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Optimization of Yeast Cultivation Factors for Improved SCP Production

Svetlana RAITA[1*], Zane KUSNERE[2], Kriss SPALVINS[3], Dagnija BLUMBERGA[4]

[1–4]*Institute of Energy Systems and Environment, Riga Technical University, Azenes iela 12/1, LV–1048 Riga, Latvia*

***Abstract* – Yeast Single-Cell Proteins (SCP) production using various agro-industrial by-products and wastes have significant potential as an alternative to the soy meal, and fish meal protein used for livestock and aquaculture feeds. The use of organic wastes as a substrate in the fermentation processes can be accepted as one of the solutions to reduce the total price of the culture and an environmentally friendlier method of removing these residues. This review article focuses on the yeast biomass yield and protein content increase strategies, which is impossible without understanding metabolic pathways and switching mechanisms. The present work discusses optimization strategies for protein-enriched yeast biomass production, such as fermentation medium composition, including a selection of carbon and nitrogen sources and their ratio, supplemented trace elements, and cultivation conditions such as pH, temperature, time of cultivation, and inoculum size. This review summarizes the theoretical knowledge and experimental results of other researchers that provide an overview of the achievements of the last decades in the production of SCP.**

***Keywords* – Amino acids; carbon to nitrogen ratio; cultivation conditions; single-cell protein; yeast**

Nomenclature		
h	Hours	–
g_{dw}	Gram of the dry biomass	–
μ_{max}	The maximum specific growth rate of biomass	h^{-1}
v/v	Volume per volume	%

1. INTRODUCTION

The aquaculture industry's importance in providing humankind with high–quality animal protein gradually increases over time. To meet the growing demand for aquafeed, alternative protein sources are essential for the sustainable development of the aquaculture industry. Since conventional aquaculture feed sources such as fishmeal and plant origin protein are limited and have a negative impact on the environment [1]–[4]. Single–cell protein (SCP) is widely used as a valuable feed additive, replacing costly conventional sources like soymeal and fishmeal [5]–[7]. Single–cell protein is microbial biomass produced by various microorganisms such as algae, bacteria, fungi, yeast, and protists, which can metabolize different carbon and nitrogen sources [8], [9]. The production of single-cell proteins involves the growth of microorganism in a fermenter and includes downstream processes such as

* Corresponding author.
E-mail address: svetlana.raita@rtu.lv

separation and concentration of microbial biomass, drying, and mixed up with animal feed or directly used [10], [11].

Yeast dried biomass is commonly used as a supplement in animal feed due to its relatively high protein, amino acid, energy, and micronutrient content compared with feed grains and oilseed meals [12]. Yeast has various advantages including larger size, thus easier harvesting, and lower nucleic acid content, which reduces the cost of post-treatment [13]. Yeasts are able to convert inexpensive non-edible by-products from the food processing and agricultural industry into high-value protein with limited dependence on arable land, water, or changing climatic conditions [14], [15]. Substrate for production of SCP should be accessible, nontoxic, abundant, renewable, low-cost, and able to support rapid growth and proliferation of the organisms resulting in high-quality biomass [16], [17]. Common sources of biomass for SCP are represented by fruit wastes [18], molasses [19], dairy industry by-products [20], [21], industry wastewater [22], [23], glycerol [24], natural gas, ethanol, methanol [25], etc.

