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Single cell protein production by Wickerhamomyces anomalus CCC32 using crude glycerol

Produção de proteína unicelular por *Wickerhamomyces anomalus* CCC32 a partir de glicerol bruto

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Abstract

The objective of this work was to evaluate the production of single cell protein (SCP) by five yeasts using crude glycerol as carbon source, to select the most promising strain and optimize biomass and crude protein production by central composite design (CCD). *Wickerhamomyces anomalus* CCC32 was the most promising strain for SCP production with biomass production of 20 g L^{-1} and crude protein concentration of 33% after 84 h using crude glycerol 25 g L^{-1}, $NaNO_3$ 3.5 g L^{-1} and 6.5 g L^{-1} of urea, at 30 °C and 150 rpm. Crude glycerol proved to be an excellent substrate option for SCP production, adding value to this residue. *W. anomalus* CCC32 showed promising characteristics for SCP production as fast growth and biomass production with high crude protein content.

Keywords: Agro industrial wastes. Fungi. Fermentation.

Resumo

O objetivo deste trabalho foi avaliar a produção de proteína microbiana, ou proteína unicelular, por cinco leveduras utilizando glicerol bruto como fonte de carbono, selecionar a cepa mais promissora e otimizar a produção de biomassa e proteína bruta por meio de delineamento composto central rotacional (DCCR). *Wickerhamomyces anomalus* CCC32 foi a cepa mais promissora para a produção de SCP, com produção de cerca de 20 g L^{-1} de biomassa e concentração de proteína bruta de 33% após 84 h fermentação, utilizando 25 g L^{-1} de glicerol bruto, 3,5 g/ L^{-1} de $NaNO_3$ e 6,5 g L^{-1} de ureia, a 30 °C e 150 rpm. O glicerol bruto mostrou-se uma excelente opção de substrato para a produção de SCP, sendo uma alternativa para agregar valor a esse resíduo. *W. anomalus* CCC32 apresentou características favoráveis à produção de SCP como crescimento rápido e produção de biomassa com elevado teor de proteína bruta.

Palavras-chave: Resíduo agroindustrial. Fungo. Fermentação.

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Introduction

Single cell protein (SCP) refers to the dried cells of microorganisms such as yeast, filamentous fungi, algae and bacteria which can be used as a protein sources for human and animal food (SISMAN *et al*., 2012; NAJAFPOUR, 2015). SCP has many applications in the food industry because of their high protein content, high percentage of essential amino acids, vitamins and other nutrients such as mineral salts (ANAPUMA; RAVINDRA, 2000; GAO *et al*., 2007; WANG *et al*., 2009). Thus, it can substitute conventional protein sources like soybean and fish meal (ANAPUMA; RAVINDRA, 2000), having a promising biotechnological application, which is even more advantageous when produced using agro industrial residues as substrates (PONSANO *et al*., 2003).

Some yeasts like *Saccharomyces cerevisiae*, *Candida utilis*, *C. tropicalis*, *Cryptococcus aureus* and some species of the genus *Hansenula*, *Pichia* and *Torulopsis* can be used for SCP production. Their biomass has high levels of protein, besides being a source of complex B vitamins, minerals and other components that stimulate resistance to diseases when used feeding for marine animals (ZHENMING *et al*., 2006). *S. cerevisiae* in addition to having high crude protein content, shows similar amino acid profile to those described in fish meal and can be used in aquaculture (VIDAKOVIC *et al*., 2015). According Juszczyk *et al*. (2013) the biomass formed by the strain *Yarrowia lipolytica* S6 produced in crude glycerol as carbon source, exhibited high crude protein concentration ($42-45\%$) and essential amino acids such as lysine, threonine and phenylalanine which can be used as a high quality nutritional component for animal feed.

