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ORIGINAL PAPER

Application of Industrial Wastes for the Production of Microbial Single-Cell Protein by Fodder Yeast *Candida utilis*

Agnieszka Kurcz[1] · Stanisław Błażejak[1] · Anna M. Kot[1] · Anna Bzducha-Wróbel[1] · Marek Kieliszek[1]

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Abstract
Purpose The aim of this study was to determine the use of glycerol as a source of carbon and the use of deproteinized potato wastewater as a source of nitrogen and mineral components for the production of biomass by fodder yeast *Candida utilis*.
Methods The yeast strain *Candida utilis* ATCC 9950 was used in this study. Experimental media contained potato wastewater and glycerol at concentrations of 5, 10, 15, 20, and 25% (w/v). Control medium was YPD and potato wastewater. During the 72 h of cultivation (200 rpm, 28 °C), the optical density (spectrophotometric method), biomass yield (weight method), glycerol (chemical method), and protein (Kjeldahl's method) concentration in the experimental media, protein content in the biomass (Kjeldahl's method) and chemical-oxygen demand index (dichromate method) in selected culture media were determined.
Results *Candida utilis* was able to grow in the medium composed of potato wastewater and glycerol. Addition of 5% of glycerol gave a biomass yield above 30 $g_{d.w.}$/L and the efficiency of protein biosynthesis was 12.2 g/L. The results showed that the cultivation *C. utilis* in this medium caused a significant reduction of glycerol (83%), nitrogen present in potato wastewater (51%), and a high degree of COD index reduction (91%).
Conclusions The study demonstrated that potato wastewater supplemented with glycerol may be successfully used as a carbon source in the production of fodder yeast *C. utilis* ATCC 9950 biomass. In addition, the results show that these two troublesome industrial wastes could be efficiently used in such processes.

Keywords SCP · Glycerol · Potato wastewater · Wastes utilization

Abbreviations

COD	Chemical oxygen demand
SCP	Single-cell protein
PW	Potato wastewater

Introduction

The concept of biomass production and the use of microorganisms as a food ingredient for humans and animals were established at the beginning of the twentieth century. The term "single-cell protein" (SCP) were coined at the Massachusetts Institute of Technology in 1966 to introduce the idea of using the biomass of unicellular organisms as a food source [1]. The idea was conceived as a consequence of rapid population growth, particularly in developing countries. Thomas Robert Malthus, a British demographer and economist, said that "although the human population increases at a geometric rate, whereas the food supply growths only at an arithmetic rate". Such a criterion creates a gap between the supply and demand for proteins [2], which urges us to look for new, alternative sources of proteins. It is also desirable that the food produced should be highly nutritious, cheap, and rapidly synthesized. Proteins of microbial origin fit these criteria,

✉ Anna M. Kot
kotannamaria@gmail.com; anna_kot@sggw.pl

[1] Department of Biotechnology, Microbiology and Food Evaluation, Faculty of Food Sciences, Warsaw University of Life Sciences, Nowoursynowska 159C, 02-776 Warsaw, Poland

and among the various groups of microorganisms, yeast strain *Candida utilis* has gained wide attention.

Synonyms for *Candida utilis* species include *Pichia jadinii*, *Saccharomyces jadinii*, *Torula utilis*, and *Cyberlindnera jadinii* [3, 4]. This yeast fulfills all the criteria of fodder yeast. It is characterized by a high content of protein in the dry matter, has the ability to use a variety of nutrients in the medium, and has a shorter generation time [5]. Moreover, *Candida utilis* yeasts have been approved by FDA (Food and Drug Administration) as safe for consumption and have been added to the American GRAS list (Generally Recognized as Safe). To this end, this yeast biomass, as well as their metabolites, may be used both in the food industry and in the fodder production [6].

Studies have shown that the yeast protein has a high content of essential amino acids, particularly lysine [7, 8]. The cell biomass obtained from the *Candida utilis* yeasts contains vitamins B complex, such as riboflavin, folic acid, and nicotinic acids [8, 9]. This species is also able to synthesize ergosterol, a compound that belongs to the steroid family, which is an immediate precursor of vitamin D_2 [10]. Furthermore, due to its ability to bind metal ions from the culture medium, often in amounts exceeding the natural demand, this fodder yeast may be used to produce protein and mineral preparations that can be readily consumed by humans [8].