The selection of yeast strain, fermentation medium composition, trace element supplementation, cultivation conditions, and harvested biomass treatment can all alter the final SCP composition [14], [26]–[28]. The protein content in yeast cells can range from 10 % to over 79 % of dry matter (see Table 1). Yeast biomass is also composed of lipids (0.5–8 %), carbohydrates (18–43%), minerals, and vitamins. Yeast biomass is rich in B vitamins, calcium, phosphorus, potassium, magnesium, copper, iron, zinc, manganese, and selenium [14], [23], [25], [29]. SCP is rich in certain essential amino acids, such as lysine and methionine, which are limited in most plant and animal sources. Studies have shown that yeasts such as *Saccharomyces cerevisiae*, *Candida utilis*, *Candida tropicalis*, *Yarrowia lipolytica* and *Kluyveromyces marxianus* have suitable amino acid composition as protein sources in diets for aquaculture [19], [24], [30]–[33]. Protein–rich yeast biomass can be used as an additive supplemented to the leading aquaculture and livestock diets instead of other sources, such as fish meal, soybean meal, and other plant–based protein sources [5], [34]–[36].

Although yeast SCP has various advantages, it also has some limitations. The main challenge is the nucleic acid content in cells. The biomass of most microorganisms contains 4–20 % of nucleic acid [1], [37]. Most yeasts contain 5–8 % of nucleic acid in dry matter which is less than bacteria contain 8–15 % [37], [38]. The consumption of single–cell protein with high contents of nucleic acids could cause serious problem with health, therefore, yeast biomass needs a reduced number of nucleic acids to acceptable level about 1 % for using as feed or food. SCP with high nucleic acid content is recommended only for feeding animals with short life spans [10], [37]. Another challenge can be considered the poor digestibility of the SCP, since yeast has a complex and thick cell envelope [39]. Therefore, for improvement of yeast SCP digestibility and reduction or removal of nucleic acids content, the cell wall of yeast should be processed by mechanical disruption, chemical, physical, enzymatic treatment, or combinations of these methods [37], [39]. Despite the increase in total production costs due to the use of methods for disrupting the walls of yeast cells, the final processed yeast presents nutrient digestibility coefficients similar to those of high quality protein for the food and feed industry [40].

Optimization of yeast cultivation factors is an extremely important step to obtain the highest efficiency of protein production and an appropriate amino acid profile in yeast cell biomass [37]. The present work discusses the optimization strategies for protein–enriched yeast biomass production that may result in a balanced, renewable, high–protein ingredient that is a suitable alternative to classic feed proteins.

2. INFLUENCING FACTORS ON YEAST SCP PRODUCTION

The concentration of protein and amino acids profile in yeast cells mainly genetically predetermined [16]; however, to fully exploit the biotechnological potential of the selected yeast strain, it is necessary to optimize microbial fermentation process [41]. The yield and productivity of SCP production are strongly dependent on culture medium composition, environmental conditions, and selected microorganisms [6], [42]. According to the literature, the main factors that affect yeast biomass growth and SCP content are pH, temperature, time of cultivation and requirements for carbon, nitrogen, and trace elements. [32], [43]–[46].

Microorganisms can respond to environmental changes and the availability of nutrients and trace elements in the fermentation medium. This ability is essential for the metabolism, growth, and reproduction of microorganisms. Yeast metabolism is a set of complex enzymatic reactions to substrate components penetrating the cell wall membrane. Therefore, it is crucial to consider that the lack of necessary elements in the fermentation medium or unfavourable conditions turns on the survival mechanisms in cells, which provokes the inhibition of anabolic processes, such as protein synthesis [47], [48].

2.1. Selection of yeast species

Various yeasts species have been used for numerous industrial applications. SCP qualifies as an excellent source of nutritive proteins, but other cellular components synthesized during fermentation also add value to the resulting biomass [6]. Besides SCP production, *Yarrowia lipolytica* is widely used in the production of lipids, erythritol and citric acid [49], *Saccharomyces cerevisiae* for bioethanol and ergosterol production [50], [51], *Kluyveromyces marxianus* for hydrolytic enzymes such as inulinase, lactase, pectinase, lipase production [52], Rhodotorula sp. for carotenoids such as β-carotene, γ-carotene, torulene and torularhodin [53], Candida sp. for biosurfactants and lipases production [54]–[56], *Pichia kudriavzevii*, *Blastobotrys adeninivorans* and *Wickerhamomyces anomalus* for biogas production [57]. Consequently, the production of SCP from yeast biomass can be widely used in related industries for the production of other cellular metabolites. For example, in the study by Ohlsson *et al.* [57] biomass of three yeast species, *P. kudriavzevii*, *B. adeninivorans* and *W. anomalus,* were examined for yeast SCP and biogas co–production. The authors concluded that this technology has potential after further optimizing cultivation parameters [57].

