



Full Length Research Paper

Determination of optimal concentration of minerals salts and nitrogen sources for *C. utilis* biomass growth in tubers hydrolysate

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The deficiency of protein in human food and animal feed is well recognized due to the rapid growth of population. It is therefore, important to increase protein production by utilizing all the available ways and means. In the light of this, an attempt was made in this study by selecting *Candida utilis* NOY1. This yeast was used to determine the optimum concentration of different minerals salts and nitrogen source on growth. This study aimed at improving the medium composition for efficient and high yield yeast biomass production using tubers wastes. Proximate analysis of the biomass revealed that the protein and nucleic acid content were 54.8 and 4.6%, respectively. Amino acid profiles were found to be comparable to those of the Food and Agriculture Organization of the United Nations reference. This study shows that tubers wastes supplemented with peptone and yeast extract could be used as a good production medium for large scale production of yeast biomass and *C. utilis* NOY1 possesses a high protein value and can be used as a better choice for single cell protein production.

Key words: Mineral salts, *Candida utilis*, biomass, tubers, wastes.

INTRODUCTION

Fermentation comprises the oldest and largest application of microbial technology. It is a biotechnological application with modern forms of industrial production utilizing living organisms, especially

microorganisms, and their biological processes. It involves conversion of carbohydrates and related components to end products such as acids, alcohols and carbon dioxide (Bamforth, 2005). The main organisms

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employed in food fermentations are lactic acid bacteria and yeast. Yeasts are common microorganisms which can grow on fruit and vegetable processing wastes. Several studies were done on bio-transformation of different agricultural waste into value-added products such as yeast biomass from salad oil manufacturing wastewater (Zheng et al., 2005a), ethanol production from non-sterilized beet molasses (Patrascu et al., 2009), biomass production from rice straw and tubers wastes hydrolysate (Zheng et al., 2005b; Ouédraogo et al., 2012a), and cultivation of the yeast on pineapple cannery effluent (Rosma et al., 2005). Yeast from genera *Candida*, *Saccharomyces*, *Torulopsis* and *Lipomyces* are able to transfer carbon source from carbohydrate to lipid, while the protoplasm contains high quantity of glyceride, phospholipid and ergosterol. Elevated levels of inorganic electrolytes in an otherwise satisfactory liquid growth medium have been found to influence several parameters of yeast activity. First, cell growth and multiplication: (a) the number of viable yeast cells per unit volume of liquid growth medium decreases as salt content increases, (b) the biomass of the culture (that is, the total weight of yeast cells per unit volume of liquid growth medium) decreases as salt content increases, and (c) the length of the lag phase (that is, the incubation period between inoculation of the culture and detectable initiation of cell growth) lengthens as salt concentration increases. Second, utilization of the primary carbon and energy source is reduced. Third, change in concentration of metabolic products: (a) there is a decrease in the production of ethanol as salt content increases and (b) there is an increase in the concentration of other fermentation products (such as glycerol, acetaldehyde, etc.) as salt content increases. The yeast *Candida utilis* has been used for the production of a number of biologically useful materials, such as amino acids, RNA, glutathione, NAD, and coenzyme A (Boze et al., 1992; Rosma et al., 2005). It has long been recognized as a useful food resource through the production of single cell protein (SCP) and is approved as a food additive by the FDA. *C. utilis* NOY1 can assimilate cheap biomass-derived sugars, such as sugar molasses and spent sulfite liquor, and a broad spectrum of compounds as nitrogen sources.

The main objective of this study was to determine the optimal concentration of minerals salts and nitrogen sources for *C. utilis* biomass growth in tubers hydrolysate medium and to evaluate the nutritional value of this biomass.

MATERIALS AND METHODS

Organism

The fermenting organism was *C. utilis* NOY1 isolated from potatoes wastes in Burkina Faso. Some of this yeast strain growth characteristics are known. Optimal pH and temperature are respectively 5 and 30°C (Ouédraogo et al., 2012b).