SCP production from agro-industrial wastes makes this process economically attractive, as it reduces production costs and contributes to recycle wastes (ANAPUMA; RAVINDRA, 2000; SISMAN *et al*., 2012). Several substrates are used for SCP production, such as sugarcane molasses, whey, beet vinasse and crude glycerol, among others (RITALA *et al*., 2017). Crude glycerol, a by-product generated from biodiesel production in a proportion of 10% (v/v), consists of glycerol, salts (such as potassium and sodium) and residual alcohol (methanol or ethanol) (da SILVA *et al*., 2015). Crude glycerol can be refined obtaining pure glycerol, which is widely used in the pharmaceutical and cosmetic industries (GALANAKIS, 2012; UPRETY *et al*., 2016). However, the refining process consumes a lot of energy and has high cost, making this process impracticable for small and medium producers of biodiesel (UPRETY *et al*., 2016). On the other hand, its disposal requires adequate treatment, as it can impact the environment, generating additional costs to the biodiesel production chain (LUO *et al*., 2016). Therefore, the development of processes to convert low-cost crude glycerol into industrial products is desirable, since it may be an alternative for its biotechnological enhancement; besides adding value to the biodiesel production chain (YANG *et al*., 2012).

Crude glycerol has advantages over other substrates, such as its lower cost and large availability, and can be used substituting traditional carbohydrates such as sucrose, glucose and starch to obtain value-added products such as high-gross-rate microbial biomass (PAPANIKOLAOU *et al*., 2008; QUIN *et al*., 2017). The objective of this work was to evaluate the production of yeast SCP using crude glycerol as a carbon source, in order to select the most promising yeast and to optimize the production of biomass and crude protein.

Material and methods

Microorganisms and substrate

Yeasts Candida (Metschnikowia) pseudointermedia CAC01, *Rhodotorula mucilaginosa* CCC31, *Wickerhamomyces anomalus* CCC32, *Aureobasidium pullulans* EBJ31 and *Trichosporon asahii* EPB13 belong to the collection of cultures of the Laboratory of Biotechnology of Microorganisms from the State University of Bahia – Campus Senhor do Bomfim, Bahia, Brazil. *C. (Metschnikowia) pseudointermedia* CAC01 was isolated from contaminated commercial coconut water (latitude 10° 28" 15' S and 40° 10" 12' W). *R. mucilaginosa* CCC31 and *W. anomalus* CCC32 were isolated from cochineal (*Dactylopius* sp.) on leaves of the ornamental plant *Ardisia crenata* ("*cafezinho*") (Both latitude 10° 28" 15.9' S and longitude 40° 10" 12 W). *A. pullulans* EBJ31 and *T. asahii* EPB13 were isolated from soil containing organic material such as decaying vegetables and goat droppings. (latitude 10° 12" 53.3' S and longitude 40° 12" 59.8' W). The strains were maintained in glycerol P.A 40% (p/v) at -18 °C for preservation. The crude glycerol used in the experiments was composed of 81% glycerol (p/v) and was obtained from a biodiesel plant located in the city of Candeias, Bahia, Brazil.

Fermentative assays

The fermentative and inoculum media were prepared using Malt extract (ME) modified containing (g L^{-1}): yeast extract (3.0), malt extract (3.0), peptone (5.0) and crude glycerol (20.0). Yeasts were cultured in 50 mL medium, agitated at 150 rpm, 30 °C, during 24 h to obtain the inoculum. Then, 10% of the inoculum was transferred into 125 mL Erlenmeyer-flasks containing 25 mL of medium, pH 5.0, adjusted with 2 M HCl. The flasks were incubated in shaker at 30 °C and 150 rpm for 96 h and samples were taken every 24 h.