Fodder yeasts, a class of wild yeast, consist of a rich enzymatic system that enables them to grow on various carbon sources. They are able to assimilate many organic compounds other than carbohydrates, such as alcohols, organic acids, and hydrocarbons [7]. This provides a possibility for various waste materials, such as molasses and vinasse, wood hydrolysates, waste sulfite liquors, starch and lignin–cellulose wastes, whey or post-spirit decoction from the industrial distilleries, to be used as media for growing yeasts [5, 11]. The use of waste materials not only reduces the cost of yeast biomass production but also enables their economic utilization.

In addition, the wastes of the potato- and starch-processing plants can be used as nitrogen sources [12, 13]. The production of potato starch generates a large amount of wastes, mainly potato wastewater (about 600 tons per 1000 tons of potatoes processed). In order to use this, for example as a fertilizer, it has to be first subjected to a coagulation process (deproteinization). This method may raise some environmental concerns due to the high pollution load of deproteinized potato wastewater. As an alternative [14] such as biotechnological processes [13, 15] could be used to dispose this waste.

Keeping in mind the ecological and economic impact, studies in recent years are using glycerol in the production of fodder yeast biomass. Glycerol is a good source of carbon for some microorganisms, and is the precursor of many valuable metabolites (e.g., dihydroxyacetone, propionic acid, and 1,3-propanediol) [16]. Previous studies have shown that glycerol can be successfully used in the production of fodder yeast biomass, where it acts as the only source of carbon [11, 17, 18].

The aim of this study was to examine the growth and protein production ability of *Candida utilis* ATCC 9950 yeasts in media containing different concentrations of glycerol as the sole source of carbon and potato wastewater as a source of nitrogen and minerals.

Materials and Methods

Microorganisms

Candida utilis ATCC 9950, a strain of folder yeast was used in this study, and was obtained from the American Type Culture Collection.

Potato Wastewater

Deproteinized potato wastewater was obtained from the starch factory PEPEES SA in Łomża (Poland). Dry matter content was determined by weight method (105 °C/24 h). Total protein content was determined by the Kjeldahl method and the factor 6.25 was used to convert nitrogen per protein [14]. The content of reducing substances was determined spectrophotometrically ($\lambda = 550$ nm) with the use of 3,5-dinitrosalicylic acid [19]. The chemical-oxygen demand index was determined using the dichromate method (Hach Lange analyzer and cuvette tests LCK014) in Water Center of the Warsaw University of Life Sciences.

Dry matter content in the deproteinized potato wastewater was found to be 3.58%. It included about 0.3% of reducing substances, constituting the carbon source for the yeast, and 0.9% of total protein being the source of nitrogen. The COD index, characterizing the degree of wastewater contamination, was 28.5 g O_2/L.

Culture Media and Conditions

Candida utilis yeast was cultivated in two control media: one medium consisted of YPD (BTL, Poland) containing 2% glucose, 2% peptone, and 1% yeast extract (w/v) [20], and the other medium contained only potato wastewater. The experimental media included potato wastewater with varied amounts of glycerol as a source of carbon (Avantor Performance Materials, Poland) –at concentrations 5, 10, 15, 20, and 25% (w/v). The pH value of all media was 5.0.

The control and experimental media were inoculated with 10% (v/v) 24-h *inoculum* of the yeast proliferated at a

temperature of 28 °C in YPD medium (pH 5.0). Ten milliliter of inoculation culture was collected and centrifuged (2600×*g*/10 min; Eppendorf Centrifuge 5804 R; Germany). The supernatant was discarded, and the tube was thoroughly rinsed with 10 mL of sterile distilled water to discard the remaining biomass. The suspension obtained was again centrifuged using the same procedure and the supernatant was removed. A total of 10 mL of the culture media (control or experimental) was added to the centrifuged yeast biomass. The content was thoroughly mixed and the suspension was quantitatively transferred to a 90-mL flask.

Control and experimental cultures were carried out in 500-mL flat-bottomed flasks containing 100 mL of medium at 28 °C for 72 h in a reciprocating shaker (Edmund Bühler SM-30 Control, Germany) at an amplitude of oscillation of 200 cycles/min. The experiment was carried out in triplicate.