Medium and fermentation process

The culture medium used for the minerals salts and nitrogen effect on yeast strain was a semi synthetic medium (Lagzouli et al., 2007a). The media (broth and agar medium) were sterilized by autoclaving for 15 min at 121°C, initial pH being fixed before sterilization at 5 with HCl 0.1 N. Chloramphenicol (0.5 g/L) sugars, salts and nitrogen sources sterilized by filtration with Millipore filter (0.45 µm) were aseptically added. Yeasts strains isolated on SABOURAUD CAF AGAR were mended in the semi synthetic broth. After 24 h incubation at 30°C, the yeast isolates were streaked on the surface of the solid SABOURAUD medium. Depending on the parameters to test and using the various concentrations of minerals salts and nitrogen, fermentation was poured into a fermentor covered with aluminium foil. The fermentor and contents were cooled to 30°C and the suspension of yeast cells was added. The loaded fermentor was then placed in a 30°C for 72 h in constant temperature bath, and the mixture was stirred at 150 rpm to keep the cells dispersed. To determine the effect of mineral salts and nitrogen sources on yeast growth, each parameter varies according to the different concentrations tested. At the same time, the other parameters remain constant.

Effects of initial $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ concentration on biomass production

In order to determine the effects of initial $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ concentration of the fermentation medium on the growth and yeast biomass production kinetics of the yeast strains, $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ concentration was changed between 0.1 and 0.5 g L⁻¹ (Ibrahim and Lee, 1993).

Effects of initial $(\text{NH}_4)_2\text{SO}_4$ concentration on biomass production

To determine the effects of initial $(\text{NH}_4)_2\text{SO}_4$ concentration of the fermentation medium on the growth and biomass production kinetics of the *C. utilis*, varies concentrations of $(\text{NH}_4)_2\text{SO}_4$ were taken from 0.4 to 0.8 g L⁻¹ (Ibrahim and Lee, 1993).

Effects of initial MgSO_4 concentration on biomass production

For the optimization of initial MgSO_4 , concentrations of MgSO_4 ranged between 0.02 and 0.12 g L⁻¹ were taken and fermentation was carried out. After 72 h of incubation, cell biomass was evaluated (Ibrahim and Lee, 1993).

Effects of initial FeSO_4 concentration on biomass production

In order to determine the effects of initial FeSO_4 concentration of the fermentation medium on the growth and yeast biomass production kinetics of the yeast strains, FeSO_4 concentration was changed between 0.001 to 0.005 g L⁻¹ (Ibrahim and Lee, 1993).

Effects of initial KCl concentration on biomass production

The effects of initial KCl concentration of the fermentation medium on the growth and yeast biomass production kinetics of the yeast strains were determined by changing KCl concentration between 0.7 and 1.2 g L⁻¹ (Ibrahim and Lee, 1993).

Effect of nitrogen source (peptone and yeast extract) on *C. utilis* NOY biomass production

Two nitrogen sources such as yeast extract and peptone, respectively were amended separately into the basal medium at different concentrations of (0, 0.25, 0.5, 0.75 and 1%) (W/V). Inoculation was done and the cultures were incubated at room temperature (30°C). After 72 h incubation in an optimal condition, the biomass production was quantified by measurement of the optical density at 540 nm and yeast dry biomass was evaluated (Ibrahim and Lee, 1993).

Biomass of yeast cells

After 72 h of fermentation, the concentration of yeast cells in the fermenting mash was measured by the turbidimetric (absorbancy) method. Cells were harvested by centrifugation at 16000 rpm for 20 min (Olsson and Nielsen, 1997), washed twice with distilled water and dried in an oven at 50°C for 48 h. After 48 h, dry cells were weighed. A standard curve of optical density versus yeast dry weight (grams per liter) that covered the appropriate range of concentrations was made with a series of six suspensions prepared from the dry, packaged yeast cells.

Optimization of yeast biomass culture conditions and biomass value evaluation

Fermentation medium and process

The inoculum for 900 ml of growth medium was prepared by the suspension. A synthetic medium was used for yeast culture. The fermentations were carried out in 2-L Pyrex glass jars closed with aluminum foil, with approximately 900 ml of working volume. Growth medium, a synthetic medium was the basal fermentation medium. This synthetic medium is composed of tubers wastes hydrolysates with supplement (0.2 g.L⁻¹ KH₂PO₄; 0.02 g.L⁻¹ MgSO₄; 0.77 g.L⁻¹ CH₄NO₂; 0.5% yeast extract) and 1 ml of oligoelements solution. All supplemented substances were used in their optimum concentration tested. The initial pH of the medium was adjusted to 5 with 0.1 M of HCl before sterilization. The inoculum was prepared in Erlenmeyer flasks in a volume corresponding to 10% of fermentation broth medium (90 ml) and incubated 30°C at 150 rpm during 16 h of incubation (Lagzouli et al., 2007b). Pre-culture medium was made by transplanting previously cultivated yeast strains on solid medium by taking pure colonies of yeast in 100 ml of liquid medium. The final fermentation medium contained 5 g.L⁻¹ of glucose.