According to the studies summarized in Table 1, yeasts of the genus Candida are most often used for protein production, which are able to accumulate approximately 39–79 % of protein in dry cell biomass. Yeasts, such as *Candida utilis, Candida tropicalis, Candida pararugosa,* and *Candida guilliermondii*, are capable of utilizing various carbon containing substrates, making them an excellent source of protein [19], [22], [34], [58]–[60]. *Yarrowia lipolytica*, another specie of yeast, commonly used for single-cell oil production, however, can also be used for single–cell protein production [24]. Studies report high protein content in the cells of the preceding species, about 46–71 % [24], [46]. Other yeast species such as *Rhodotorula glutinis*, *Pichia stipitis*, *Blastobotrys adeninivorans* and *Wickerhamomyces anomalus* show satisfactory results, 30–46 % of protein content [28], [34], [57]. However, when evaluating the protein content of yeast species, biomass yield and the factors influencing them should be considered, which is described in the sections below.

2.2. Carbon source

Production of single-cell protein by fermentation process has been mentioned by many researchers (see Table 1). It is well established that most yeasts use sugars as their main

carbon and energy sources; however, there are some yeasts that can utilize non–conventional carbon sources such as starch, alcohols, polyols, hydrocarbons, and fatty acids [61]. For example, *Y. lipolytica* can metabolize a limited range of hexose sugars such as glucose, fructose, and mannose, but it can utilize acetate, alcohols, and hydrophobic substrates, including oils, alkanes, and fatty acids [49]. The ability of oleaginous yeast to utilize hydrophobic substrates is due to the presence of specific enzymes [62]. Noteworthy, the biosynthesis of protein in oleaginous yeasts such as *Y. lipolytica, R. glutinis, C. tropicalis* [63] cells is competitive to the lipid accumulation [15] therefore, it is especially important to observe certain culture conditions such as the ratio of carbon to nitrogen, as described below.

A number of agro-industrial waste and by-products have been used for the production of SCP and other metabolites, including glycerol [24], cheese whey [20], waste milk [21], different fruits peels [18], industrial waste cooking oil [64], salad oil manufacturing wastewater [30], potato processing wastewater [23], [65], olive mill wastewater [22], organic fraction of municipal solid waste [57]. Table 1 summarizes studies with the biomass and protein content using different substrates, cultivation conditions, and yeast species.

For microbial cultivation, a single carbon source is often used, although the use of multiple substrates can positively affect biomass yield and improve protein concentration. Generally, microbial metabolism varies significantly when fermentation medium is presented with mixed carbon substrates compared to a single carbon source, as different nutrients interact in complex ways within the metabolic network [45]. It is important to choose suitable substrates in the optimal ratio for each species of microorganism. Kurcz *et al.* [60] found that the addition of 5 % glycerol to the potato wastewater medium increased yeast *C. utilis* biomass and protein yield compared to the glycerol–free medium, but when glycerol concentration in the medium is above 10 %, the opposite effect is viewed, biomass yield and protein content decreased. The authors suggest that a higher concentration of glycerol inhibits the growth of *C. utilis*. On the other hand, the authors suggest that part of the glycerol assimilated by the yeast was probably used in the biosynthesis of other cell components, which led to a decrease in the proportion of protein components of the yeast biomass as a consequence [60].