Evaluation of different nitrogen sources for biomass and crude protein production

To test the influence of different nitrogen sources on the selected yeast was a medium containing (g L^{-1}): K_2HPO_4 (1.0), NaCl (1.0), $MgSO_4$ (0.2), crude glycerol (20.0) and different nitrogen sources at 10 g L^{-1}: yeast extract, corn steep liquor, peptone, sodium nitrate (NaNO3), ammonium sulfate ($(NH_4)_2SO_4$) and urea (CH_4N_2O) was used. The fermentation assays occurred in 250 mL Erlenmeyer-flasks containing 50 mL of medium, at 150 rpm, 30 °C during 24 h. All the tests were performed in triplicate and the results obtained were statistically evaluated by analysis of variance (ANOVA) and Tukey's test at 5% of probability using the software R studio 3.3.4.

Analytical methods

The samples were centrifuged at 5.000 rpm for 20 min and the biomass was used to determine cell growth and crude protein content; the supernatant was frozen for further analysis of glycerol and alcohol by High Performance Liquid Chromatography (HPLC, Dionex model Ultimate 3000, Germany) equipped with RI detector and Rezex ROA H+ column (300 x 7.8 mm). The temperature was kept up at 80 °C and mobile phase of H_2SO_4 0.005 M with flow of 0.6 mL min^{-1}.

Cellular growth (g L^{-1}) was determined using standard curve relating dry mass x optical density at 600 nm. To determine the crude protein concentration, the biomass was washed with distilled water and dried at 65 °C. Then, 0.1 g of the dried biomass was digested with a mixture of 3.5 mL concentrated sulfuric acid and 3.0 mL hydrogen peroxide 30% (v/v) at 350 °C for 1.5 h. The total protein content of the biomass was determined by the phenol-hypochlorite method, adopting the factor of 6.25 for the conversion from the total nitrogen contents (WEATHERBURN, 1967; FELKER, 1977).

Optimization of biomass and crude protein production using Central Composite Design (CCD)

The optimization of biomass and crude protein production was performed using the response surface methodology. Biomass (g L^{-1}) and crude protein production (%) were dependent variables and the crude glycerol, sodium nitrate and urea were independent variables. A complete factorial planning matrix 2^3 was constructed by means of Central Composite Design (CCD), totalizing 17 assays (RODRIGUES; IEMMA, 2009). Two levels were selected, one higher (+1) and an inferior one (-1), in addition to two axial points (+1.68 and -1.68) and a central point (0) which was performed in triplicate. This model is represented by a second-order polynomial regression, given by equation (1)

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2, \quad (1)$$

where Y is the predicted response of biomass production and/or crude protein production; X_1, X_2 and X_3 are the coded forms (glycerol, sodium nitrate and urea, respectively); b_0 refers to the point of intersection; b_1, b_2 and b_3 are linear coefficients; b_{12} coefficient of double interaction; b_{11}, b_{22} and b_{33} are quadratic coefficients. The values of the studied levels were calculated through equation (2)

$$X_n = \frac{X - X_0}{X_{+1} - X_{-1}}, \quad (2)$$

where X_n is the encoded value; X is the real value of the independent variable; X_0 is the actual value of the center point; X_{+1}, is the value of the upper level; X_{-1} is the value of the lower level.

The inoculum used in the experimental design trials was prepared in 125 mL Erlenmeyer-flasks containing 25 mL of (g L^{-1}): K2HPO4 (1.0), NaCl (1.0), $MgSO_4$ (0.2), yeast extract (1.0), $NaNO_3$ (5.0), urea (5.0) and crude glycerol (20.0), at 150 rpm, 30 °C, for 24 h. The fermentative assays were carried out in 250 mL Erlenmeyer-flasks containing 50 mL of the composition media described above and 10% (v/v) inoculum, incubated in shaker under the same conditions. The concentration of crude glycerol, sodium nitrate and urea were adjusted according to each experimental design to be presented in Table 2. The results were evaluated using the software Statistic 7.1.