Biomass Yield and Optical Density of the Culture (OD)

To determine the yield of biomass, 10 mL of medium was collected, centrifuged (5600×*g*/10 min), and then washed with 10 mL of distilled water to wash out to remove any glycerol. The resulting biomass was dried (85 °C/24 h) until a constant weight was obtained. The dried biomass was weighed and the results were expressed in $g_{d.w.}$/L of culture medium [21].

The optical density of the culture (OD) was determined by spectroscopic method ($\lambda = 600$ nm) based on a previous work [21].

Determination of Total Protein Content

The purpose of this analysis was to determine the total protein content in the biomass of the yeast cell, as well as the changes in total protein content in the control and experimental media of the studied yeast culture. The analysis was carried out following Kjeldahl's method, and the nitrogen content was converted into total protein content using the coefficient factor 6.25 [14, 22].

Determination of Total Glycerol Content

Glycerol concentration was analyzed at particular time period during cultivation to determine the degree of its utilization as a carbon source by yeasts. The reaction involves oxidation of two adjacent hydroxyl groups present in the glycerin molecule affected by *meta*-periodic acid activity. As a result, the carbon chain is broken, and one molecule of formic acid and two molecules of formaldehyde are formed. The formic acid thus formed was determined by acid–base titration according to Branch Standard [23].

Determination of COD Index

The COD index was determined before and after cultivation in order to assess the degree of utilization of potato wastewater and glycerol. This index has been determined following the method described in "*Potato wastewater*".

Statistical Analysis

Statistical analysis was performed using STATGRAPHICS Plus v.5.1 software. One-way analysis of variance (ANOVA) and Tukey's test ($\alpha = 0.05$) were used to establish how various types of medium affected the differentiation in yeast growth (in the range of biomass yield), the protein content in the cells, as well as the degree of utilization of glycerol and nitrogen compounds from experimental media.

Results and Discussion

Characteristics of the *C. utilis* Yeast Growth

The growth of *Candida utilis* in control and experimental media was determined by calculating the biomass yield. The biomass yields obtained during 72 h of culture are presented in Fig. 1.

In all culture variants, the initial biomass yield (directly after media inoculation) was similar and ranged from 1.00 to 1.29 $g_{d.w.}$/L. In case of the control YPD medium, an intense growth of biomass yield up to 13.00 and 15.93 $g_{d.w.}$/L after 24- and 48-h of cultivation, respectively, was found. However, after 72 h, a decrease in biomass yield was recorded (12.57 $g_{d.w.}$/L). This may be due to yeast cell autolysis. Because optimal yeast development was observed at this medium composition [20], the results obtained may be considered as typical for the studied *Candida utilis* ATCC 9950 strain.

In the medium composed only potato wastewater, it was observed that biomass yield increase from 1.18 to 5.65 $g_{d.w.}$/L after the first day and the yield remained at the same level until the end of the yeast cultivation. The decreased growth and the corresponding decrease in biomass yield, compared to YPD medium, might be probably due to the low sugar content (0.3%) that was the only available carbon source for the yeast. The medium with potato wastewater and 5% glycerol showed an intense increase in biomass yield. The biomass yield obtained on the first day was 7.24 $g_{d.w.}$/L, and after 48 h of cultivation was 18.51 $g_{d.w.}$/L. This value was higher than the yield obtained in

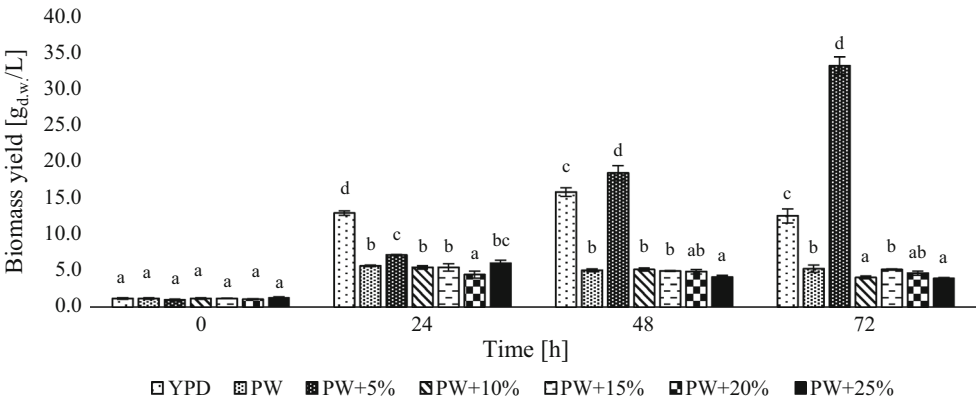

Fig. 1 The yield of *Candida utilis* ATCC 9950 yeast biomass obtained during submerged culture in control and experimental media (*PW* potato wastewater; 5–25%, glycerol content). The *same-letter* index means lack of significant difference (Tukey's test performed at the same hours of cultivation, $\alpha = 0.05$)