Nutritional value of SCP

Nutritional value of the product was estimated on the basis of total protein content, amino acid profile, total carbohydrates and ash content on the dried yeast biomass. The concentration of yeast cells in the fermenting mash was measured using the turbidimetric (absorbance at 600 nm) method and by determining dry weight of yeast cells (Lagzouli et al., 2007b). Nitrogen content of yeast biomass was determined by micro-Kjeldhal method. Total carbohydrates and ash were analyzed using the procedure mentioned in AOAC methods (AOAC, 2006). The crude protein values were obtained by multiplying the nitrogen content by 6.25 (Mateles and Tannenbaua, 1968).

The total amino acid content analysis was carried out after hydrolysis with 6N HCl at 110°C for 24 h in a Biotronic LC-300 Amino Acid Analyser and lipid by chloroform-methanol method (Badid et al., 2001). Tryptophan was determined after

methanesulphonic acid hydrolysis. Cysteine was determined by performic acid oxidation. Nucleic acid was determined according to the method described by Sambrook et al. (1989).

Statistical analysis

Analysis was performed in triplicate except for total nitrogen content analysis, where duplicate analysis was carried out. The data was subjected to Duncan's Multiple Test of Statistical Package for Social Science, Version 12 (SPSS Inc., Illinois USA), where $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Effect of mineral salts on *C. utilis* NOY biomass production

Yeast biomass production depends on minerals salts concentrations. For the different minerals salts tested, yeast biomass increases when minerals salts concentration increases till they reach a maximum value. After that, increasing minerals salts concentration is not a limited factor for biomass production, leading to a decreasing yeast biomass. Various mineral salts [(NH₄)₂SO₄, MgSO₄.7H₂O, FeSO₄.7H₂O, KCl, KH₂PO₄] at different concentrations were tested on the biomass formation by *Candida utilis*. The effect of the different mineral salts is as shown in Figure 1a, b, c, d, e and f.

MgSO₄.7H₂O at the concentration of 0.07 g/L was found to be the most effective, enhancing biomass production to about 8.29 g.L⁻¹. Other mineral salts resulted in varied quantities of biomass production from 2.36 to 7.57 g.L⁻¹. Phosphate can enhance or suppress the production of growth at different concentrations.

According to Pathissery et al. (2013), the optimum magnesium sulphate concentration was found to be 0.25%. A further increase of phosphate showed no significant effect on cell yield. In the present study, the optimum phosphate concentration was found to be in the range 0.2 to 0.3%.

Based on the experiment results, the conclusion that can be drawn was mineral salts may exhibit enhancing or inhibiting effects depending on the concentration. In most cases, higher concentration of mineral salts was inhibitory for biomass formation.

The bio-elements are one of the important factors affecting biomass production in several microorganisms (Stanly et al., 2013). Some of them such as K⁺, Mg²⁺ and Zn²⁺ ions played a significant role in the increase of biomass production (Stanly and Pradeep, 2013). Metal ions have been shown to significantly affect production of various industrially important products like vitamins, biomass, organic acids, enzymes, etc (Wei et al., 2007). Production of extracellular enzymes is greatly influenced by the presence of various components in the media that include inducers, carbon and nitrogen substrates and trace elements. Yeasts require a range of metals for