Results and discussion

Evaluation of biomass and crude protein production using crude glycerol

The kinetic of crude glycerol fermentation by yeast evaluated for 96 h is shown in Figure 1. The yeast *W. anomalus* CCC32 consumed the entire substrate in less than 48 h, followed by *A. pullulans* EBJ31 and *C. pseudointermedia* CAC01 (72 h); *R. mucilaginosa* CCC31 consumed crude glycerol after 96 h and *T. asahii* EPB13 did not use glycerol.

The strain *W. anomalus* CCC32 reached the highest biomass production, of around 21.5 g L^{-1}, after 72 h; strains *A. pullulans* EBJ31, *C. pseudointermedia* CAC01 and *R. mucilaginosa* CCC31 produced less biomass and showed slowercellular growth, probably due to the slower consumption of glycerol; *T. asahii* EPB13 was the strain that ehxibited the lowest biomass production (5.24 g L^{-1}), suggesting that it growth using other nutrients from culture medium than glycerol.

The maximum crude protein production occurred after 24 h for all strains and there was no statistical difference ($p > 0.05$) between *A. pullulans* EBJ31, *C. (Metschnikowia) pseudointermedia* CAC01 and *W. anomalus* CCC32. However, after 24 h *W. anomalus* CCC32 produced approximately twice as much biomass (12.97 g L^{-1}), statistically differing from other strains ($p < 0.05$), which resulted in a higher yield of protein production ($Y_{p/s}$), 0.05 g g^{-1}.

Considering that *W. anomalus* CCC32 ehxibited fast growth in medium containing crude glycerol, higher biomass production, faster glycerol consumption and higher protein yield, it was the strain selected for the biomass optimization and crude protein production tests.

Yeast *W. anomalus* shows several biotechnological interest and industrial applications, as for biological control in fruits (OLSTORPE *et al*., 2010; ORO *et al*., 2014; CAPPELLI *et al*., 2014), production of biofuels (WALKER, 2011), polyhydroxyalcanoates (OJHAA; DAS, 2018), biosurfactants (SOUZA *et al*., 2018), aromatic and sensory compounds that improve the taste of foods such as cocoa and chocolate (KONÉ *et al*., 2016), beer (BASSO *et al*., 2016), oriental foods (ZHA *et al*., 2018), among others. In addition, this yeast is classified as biosafety level 1, which makes it safe for many different industrial applications (SUNDH; MELIN, 2010).

Nitrogen sources for SCP production by W. anomalus CCC32

In addition to the carbon source, the production of biomass and protein depends on the specific characteristics of each strain and its ability to assimilate different carbon and nitrogen sources. Therefore, it is common to observe high protein production in yeast biomass using different nitrogen sources. Li *et al*. (2017) reported that ammonia, urea, peptone, yeast extract, corn steep liquor, among others, increased yeast cell growth, but not all sources contributed in a similar way for each species. In the case of *W. anomalus* CCC32 strain the yeast extract led to the higher production of biomass and crude protein, differing statistically from other nitrogen sources ($p < 0,05$), see Table 1, as described by Gao *et al*. (2007) and Gao *et al*. (2012) for production of SCP by *Cryptococcus* aureus G7a and *Candida tropicalis*, respectively. The yeast extract contains vitamins, minerals and amino acids that promote cell growth (LI *et al*., 2011). However, sodium nitrate also favored the production of biomass, not differing statistically from peptone ($p > 0.05$). On the other hand, urea favored the production of crude protein, whith 32.5%, and 4.54 g L^{-1} biomass. Patelski *et al*. (2015) reported that combination of urea and dibasic phosphate increased production of crude protein by *Candida tropicalis* and *Trichosporon cutaneaum*, while for protein production was favored by urea as the only nitrogen source for *S. cerevisiae*. Therefore, according to these authors, the nitrogen source must be carefully chosen because it influences both quantitatively and qualitatively the production of protein.