YPD medium (15.93 $g_{d.w.}$/L). A further increase in biomass yield was observed during the subsequent hours of cultivation and it reached a value of 33.25 $g_{d.w.}$/L after 72 h. The total biomass yield in this medium increased by more than twofold compared to the yield in the control YPD medium and by more than sevenfold compared to the medium that composed only of potato wastewater. Based on this result, it can be concluded that addition of glycerol, an additional carbon source in the medium with potato wastewater, beneficially affected the growth of the studied yeast strain *Candida utilis*. Moreover, the medium that composed of potato wastewater with 5% glycerol resulted in a higher growth of yeast than the control YPD medium, which is considered optimal.

The biomass yield obtained from all the other media with glycerol concentrations that ranged from 10 to 25% was similar to that obtained from medium that consisted of only potato wastewater. In all variants of these four media, an initial value of biomass yield (from 1.15 to 1.29 $g_{d.w.}$/L) increased up to 4.49–5.49 $g_{d.w.}$/L. Therefore, *Candida utilis* ATCC 9950 yeasts grown in media with higher glycerol concentrations (above 10%) were unable to utilize the carbon source and assimilated mostly the components of potato wastewater. This might be probably due to the high osmotic pressure of the culture environment, which reduced the vitality of the studied yeast [14].

Based on the results of the optical density (OD), growth curves were determined for *C. utilis* yeast during cultivation in the control and experimental media.

The logarithmic phase lasted for 24 h and a OD value of 2.145 was obtained for control medium that consisted of potato wastewater. However, the yeast growth was found to be much lower than in the control medium YPD (OD = 2.727). After 24 h, the yeast had entered stationary phase in moth media and the OD values remained the same until the end of the cultivation. A different scenario was observed for the medium with potato wastewater and 5% glycerol. After 24 h of cultivation, the optical density was 2.245. Between 24 and 30 h the rate of growth decrease. In turn, after 30 h, a reacceleration in growth and transition to the next logarithmic phase were observed (Fig. 2). In this medium diauxic growth caused by the presence of at least two carbon sources was observed. *C. utilis* yeast utilized reducing sugars from the medium reaching the end of the logarithmic phase after 24 h of cultivation, as in the case of the medium that composed entirely of potato wastewater. However, no stationary growth phase was observed for the medium with 5% of glycerol. During a brief phase of slow growth (between 24 and 30 h), the yeasts probably oriented their metabolism to use other source of carbon such as glycerol present in the medium. After 72 h of cultivation, the OD value was 1.5-fold higher than in the medium that contained only potato wastewater.

The growth curves obtained for the media with higher glycerol concentration (above 10%) was found to be very similar to that obtained for the culture medium with potato wastewater. This result is in agreement with the results obtained for biomass yield, which indicates that the yeasts in an environment with higher concentration of glycerol are not able to assimilate this compound from the culture medium.

Błażejak et al. [24] used glycerol and potato wastewater as the media to cultivate oleaginous yeast *Rhodotorula gracilis*. The highest biomass yield of the studied yeast was obtained in the medium with 5% glycerol addition. Increasing concentrations of glycerol showed a reduction in biomass yield. These authors also reported that higher glycerol concentrations inhibited the yeast growth in the culture medium.