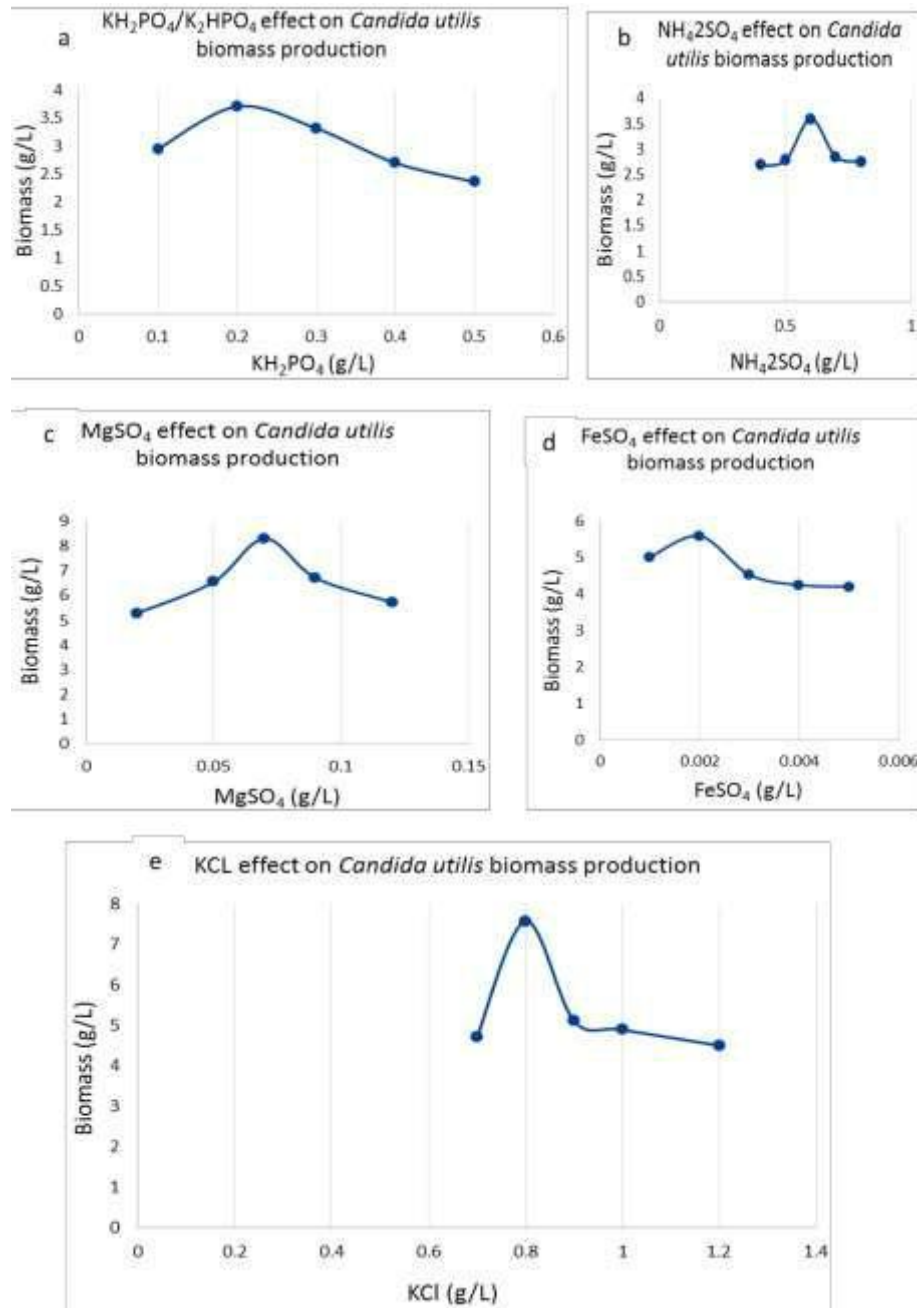


Figure 1. Effect of minerals salts on *Candida utilis* NOY1 biomass production.

optimal growth, metabolism and fermentation performance. The requirement for metal ions varies, so widely with the different strains that it is necessary to adjust the composition of the medium to avoid the inhibitory effects of others.

Effect of nitrogen source on *C. utilis* NOY biomass production

Growth of *C. utilis* was found to be influenced by the

concentration of nitrogen source in the medium. With peptone, the maximum growth of yeast biomass (3.25 g.L^{-1}) was observed at the concentration of 0.75%. However, with yeast extract, the concentration of 0.5% gave the maximal yeast biomass (4.56 g.L^{-1}). A gradual increase in growth could be observed with the increase in nitrogen source. However, after the optimum concentration, nitrogen source was found to have adverse effect and resulted in lesser growth. The different effect of yeast extract and peptone in *C. utilis* biomass production is as shown in the Figure 2.