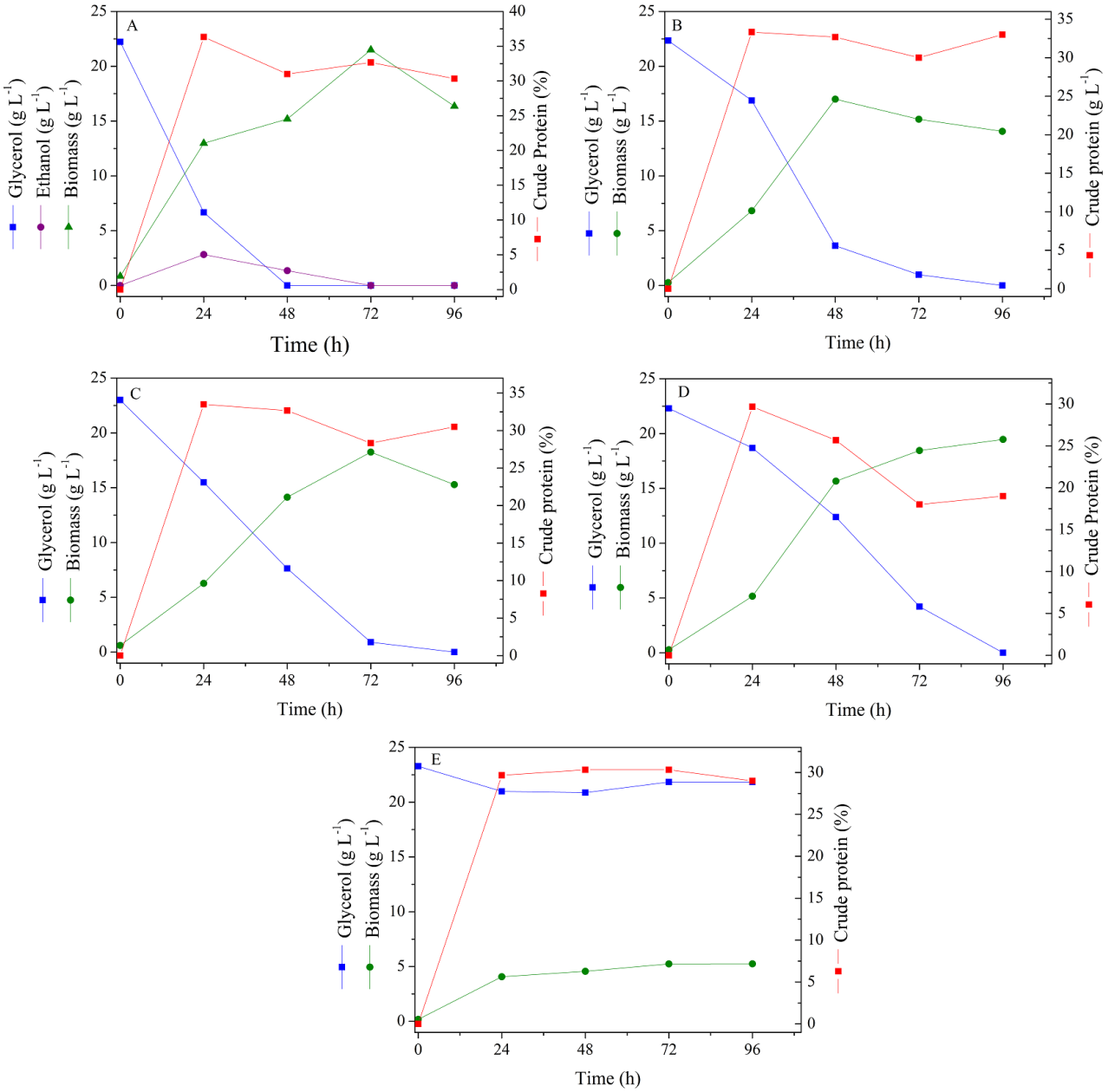
Table 1 – Biomass (g L^{-1}) and crude protein (%) production by *W. anomalus* CCC32 after 24 h fermentation in different nitrogen sources.