TABLE 1. DIFFERENT SUBSTRATES USED AS A CARBON SOURCE AND SCP CONTENT IN DIFFERENT YEAST SPECIES

Yeast specie	Carbon substrate	Cultivation conditions			DCW, g/L	SCP, %	Ref.
		Cultivation time, h	T, °C	pH			
Saccharomyces cerevisiae	Glycerol	48	28	5.5	n/d	47.9	[24]
	Mango residue	30	30	4.0	15.28	79.1	[41]
Candida utilis	*Opuntia ficus-indica* hydrolysate	50	35	5.0	12.2	14.0	[58]
	Potato wastewater	48	28	5.0	5.65	48.9	[60]
Candida tropicalis	Soy molasses	30	30	5.5	10.83	56.4	[19]
	Sugarcane bagasse hydrolysate	96	30	5.0	16.97	60.1	[59]
	Sugar beet pulp	10	30	4.5	16.21	47.8	[34]
Candida pararugosa	Olive mill wastewater	96	30	n/d	21.68	39.4	[22]

Candida guilliermondii	Sugar beet pulp	10	30	4.5	15.5	49.2	[34]
Yarrowia lipolytica	Glycerol	48	28	5.5	n/d	46.7	[24]
	Waste cooking oil	120	28	n/d	57.37	12.6	[64]
	Olive fruits wastes	n/d	30	5.0	14.40	71.0	[46]
Kluyveromyces marxianus	*Opuntia ficus-indica* hydrolysate	50	40	5.0	11.1	10.0	[58]
Rhodotorula glutinis	Potato wastewater and 5 % glycerol	72	28	5.0	19.24	40.5	[42]
Pichia stipitis	Sugar beet pulp	10	30	4.5	19.54	45.6	[34]
Pichia kudriavzevii	Biogas substrate	12–15	30	7.0	7.36	32.7	[57]
Schwanniomyces etchellsii	Olive mill wastewater	96	30	n/d	15.11	35.9	[22]
Blastobotrys adeninivorans	Biogas substrate	12–15	37	7.0	14.83	30.5	[57]
	Spruce sugar hydrolysate	28	30	5.0	27.62	42.45	[28]
Wickerhamomyces anomalus	Biogas substrate	12–15	30	7.0	7.03	22.6	[57]
	Spruce sugar hydrolysate	24	30	5.0	29.78	41.22	[28]

Note: DCW – dry cell weight (grams per liter of medium); SCP – single–cell protein content (% of DCW); n/d – not defined.

2.3. Nitrogen source

During protein synthesis, nitrogen is one of the significant factors due to the structure properties of proteins [37]. Different sources of nitrogen like ammonia, ammonium salt, nitrate, urea, and organic nitrogen in different substrates such as potato and starch processing wastes and cheese whey, are consumed by microorganisms [1], [16], [32]. Yeasts are capable of utilizing a range of different inorganic and organic sources of nitrogen for incorporation into the structural and functional nitrogenous components of the cell, such as amino acids, peptides, proteins, polyamines, nucleic acids, and vitamins [47]. While yeast cells can use a variety of nitrogen–containing compounds as the sole nitrogen source, they show a hierarchical preference for those sources. Therefore, the growth rate and the type of synthesized metabolite depend on the quality and amount of available metabolizable nitrogen [66]. Most yeast strains prefer glutamine or ammonia but will use other nitrogen sources, although with a lowered growth rate [66]. This is due to the fact that yeasts can use ammonium ions as the sole source of nitrogen since they possess genes encoding enzymes for the biosynthesis of all amino acids. The ammonium ions that are either supplied as a nutrient or are derived from the catabolism of other nitrogenous compounds can be directly assimilated and then serve as donors of the amino acid synthesis [47].