Yen et al. [25] used a medium composed of 3% glycerol as carbon source and the main waste from rice wine production (decoction, so called "thin stillage") as a source of nitrogen compounds and minerals for producing *Rhodotorula glutinis* BCRC 22360 yeast biomass. The biomass yield obtained was compared to the control culture, which contained 3% glycerol like the experimental medium but the decoction was replaced by yeast extracts and mineral salts. After 48 h of cultivation, a higher biomass yield was

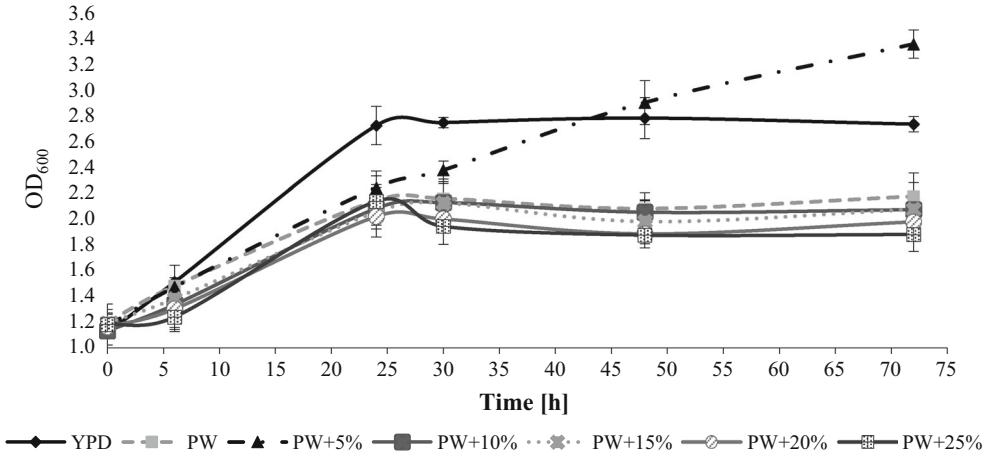

Fig. 2 Changes in optical density of culture *Candida utilis* ATCC 9950 yeast during cultivation in control and experimental media (*PW* potato wastewater; 5–25%, glycerol content)

obtained from the medium containing waste decoction—about 9.2 $g_{d.w.}$/L. In turn, biomass yield obtained from the control medium was 8.1 $g_{d.w.}$/L. The authors emphasized that the decoction contained high amounts of nutrients (proteins, amino acids, growth factors) which resulted in a higher biomass yield of the studied yeast compared to the standard medium containing yeast extract. Therefore, appropriately selected industrial waste may be used as substitutable sources of nitrogen and nutrients for yeast biomass production.

Protein Content of the *C. utilis* Yeast Biomass

Protein is one of the major components of yeast cell biomass and an important parameter in the evaluation of the culture method, especially in the case of fodder yeasts and SCP production [26]. Therefore, in this study, the protein content was determined both in control and experimental media, and the protein yield was calculated per liter of the medium.

A decrease in the protein content of the yeast biomass was found for most of the media during cultivation. The lowest changes were recorded for control YPD medium, where protein content decreased by up to 43.5 g/100 $g_{d.w.}$ after 72 h. Similar protein content was obtained from the culture medium that contained potato wastewater. After 72 h of cultivation, protein content decreased by up to 41.7 g/100 g $g_{d.w.}$. A similar tendency was observed for the glycerol media at concentrations of 5 and 10%. In turn, a slight increase in the protein content was observed for glycerol media at concentrations of 15, 20, and 25% at 72 h of cultivation (Table 1).

However, it should be highlighted that lower values of total protein of cell biomass of *Candida utilis* yeasts were observed for the media with glycerol compared to YPD medium and medium composed only of potato wastewater. In these two control media, the protein content remained above 40 g/100 $g_{d.w.}$. In turn, in the experimental medium with 5% glycerol, the protein content obtained after 72 h of cultivation was 36.7 g/100 $g_{d.w.}$, whereas in that with higher glycerol concentrations (10, 15, and 20%), the protein content was low, ranging from 32 to 36 g/100 $g_{d.w.}$. In the case of medium supplemented with 25% glycerol, it was even lower (28–30 g/100 $g_{d.w.}$). Therefore, it might be argued that an increasing concentration of glycerol negatively affected the biosynthesis of protein compounds, reducing their presence in the dry matter of the studied yeast cell biomass. In the case of medium that had a lowest concentration of glycerol (5%), a lower percentage of total protein was observed compared to that of the YPD medium and the medium that contained only potato wastewater. However, it is worth noting that the medium with 5% glycerol resulted in a high biomass yield (33.25 $g_{d.w.}$/L) which in turn resulted in a high protein yield (12.2 g/L). Based on this result, it can been concluded that the most effective medium for SCP synthesis was the medium with 5% glycerol addition, as a relatively high amount of yeast protein was obtained.