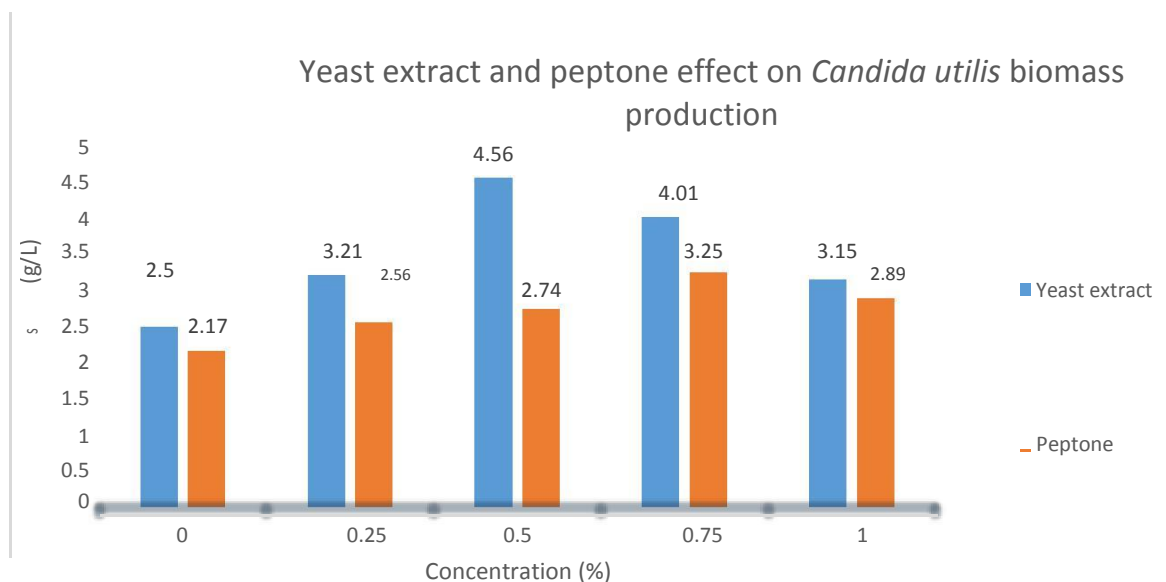


Figure 2. Effect of nitrogen source on *Candida utilis* NOY1 biomass production.

Media composition plays a vital role in the improvement of efficiency and economics of microbial fermentation (Nancib et al., 2001). As biomass of yeast are mainly contributed by carbon, hydrogen, oxygen and nitrogen sources from the provided medium (Bamforth, 2005), hence, when the biomass yield increases with nitrogen supplementation, substrate usage will increase concurrently. From the data obtained, similar trend of substrate consumption for all type of nitrogen supplements can be observed.

Generally for bacteria, a concentration of 0.5 to 1% is incorporated in media. In the present study also, the optimal concentration falls within this range. Yeast growth medium require more amount of yeast extract in it than that required in bacteriological media. The presence of hydrolyzed yeast components would be definitely supporting good growth. It was found that cell dry biomass of *C. utilis* was increased with the addition of peptone and yeast extract to the medium, and reached to a maximum when it was 0.5 and 0.75% (Figure 2). In this study, from the two nitrogen sources, yeast extract gave the best biomass concentration (4.56 g.L^{-1}).

Nutritional value of *C. utilis* biomass

The amino acid composition of the yeast biomass is shown in Table 2. The amino acids were fairly well represented when compared with those of the FAO reference.

Chemical composition and amino acid analysis of the biomass of *C. utilis*

Elementary analysis of the biomass is shown in Table 1. Protein, lipid and carbohydrate content were 54.8, 15.12

Table 1. Nutritional value of *Candida utilis* NOY biomass

Components	Nutritional value
Protein (% N \times 6.25)	54.8 ± 0.12
Lipid	15.12 ± 0.98
Carbohydrate	2.8 ± 0.2
Ash	8.1 ± 0.18
Amino acids	36.12 ± 1.18
Nucleic acid	4.6 ± 1.26

Table 2. Amino acid analysis of *Candida utilis* NOY1 biomass.