In the study by Arous *et al.* [22] preferred carbon source for cultivation of *S. etchellsii* and *C. pararugosa* was ammonium salts (ammonium chloride and ammonium sulphate), in comparison with the addition of potassium nitrate, soy protein, and yeast extract gave 3–8 times lower biomass yield [22]. In another study by Umesh *et al.* [32], the highest biomass

yield and protein content was obtained from *S. cerevisiae* when the medium contained beef extract and yeast extract as nitrogen sources over ammonium nitrate, ammonium sulfate, urea, and sodium nitrate [32]. Between inorganic nitrogen sources preferable for protein production by *C. utilis* were ammonium sulfate, urea, and ammonium chloride compared with potassium nitrate and sodium nitrate [44]. Interestingly, the selection of nitrogen sources can significantly improve the utilization of xylose by yeast. Wu *et al.* [67] report that urea efficiently improved xylose consumption by *C. intermedia* in corncob and silver grass straw hydrolysate compared to ammonium nitrate, ammonium chloride, and diammonium hydrogen phosphate had a negative effect on xylose consumption [67].

2.4. Carbon to nitrogen ratio

The initial carbon to nitrogen (C:N) ratio is a very important factor for substrate reduction, biomass production, and protein content [22], [30], [68]. C:N ratio of 10:1 is reported as the most appropriate result as the same ratio is presented in the microorganisms. A higher ratio will cause the disappearance of nitrogen before all carbon is consumed and the required biomass will not be obtained [1]. When nitrogen is limited, yeast cells slow their growth, while in the extreme case of nitrogen depletion, cells stop growing even with all other nutrients available in excess and enter a nitrogen–specific passive state [66]. At the ratio of 1:1, most nitrogen cannot enter cells and will be wasted [1]. For oleaginous yeasts, mechanisms are similar, i.e., with distinction at low nitrogen level, yeasts switch the metabolic pathway of protein synthesis to lipid synthesis [69]. Zheng *et al.* [30] recommended a C:N ratio from 5:1 to 8:1 for SCP production of *C. utilis* OZ993, at which protein content results in 48–49 %. However, between these ratios, the highest biomass was achieved at 5:1 C:N ratio. On the other hand, lower C:N ratios adversely affected the cellular protein content of *C. utilis*. There was a substantial reduction in the protein level of cells, from 49 % to 18 %, when the C:N ratios gradually declined from 5:1 to 1:1 [30].

A similar conclusion was achieved in other researchers works. Arous *et al.* [22] report that the optimal C:N ratio was 8:1–10:1 for *S. etchellsii* and *C. pararugosa* with higher biomass production on oil mill wastewater–based medium with ammonium chloride supplementation [22]. In the study by Spalvins *et al.* [64], the highest SCP content in *Y. lipolytica* biomass was observed at 5:1–10:1 C:N ratio cultivated on waste cooking oil contained medium [64].

2.5. Supplementation of trace elements

An important role in SCP production is the trace elements addition to the fermentation medium. Yeasts require a range of metals for optimal growth, metabolism, and fermentation performance. The requirement for metal ions varies widely with the different strains, so it is necessary to adjust the composition of the medium to avoid the inhibitory effects of trace elements on the growth of selected microorganisms [31]. The most requested is phosphate, magnesium, calcium, potassium, zinc, and iron [12], [34], [37], [55], [70]. Yeast cells are starved for phosphate and sulphur arrest in a quiescent state in which fermentation of glucose is suppressed: external glucose is not depleted [66]. Daskalaki *et al.* [71] report that *Y. lipolytica* completely assimilates existing nitrogen in the medium within 48 hours; however, when the amount of carbon in the medium is depleted, the addition of nitrogen and magnesium causes an increase in the protein content of the biomass [71]. This mechanism is explained in the work of Dourou *et al.* [72], where the life cycle of oleaginous microorganisms has been described. After the depletion of the carbon source in the medium or due to a low uptake rate, the oleaginous microorganisms utilize their own storage lipids as an energy source for

maintenance purposes or as an intracellular carbon source for the production of new lipid–free cell components, provided that essential nutrients are available in the fermentation medium [72].

