is the central point (200 rpm agitation and 1.0 vvm aeration), which coincide with the best conditions for *Y. lipolytica* NRRL YB-423 growth.

Fatty acid profile

Table 4 shows the fatty acid profile of the lipid fraction obtained from the biomass of *Y. lipolytica* NRRL YB-423, cultivated in a medium containing raw glycerol, at the CCD central point conditions (200 rpm agitation and 1 vvm aeration). The proportion of saturated fatty acids obtained for *Y. lipolytica* NRRL YB-423 was only 15.41%, with a prevalence of heptadecanoic acid (C17:0), and the content of monounsaturated fatty acids was 19.39%, with a prevalence of cis-10-heptadecanoic acid (C17:1). The polyunsaturated fatty acids were the ones produced in larger quantity (62.55%). Of these, 49.16% corresponded to linoleic acid (C18:2 cis), but with a noticeable quantity of γ–linolenic acid (C18:3 n-6) – almost 12.75%. This composition differs greatly from yeast *S. cerevisiae* as it contains a high percentage of saturated fatty acids (42.71%), with emphasis on palmitic acid (24.60%), and a smaller proportion of polyunsaturated fatty acids (28.9% linoleic acid) (Yamada and Sgarbieri, 2005).

In comparison with data available for *Y. lipolytica*, the study conducted by Papanikolaou *et al*. (2008) can be quoted: using raw glycerol (45.9 g L^{-1}) as a carbon source, they obtained a biomass of *Y. lipolytica* ACA-DC 50109 containing 6-14% of lipids with 20.2% of linoleic acid and 44.9% of oleic acid. With yeast *Y. lipolytica* LGAM SC711 cultivated in shaken flasks, Papanikolaou *et al.* (2002) obtained similar results

Table 4. Fatty acid profile (%)* of the lipid fraction of the biomass of *Y. lipolytica* NRRL YB-423

Saturated Fatty Acids		Monounsaturated Fatty Acids		Polyunsaturated Fatty Acids	
Fatty Acid	Relative amount (%)	Fatty Acid	Relative amount (%)	Fatty Acid	Relative amount (%)
Pentadecanoic (C15:0)	0.38 ± 0.01	Cis-10-heptadecanoic (C17:1)	9.31 ± 0.02	Linoleic (C18:2 cis)	49.16 ± 0.04
Palmitic (C16:0)	0.41 ± 0.02	Oleic (C18:1 cis)	1.78 ± 0.01	γ-linoleic (γ C18:3 n-6)	12.75 ± 0.03
Heptadecanoic (C17:0)	12.19 ± 0.04	Elaidic (C18:1 trans)	6.36 ± 0.01	β-linolenic (α C18:3 n-3)	0.14 ± 0.01
Stearic (C18:0)	0.12 ± 0.01	Cis-11-eicosenoic (C20:1 n-9)	1.03 ± 0.02	Cis-11,14-eicosadienoic (C20:2)	0.39 ± 0.02
Arachidic (C20:0)	0.17 ± 0.01	Erucic (C22:1 n-9)	0.44 ± 0.02	Cis-13,16-docosadienoic (C22:2)	0.11 ± 0.01
Tricosanoic (C23:0)	0.23 ± 0.01	Nervonic (C24:1 n-9)	0.46 ± 0.01		
Lignoceric (C24:0)	1.90 ± 0.20				

Not identified: 2.70 ± 0.18%.
* Mean value ± standard deviation of three determinations.

in relation to the fatty acid composition (21±3 and 47±4% for linoleic acid and oleic acid, respectively) from a medium containing 30 g L^{-1} of glycerol and a C/N molar ratio of 100.

Meanwhile, Beapoulos *et al.* (2009) claim that *Y. lipolytica* accumulates lipids at a smaller proportion in relation to other yeasts, but it is the only one capable of accumulating a high proportion of linoleic acid (over 50%), corroborating the results obtained in the present study. Furthermore, it is important to highlight that medium composition influences fatty acid composition (Athenstaedt *et al*., 2006). Sources of carbon, nitrogen, metallic ions, temperature and pH may affect the composition. This implies that the composition of lipids can be manipulated, leading to high percentages of fatty acids of interest (Dyal and Narine, 2005).

Gutierrez and Silva (1993) claim that supplemental essential fatty acids, such as linoleic acid, are required in animal feed, with fish farming (Santos *et al*., 2007), poultry breeding (Uliana *et al*., 2001; Barreto *et al.*, 2006) and cattle (Ribeiro *et al.*, 2007) being points in question. This supplementation is usually performed using vegetal oils such as soy, linseed, rapeseed, rice, corn and sunflower oil, although growing interest in new sources of essential fatty acids has developed, and much attention has been devoted to microorganisms (Cazetta and Celligoi, 2005).

Therefore, based on what has been shown above, *Y. lipolytica* NRRL YB-423 has proved to be a promising source of essential polyunsaturated fatty acids, mainly linoleic acid. Therefore, it can be considered as an alternative source of this fatty acid in food formulations for animals, and could be useful in combination with other low-lipid feedstuffs such as *S. cerevisiae*. Moreover, considering the fatty acid profile, biomass of *Y. lipolytica* NRRL YB-423 could be used as feedstock for biodiesel production, but additional studies are required to improve lipid content.

Conclusion

In this work, K_La proved to be an important factor for the performance of *Y. lipolytica* NRRL YB-423 in the cultivation in a 5 L bench bioreactor for the production of biomass as a source of lipids. The best results were obtained through 200 rpm agitation and 1.0 vvm aeration (K_La of 8.29), attaining 19.14 g L^{-1} of maximum biomass concentration, with volumetric productivity of 0.36 g L^{-1} h^{-1} and lipid content of 7.87%. Furthermore, these conditions led to an improvement in yeast performance when compared to shaken flask culture. Based on the determination of the profile of fatty acids, the biomass proved to be a promising source of essential fatty acids, particularly linoleic acid (49.16%).

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