A low total protein content of the yeast biomass from the cultures grown in media supplemented with glycerol was also reported by Juszczyk et al. [11]. Their study involved a medium composed of 3% glycerol, 1% yeast extract, 0.75% peptone, and inorganic salts. *Candida utilis* ATCC 60558 yeasts growth in this medium yielded a protein content in biomass of only 23.7 g/100 $g_{d.w.}$. This value was lower compared to the values obtained from the medium that contained wastewater enriched with 5% glycerol of this study. Low protein contents in ranges 28.5–33.4 g/100 $g_{d.w.}$ were also obtained for other yeast species (*Yarrowia lipolytica*, *Candida robusta*, and

Table 1 Protein content in *Candida utilis* ATCC 9950 yeast biomass and its yield during submerged culture in control and experimental media

Type of culture medium	Time (h)			
	Protein content[a] (g/100 $g_{d.w.}$) ± SD		Protein yield (g/L) ± SD	
	48	72	48	72
YPD	46.1 ± 0.6^{e}	43.7 ± 0.7^{e}	7.3 ± 0.2^{c}	5.5 ± 0.4^{b}
PW	48.9 ± 0.7^{f}	41.7 ± 0.3^{d}	2.5 ± 0.1^{b}	2.2 ± 0.2^{a}
PW + 5% glycerol	40.8 ± 0.8^{d}	36.7 ± 0.5^{c}	7.6 ± 0.3^{c}	12.2 ± 0.4^{c}
PW + 10% glycerol	35.1 ± 0.8^{c}	32.4 ± 0.6^{b}	1.8 ± 0.1^{a}	1.3 ± 0.1^{a}
PW + 15% glycerol	35.7 ± 1.1^{c}	36.4 ± 0.9^{c}	1.8 ± 0.1^{a}	1.9 ± 0.1^{a}
PW + 20% glycerol	33.7 ± 0.9^{b}	35.8 ± 0.6^{c}	1.6 ± 0.2^{a}	1.7 ± 0.1^{a}
PW + 25% glycerol	28.4 ± 1.2^{a}	29.7 ± 1.0^{a}	1.2 ± 0.1^{a}	1.2 ± 0.1^{a}

SD standard deviation

[a] The same-letter index means lack of significant difference (Tukey's test performed at the same hours of cultivation, $\alpha = 0.05$)

Candida tropicalis). Based on this data, it may be concluded that application of deproteinized potato wastewater instead of standard sources of nitrogen compounds and nutrients (yeast extract, peptone, and mineral salts) in glycerol medium could result in a higher protein content of the cells of the studied yeast strain.

In addition, part of the glycerol assimilated by the yeast was probably used in the biosynthesis of other cell components, which in consequence led to a decrease in the proportion of protein components of the yeast biomass. The other cell components may include intracellular fat that has a nutritionally profitable fatty acids profile [27, 28] or polysaccharides that have health-promoting effects on yeast cell membrane [29].

Utilization of Culture Media Components

An important aspect that should be taken into account, in case microorganisms are used in the preparation of food components, is the application of the substrates present in the culture media. This approach allows one to determine the degree of utilization of the waste raw materials applied in the biotechnological processes [24]. Table 2 presents the degree of utilization (%) of glycerol and nitrogen compounds (calculated as total protein) from the culture media.

In experimental media with higher glycerol concentrations (from 10 to 25%), glycerol utilization by yeasts was low and ranged from 0.4% for media with 25% glycerol to 2.6–2.7% for media with 10 and 15% glycerol. It should be emphasized that the growth of yeast in media with higher glycerol concentrations was limited, which was reflected in the value of biomass yield (4.49–6.11 $g_{d.w.}$/L). Yeast growth in these media was similar to the results obtained for the medium containing only potato wastewater, which may suggest that *Candida utilis* yeasts were not able to assimilate glycerol at higher concentrations (above 10%) and they utilized only wastewater components (reducing sugars, nitrogen compounds) for their growth.