Gao *et al.* [19] showed that the addition of $CaCl_2$ in the medium is important for the production of SCP by *C. tropicalis*. The biomass production and total protein content increased when 0.05 g/L $CaCl_2$ was supplemented to soy molasses medium, where the addition of NaCl, $MgSO_4 \cdot 7H_2O$, K_2HPO_4, and $ZnSO_4 \cdot 7H_2O$ in the same amount had no notable effect [19]. In turn, the study by Nicolas *et al.* [31] provides a more detailed overview of the effect of different salt concentrations on the growth of the yeast *C. utilis*. Mineral salts KH_2PO4, $MgSO_4$, $FeSO_4$, and KCl at 0.2, 0.07, 0.002, and 0.8 g/L concentrations significantly increased biomass production [31].

Kieliszek *et al.* [48] optimized medium with 0.02 g/L selenium supplementation enriched *S. cerevisiae* and *C. utilis* biomass functional diversity in terms of protein and amino acid content. Yeasts of both strains enriched with selenium contained a large amount of glutamic acid, aspartic acid, lysine, valine, histidine, and leucine. An analysis of the amino acid composition of *C. utilis* yeast biomass enriched with selenium showed that the concentration of the most important amino acids lysine was at a higher level (8.3 %) as compared to that of the biomass obtained without the addition of this element (5.6 %). Moreover, after cultivation in the medium supplemented with selenium, the total amino acid content for both *C. utilis* and *S. cerevisiae* strains was higher and increased by approximately 12 and 5 %, respectively. However, the total protein content in the biomass of *S. cerevisiae* and *C. utilis* slightly decreased as compared to that in the control sample without additional selenium supplementation from 48.4 % and 42.1 % to 42.6 % and 37.0 %, respectively [48].

2.6. Temperature and pH

Another important factor in successful yeast cultivation is the choice of temperature and pH of medium. According to the literature, the most common temperature used for protein biosynthesis by yeast species is 25–30 °C [22], [24], [46], [64], [73]; however, for some yeasts, such *C. util*is and *K. marxianus* a temperature of 35 and 40 °C can be used [58]. Low temperature can inhibit nutrients from crossing the cell membrane, while a high temperature may inactivate enzymes of the metabolic pathway [22]. The optimal pH value for yeast growth is 4.0–7.0 [18], [34], [41], [74]; within this range, the most preferred pH of the medium for protein biosynthesis is 5.0–5.5, as described below.

Siddique *et al.* [18] report that the optimal conditions for the cultivation of *C. tropicalis* cultivated in 2 % watermelon peels hydrolysate were 37 °C and pH 7. In the other study by Gao *et al.* [19], the maximum biomass and protein content of *C. tropicalis* were attained when the initial pH was 5.5 at 30 °C. The highest biomass yield and protein content in Umesh *et al.* [32] work was obtained from *S. cerevisiae* cultivated at 25 °C and pH 5 in comparison with the lower result at 15, 37, 45 °C and pH 4, 6, 7, and 8. In the study by Jalasutram *et al.* [44], the *C. utilis* was cultivated in the range of pH 3 to 9 at 30 °C; the maximum protein concentration was obtained at 6 pH and lowest at pH 3, 4, 8, and 9. The authors note that the activity of enzymes involved in protein production decreases under strongly alkaline and acidic conditions of cultivation [44].

For *Y. lipolytica* the optimal conditions for protein production were 30 °C and pH from 5.0 to 6.0, where the highest biomass was obtained at pH 5.0. In these conditions the yeast produced 40–50 % of the protein in its dry cell weight cultivated on medium based on wastes from biodiesel production (vegetable oils, degumming, and glycerol fractions) [15]. These environmental conditions appropriate for *Y. lipolytica* cultivation in olive fruits waste–based medium is consistent with another study by Rages *et al.*, [46], which observed the highest

protein concentration of about 71 %, which has been reached at 30 °C and pH 5. With an increase in pH from 5.5 to7.5, a decrease in the amount of protein in the biomass was observed. In contrast, media with an initial pH of 6.5 resulted in a higher biomass yield, with 55 % content of protein [46]. Kot *et al.* [74] noted that the pH value in the range of 4.0–7.0 did not significantly affect the amount of protein in the *R. glutinis* biomass, which varied from 38.5 % to 41.3 % after 72 h of cultivation. However, maximum biomass production and protein content was obtained at pH 5.0 [74].