Nitrogen source	Biomass (g L^{-1})	Crude protein (%)
Yeast extract	11.65^{a*}	37.33^{a}
Sodium nitrate	10.37^{b}	22.67^{e}
Peptone	10.18^{b}	29.67^{c}
Corn steep liquor	6.75^{c}	13.33^{f}
Ammonium sulfate	6.13^{d}	26.67^{d}
Urea	4.54^{e}	32.50^{b}

*Equal letters do not differ according Scott-Knott's test at 5% significance

Source: The authors.

Figure 1 – Kinetic fermentation of crude glycerol by *W. anomalus* CCC32 (A), *A. pullulans* EBJ31 (B), *C. (Metschnikowia) pseudointermedia* CAC01 (C), *R. mucilaginosa* CCC31 (D) and *T. asahii* EPB13 (E) at 30 °C, 150 rpm, during 96 h.



Source: The authors.

Highest SCP production by *W. anomalus* CCC32 occurred with the yeast extract, however sodium nitrate and urea also showed good results. While sodium nitrate favored the production of biomass, urea favored the production of crude protein. Thus, due to the high commercial cost of yeast extract (and peptone which may increase the cost of SCP production, combinations of sodium nitrate and urea were chosen as nitrogen sources for the following fermentation tests.

Ali *et al*. (2009) and Ahmed *et al*. (2010) also reported that urea favored the production of crude protein in yeasts. Gao *et al*. (2007) substituted the yeast extract by soybean meal hydrolyzate as nitrogen source in the SCP production by *C. aureus* G7a, since the use of low cost substrates is one of the most important aspects for SCP production. The use of yeast extract and peptone in large scale greatly increases the cost of production due to its high commercial value.

Effect of crude glycerol and inorganic salts on biomass and crude protein production using Central Composite Design (CCD)

The results obtained in the CCD 2^3 showed the influence of crude glycerol, urea and sodium nitrate on the biomass and crude protein production by *W. anomalus* CCC32 after 24 h of fermentation, see Table 2. The highest biomass production was obtained in the assay 7 (9.74 g L^{-1}) and the highest crude protein production in assays 1, 5 and 8 (30%).

According to the statistical analysis, crude glycerol and urea had a significant influence on biomass production, in the linear terms ($p < 0.05$). However, only crude glycerol had a significant influence on crude protein production, in the quadratic term, that is, the increase in glycerol concentrations significantly decreased crude protein production, Table 3.

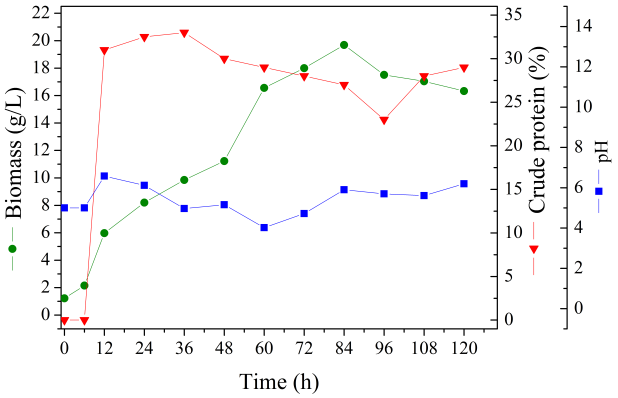
High concentrations of the carbon source in the culture medium increase osmotic pressure and, consequently, reduce cell growth (BZDUCHA-WOROBEL *et al*., 2015). In addition, the presence of contaminants in the crude glycerol can negatively influence the results, which may explain the reduction of biomass production by *W. anomalus* CCC32 in the assays where crude glycerol concentration was above 28 g L^{-1}. Kurcz *et al*. (2018) also reported a significant influence of glycerol concentration on biomass and crude protein production by *Candida utilis*. Dos Santos *et al*. (2012) observed the concentration of crude glycerol had significant effect only on the biomass production by *Yarrowia lipolytica* NRRL YB-423, but did not have a significant effect for crude protein production.