In this study, different results were obtained for medium containing wastewater with 5% glycerol. In such medium, the yeast proliferated quickly, reaching high biomass yields (33.25 $g_{d.w.}$/L after 72 h of submerged culture). This might be due to the ability of *Candida utilis* yeasts to assimilate glycerol at low concentrations (5%). The yeast utilized

Table 2 Degree of utilization of glycerol and nitrogen compounds (calculated as total protein) from the media after 72 h of submerged culture of *Candida utilis* ATCC 9950 yeasts

Type of culture medium	Degree of glycerol utilization[a] (%) ± SD	Degree of protein utilization (%) ± SD
PW	–	17.7 ± 0.8^{d}
PW + 5% glycerol	82.7 ± 3.1^{d}	51.2 ± 2.4^{e}
PW + 10% glycerol	2.6 ± 0.3^{c}	2.5 ± 0.2^{b}
PW + 15% glycerol	2.7 ± 0.6^{c}	3.8 ± 0.4^{c}
PW + 20% glycerol	1.4 ± 0.2^{b}	1.4 ± 0.1^{a}
PW + 25% glycerol	0.4 ± 0.1^{a}	2.9 ± 0.3^{b}

SD standard deviation

[a] The same-letter index means lack of significant difference (Tukey's test performed at the same hours of cultivation, $\alpha = 0.05$)

about 83% of the glycerol present in the medium, that is, much more than in the case of other cultures (about 1–3%). Błażejak et al. [24] observed that the yeast demonstrated the highest ability of glycerol assimilation when the glycerol concentration in the medium was 5%. In our study, we observed that the yeasts utilized about 86% of glycerol. In turn, when the concentration of glycerol was 10–20% in the medium, the degree of its utilization was considerably lower (even below 60%).

A similar situation may be observed for the utilization of nitrogen compounds from the culture media. The media that contained glycerol at concentrations ranging from 10 to 25% showed a lower degree of total protein utilization that ranged from 1.4 to 3.8%. In turn, in the medium that composed only of potato wastewater, the yeasts studied utilized about 18% of nitrogen compounds present in the environment. It may be argued that high glycerol concentration not only limited the ability of *Candida utilis* yeasts to assimilate the compound but also inhibited the growth and proper metabolism of this yeast. A strong covering of yeast cells with the carbon substrate was observed in the medium with higher glycerol concentration (above 10%), due to the high viscosity and density of the compound [30]. In consequence, this may hinder the uptake of nitrogen compound by yeasts from the environment.

A highest level of nitrogen compound utilization by the yeasts was observed in the experimental medium with potato wastewater and 5% glycerol. Total utilization of protein compounds from the medium after 72 h of submerged culture was 51.2%, and it was almost threefold higher compared to the medium that composed only of potato wastewater (17.7%). Enhanced total protein utilization from the medium was caused by an intense growth of the studied yeasts, which was concurrently related to the acceleration in their metabolism and an increased nutrient demand. The production of *Candida utilis* yeast biomass in the medium with potato wastewater and 5% glycerol addition enabled a considerable decrease in COD index value. COD reduction in this medium was around 91%; however, to the medium composed of potato wastewater only this index value decreased by 74%.

Conclusions

This study demonstrated that potato wastewater with addition of glycerol as a carbon source may be successfully used in the production of a strain of fodder yeast *Candida utilis* ATCC 9950 biomass. Enrichment of wastewater with 5% glycerol guaranteed a higher yield of the yeast biomass, even at levels above 30 $g_{d.w.}$/L. It was also found that glycerol when added at higher concentrations (from 10 to 25%) inhibited the growth of the studied yeast strain.

Despite the easy availability of glycerol, the biomass obtained was characterized by a relatively low content of protein, which might be due to the metabolism that focuses on the biosynthesis of other cell components and storage substances such as lipids or polysaccharides. However, the highest biomass yield, and by that the highest protein yield, was obtained in the medium with potato wastewater and 5% glycerol.

It should also be emphasized that this method of production of *Candida utilis* ATCC 9950 yeast biomass efficiently utilized troublesome industrial wastes, such as glycerol and potato wastewater, besides being characterized by its significant usage of nitrogen (51%) and glycerol (83%) from the culture medium and its high degree of COD index reduction (91%). Taking into account the intensity of this yeast biomass increase, the cultivation time should be extended to over 72 h, until the carbon substrate is completely utilized.

Compliance with Ethics Standards

Conflict of interest The authors declare no conflict of interest.

Human or Animal Context This article does not contain any studies with human or animal subjects.

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