2.7. Time of cultivation

Zakhartsev *et al.* [75] described how the temperature of microorganism cultivation affects the kinetics of key molecular processes in the cell, thereby affecting the biomass specific growth rate†. On the other hand, a specific growth rate affects the macromolecular composition of growing microbial cells. For example, in carbon limited continuous cultures of yeast *S. cerevisiae* at low biomass specific growth rate ($\mu_{max} < 0.1\ h^{-1}$) the carbohydrates content is up to 50 % and proteins content is up to 40 % of the dry biomass, whereas at high growth rate ($\mu_{max} > 0.3\ h^{-1}$), the carbohydrates content linearly decreases to 15 % and proteins content increases up to 60 % of the dry biomass [75]. This may explain the high values of the protein content in a short cultivation time as described in the studies of Patelski *et al.* [34] and Lapena *et al.* [28], were 46–49 % SCP in the biomass of *C. tropicalis*, *P. stipites*, *C. guilliermondii*, and 47–51 % in *C. jadinii*, *W. anomalus*, *B. adeninivorans* were obtained after 10 and 12 h of cultivation, respectively.

One of the highest protein content of 69% is mentioned in a study by Rages *et al.* [46], in cultured *Y. lipolytica* for 96 h. However, the amount of biomass was not high, only 13.10 g per liter. In the following days of cultivation, the amount of protein decreased, and at the time of the 8th day of incubation, it dropped to 45.63%. In turn, the maximum biomass was reached on the 6th day of cultivation [46]. In studies by Dharumadurai *et al.* [17] and Rajendran *et al.* [73] *S. cerevisiae* reached higher biomass content after 168 h of fermentation, but the maximum protein content was recorded at 72 h of fermentation at a 5 % concentration of pineapple waste hydrolysate and papaw and banana fruit juices, respectively. Other results were obtained in the Umesh *et al.* [32] work when *S. cerevisiae* was cultivated on papaya waste hydrolysate. Higher biomass and protein content was obtained on 120 h of cultivation. In another study by Kurcz *et al.* [60], protein content in *C. utilis* dry biomass was higher after 48 h of fermentation in a potato wastewater–based medium, and after 72 h it decreased from 43.5 to 41.7 %, respectively. A similar tendency was observed when glycerol was added to the potato wastewater medium in the amount of 5 % and 10 %. However, when glycerol was added to the medium in a volume of 15–25 %, the protein content was higher by 72 h, which indicates that the time of protein accumulation depends on the constituent components of the medium [60].

The cultivation time of yeasts is an important parameter for protein production, so it is necessary to perform experimental studies on the influence of the quantity and quality of the substrate on the biomass growth rate and the rate of substrate consumption in order to properly assess the optimal time for biomass harvesting.

2.8. Inoculum size

The size of the inoculum (population of microorganisms or cells that is introduced in the fermentation medium) is another important factor in starting the fermentation process and

† μ_{max} – maximum specific growth rate of biomass [g_{dw}/ (g_{dw} · h)] or [h^{-1}].