However, the analysis of variance showed that values of calculated F for biomass production and crude protein production were lower than the Ftab (3.68), although R^2 was greater than 0.7 for both responses, Table 4.

Therefore, new tests were performed varying the concentrations of glycerol from 20 to 30 g L^{-1}, setting the concentrations of $NaNO_3$ (3.5 g L^{-1}) and urea (6.5 g L^{-1}) to confirm the values of biomass and crude protein production. The average crude protein production was 33% for the different concentrations of crude glycerol, not differing statistically from each other. On the other hand, the biomass production was higher at a glycerol concentration 25 g L^{-1}, reaching 9.34 g L^{-1}, Table 5.

The fermentation kinetics was evaluated for 120 h. There was little change in pH over time, which remained constant at around 6.0, evidencing the low production of organic acids by this strain and that most of the carbon was used to produce SCP, with little release of undesirable products from the metabolism to the culture medium. The maximum biomass production was around 20 g L^{-1} after 84 h of fermentation during the exponential phase, and the maximum crude protein yield was 33% on average between 12 and 36 h, yield ($Y_{x/p}$) of 0.1 g g^{-1} and productivity (Y_p) of 0.018 g L^{-1} h^{-1}, Figure 2.

Figure 2 – Fermentation kinetics of biomass (g L^{-1}) and crude protein (%) production by *W. anomalus* CCC32 at crude glycerol 25 g L^{-1}, $NaNO_3$ 3.5 g L^{-1}, urea 6.5 g L^{-1} at 30 °C, 150 rpm during 120 h.



Source: The authors.

The results obtained with *W. anomalus* CCC32 in mineral medium as nitrogen sources, are comparable to those obtained by yeasts using high cost media such as yeast extract and peptone, which were among 17-22 g L^{-1}, on average (GALVANO *et al*., 2011; TACCARI *et al*., 2012; SANTOS *et al*., 2013). In addition, the mineral medium provided for *W. anomalus* CCC32 growth favored high biomass yield (20 g L^{-1}) in about half the time when compared with other yeast strains. Duarte *et al*. (2013) and Uprety *et al*. (2017) described maximum biomass production by *Candida* sp. LEB-M3 (around 20g/L) and *Rhodosporidium toruloides* ATCC10788 (21.16 g L^{-1}) after 168 h, respectively.

Crude protein production by *W. anomalus* CCC32 were similar to those obtained by *C. utilis* (36.7%) in medium containing potato wastewater and glycerol at 5% (w/v) after 72 h (KURCZ *et al*. 2018). However, dos Santos *et al*. (2012) described lower crude protein content (18.2%) by *Y. lipolytica* NRRL YB-423 using crude glycerol and ammonium sulfate as nitrogen source.

Table 2 – Central composite design 2^3 matrix showing codified values, real values and responses obtained for biomass and crude protein production.

Essays	Glycerol ($g\ L^{-1}$)	$NaNO_3$ ($g\ L^{-1}$)	Urea ($g\ L^{-1}$)	Biomass ($g\ L^{-1}$)	Crude protein (%)
1	-1 (28)	-1 (3.5)	-1 (3.5)	8.60	30
2	+1 (52)	-1 (3.5)	-1 (3.5)	8.08	26
3	-1 (28)	+1 (6.5)	-1 (3.5)	8.17	28
4	+1 (52)	+1 (6.5)	-1 (3.5)	7.70	28
5	-1 (28)	-1 (3.5)	+1 (6.5)	9.34	30
6	+ (52)	-1 (3.5)	+1 (6.5)	8.47	23
7	-1 (28)	+1 (6.5)	+1 (6.5)	9.74	26
8	+1 (52)	+1 (6.5)	+1 (6.5)	8.77	30
9	-1.68 (20)	0 (5.0)	0 (5.)	8.88	29
10	+1.68 (60)	0 (5.0)	0 (5.0)	8.00	27
11	0 (40)	-1.68 (2.5)	0 (5.0)	7.69	26
12	0 (40)	+1.68 (7.5)	0 (5.0)	9.20	22
13	0 (40)	0 (5.0)	-1.68 (2.5)	7.90	25
14	0 (40)	0 (5.0)	+1.68 (7.5)	8.57	21
15	0 (40)	0 (5.0)	0 (5.0)	8.77	24
16	0 (40)	0 (5.0)	0 (5.0)	8.54	25
17	0 (40)	0 (5.0)	0 (5.0)	8.54	23