Amino acid	<i>Candida utilis</i> biomass	FAO/WHO reference
Phenylalanine	4.1 ± 0.25	-
Tyrosine	2.4 ± 1.20	-
Methionine	1.58 ± 2.1	-
Cysteine	-	-
Valine	5.5 ± 0.15	5
Threonine	4.1 ± 0.58	-
Tryptophan	3.9 ± 0.78	-
Isoleucine	4.8 ± 0.99	4
Histidine	-	-
Arginine	3.2 ± 1.12	-
Leucine	7.12 ± 1.64	7
Lysine	5.14 ± 0.82	5.5
Aspartic acid	8 ± 0.72	-
Glutamic acid	15.3 ± 0.66	-
Serine	3.6 ± 1.02	-
Glycine	3.8 ± 1.96	-
Alanine	6.9 ± 0.58	-
Proline	2.8 ± 0.23	-

Data are expressed as g per 100 g protein.

and 2.8%, respectively.

The protein content of most moulds and fungi used in single cell protein production vary considerably from 26 to 55% (Litchfield, 1979). Our results were higher than the results of other authors on *Saccharomyces cerevisiae* (Hezarjaribi et al., 2016; Esabi, 2001). High protein content of 54.8% using optimum culture suggests that *C. utilis* is a good commercial case for single cell protein production.

Ash content of *C. utilis* was estimated at 8.1%. Our results were similar to those reported by Jaganmohan et al. (2013) but lower than those reported by Esabi (2001). The ash content of *C. utilis* was higher than in the present observation when SCP was produced using Ram Horn Hydrolysate (RHH) (Konlani et al., 1996) and when rice polishing was used as substrate (Ibrahim et al., 2004).

One significant result was the low content of nucleic acid of about 4.2%. In a study done by Ravinder et al. (2003), the nucleic acid content of *Aspergillus oryzae* mutants when deoiled rice bran was used as substrate for the production of SCP was higher than in the present study. *C. utilis* in the present study showed much lesser amount of nucleic acid content than nucleic acid content reported in *Candida utilis* in RHH medium and *Candida krusei* (Esabi, 2001). The nucleic acid content is a bit higher than *Kluyveromyces fragilis* when the biomass was produced on deproteinized whey supplemented with 0.8% diammonium hydrogen phosphate and 10 ppm indole-3-acetic acid (Konlani et al., 1996). The low content is a positive indication for use as a protein source in animal feeds. While most microorganisms contain nucleic acid between 6 and 15% (Goldberg, 1985), the low content in *C. utilis* is a very interesting result for animals feed.

The total amino acid is 36.12%, that is, comparable with the results of Jaganmohan et al. (2013). The details amino acid composition *C. utilis* and FAO reference protein (Anupama, 2000) are shown in Table 2. The biomass obtained from the yeast contained all the essential amino acids. The amino acids were fairly well represented when compared with those of the FAO reference. The nutritional value, which awaits proper evaluation by way of animal feeding experiments, appeared theoretically comparable to that of other fungi used for single cell protein production (Anderson et al., 1975). Essential amino acid concentrations were somewhat lower than the FAO reference protein. Among the amino acids, glutamic acid was the most abundant. It was reported that the potential nutritional value of SCP is determined with amounts of lysine and methionine amino acid (Tauk, 1982; Malathi and Laddha, 1989). Therefore, the biomass obtained may be suitable for human and animal consumption.

Based on the results gathered so far, it was strongly concluded that the biomass which is non-toxic and non-pathogenic, can be a potential source of protein. Some of the highlights of the findings will be reported elsewhere.

Conclusion

Supplementation of nitrogen source and minerals enhanced the yeast biomass production. Tubers hydrolysates supplemented with peptone, yeast extract and minerals can be used as a good fermentation medium for large scale production of yeast biomass for application in food and feed industry. Among the type of nitrogen sources evaluated, yeast extract are the best compared to peptone.

The complete acceptability of yeasts as animal nutritional supplement needs to be assessed before making any positive declaration. Research on the digestibility and acceptability of the biomass by the test animals should be performed. However, the present study in view of limited time frame, was restricted to the assessment or/and improvement of yeast protein content and nutritional profile, so that further work can be taken up in due course. Based on the results gathered so far, it was strongly concluded that the biomass which is non-toxic and non-pathogenic can be a potential source of protein.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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