Source: The authors.

Table 3 – Regression coefficients for biomass and crude protein production by *W. anomalus* CCC32.

Factors	Regression coeficients		Standard error		t(7)		*p*-value	
	Biomass	Protein	Biomass	Protein	Biomass	Protein	Biomass	Protein
Means	8.60	23.83	0.24	1.37	35.75	17.37	0.000000	0.000001
Glycerol (L)	-0.32	-0.76	0.11	0.64	-2.79	-1.17	**0.026783**	0.277173
Glycerol (Q)	-0.01	1.98	0.12	0.70	-0.06	2.80	0.957175	**0.026591**
$NaNO_3$ (L)	0.18	-0.27	0.11	0.64	1.57	-0.42	0.159364	0.684452
$NaNO_3$ (Q)	-0.005	0.57	0.12	0.70	-0.04	0.80	0.968106	0.448302
Urea (L)	0.36	-0.71	0.11	0.64	3.17	-1.1	**0.015630**	0.305302
Urea (Q)	-0.07	0.21	0.12	0.70	-0.63	0.30	0.543472	0.769702
Glycerol x $NaNO_3$	-0.006	1.87	0.15	0.84	-0.04	2.22	0.967411	0.061125
Glycerol x Urea	-0.106	0.12	0.15	0.84	-0.71	0.14	0.495000	0.886096
$NaNO_3$ x Urea	0.19	0.37	0.15	0.84	1.27	0.44	0.41787	0.669302

Source: The authors.

Table 4 – ANOVA for the regression model obtained by the response surface.

Factors	Sum of squares		df		Mean square		Fcal	
	Biomass	Protein	Biomass	Protein	Biomass	Protein	Biomass	Protein
Regression	4.01	91.29	9	9	0.44	10.14	2.6	1.79
Residual	1.21	39.65	7	7	0.17	5.66		
Total	5.22	130.94	16	16				

Source: The authors.

Table 5 – Biomass (g L^{-1}) and crude protein (%) production by *W. anomalus* CCC32 in different glycerol concentrations, 150 rpm, 30 °C for 24 h.

Crude glycerol (g L^{-1})	Biomass (g L^{-1})	Crude protein (%)
20.0	8.43^{bc}	31.0^{ab}
22.5	8.35^{c}	30.0^{b}
25.0	9.34^{a}	33.0^{a}
27.5	8.7^{bc}	30.0^{ab}
30.0	8.97^{ab}	31.3^{ab}

*Equal letters at the same column do not differ according Tukeys test at 5% significance.

Source: The authors.

Conclusion

Almost all the yeasts studied in this work ehxibited good growth rates and SCP production, evidencing that crude glycerol can be used as a carbon source for biomass production. Besides, *W. anomalus* CCC32 was the strain that showed the best characteristics for production of SCP from crude glycerol as fast growth and production of biomass with high protein content a short time period. The production of biomass and crude protein in mineral medium has shown to be promising for SCP production, which can make the process economically more advantageous. The conversion of crude glycerol into higher value-added products, such as SCP, is important for the valorization and biotechnological application of this by-product from biodiesel industry.

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