influencing single–cell protein production [22], [41], [44], although studies describing the effect of different inoculum sizes on protein production are rare. The optimum inoculum size varies for different microorganisms [16], and depends on the total concentration of dissolved oxygen and nutrients in the fermentation medium [22], [44]. For example, in a study by Jalasutram *et al.* [44], for *C. utilis* inoculum ranging from 2 to 10 % v/v protein production was increased with the increase of inoculum size with an optimum at 6 %, after which the level of SCP production was decreased. The authors note that cultivated yeast with an initial inoculum concentration of 6 %, dissolved oxygen, and consumed oxygen were at an equilibrium level [44]. Similar results were obtained in Arous *et al.* [22], when the effect of inoculum size 2 %, 5 %, 7 %, and 10 % on the biomass production of *C. pararugosa* and *S. etchellsii* strains were tested. Under optimized cultivation conditions, the addition of 2 to 5 % inoculum of *C. pararugosa* led to maximum biomass production after 4 days of incubation. 7 % inoculum size addition caused a drop in yeast biomass to approximately 28 % as compared to biomass obtained with 5 % inoculum size. Similarly, a slight decrease in the biomass production of *S. etchellsii* was observed when the inoculum size exceeded 7 %; however, there was no significant difference in biomass production of this strain when different inoculum sizes were tested. The authors concluded that with large inoculum sizes (higher than 7 %), the nutrients in the growth medium might be rapidly consumed which results in low rate of yeast growth and cell survival [22]. In another study by Somda *et al.* [41], the optimization of SCP production of *S. cerevisiae* was based on two parameters: a substrate concentration of 5 to 10 % (g/L) and an inoculum size of 2 to 12 % (v/v). The highest protein content of 79 % was achieved at 8 % inoculum and 8 % mango residues concentration [41].

Thus, to optimize the protein production process, it is necessary to comprehensively consider the influence of different cultivation parameters, taking into account not only the composition of the fermentation medium, but also the preferences and characteristics of each species of yeast.

3. SCP AMINO ACID COMPOSITION

Yeast biomass has been successfully used in fish feed formulations, both at low and at higher inclusion amount as fishmeal replacement [57], [76]. The nutritional value of yeast primarily depends on the content of protein and its amino acid composition, as well as on the content of lipid, vitamin and minerals [4], [24]. Amino acids are significant biomolecules that serve as protein building blocks and are intermediates in various metabolic pathways in organisms [77]. According to the FAO report [78], the protein and amino acids requirements for different animal species are different. Fish diets have higher protein requirements than mammalian diets, and protein requirements decrease with age [78]. Essential amino acids required in aquatic animal diets are lysine, methionine, and threonine, and these three amino acids are limited in plant–based feed such as soybean, corn, and rice [5], [35], [36]. Single-cell proteins are generally well digested by fish and crustaceans and comprise a protein content and amino acid profiles similar to fishmeal [14], [79].

Table 2 summarizes the composition of amino acids in SCP of yeast biomass compared with other sources. The biomass of yeasts like *C. utilis*, *C. tropicalis*, *Y. lipolytica* and *S. cerevisiae* is rich in lysine, threonine, valine, and leucine [19], [24], [30], [31]. Although the concentration of essential amino acids in yeast protein varies between species and strains of the same species and depends on the substrate used in the studies, added trace elements and cultivation conditions [33], [48].

TABLE 2. ESSENTIAL AMINO ACID COMPOSITION OF DIFFERENT PROTEIN SOURCES

Protein source	Essential amino acids content, g/ 100 g of protein									Ref.
	Lys	Thr	Val	Met	Ile	Leu	Phe	His	Arg	
C. utilis	7.8	4.7	4.0	1.0	4.1	7.9	3.4	1.5	4.4	[30]
	5.14	4.1	5.5	1.58	4.8	7.12	4.1	n/a	3.2	[31]
C. tropicalis	6.91	4.35	4.58	2.27	4.00	6.24	3.71	n/a	n/a	[19]
Y. lipolytica	6.2	4.2	4.7	1.4	4.0	7.1	3.9	2.5	4.8	[24]
S. cerevisiae	6.5	4.6	4.9	1.4	3.7	6.4	3.3	2.4	4.7	[24]
	2.5	3.3	3.1	3.2	2.6	2.9	3.0	2.8	1.4	[32]
K. marxianus	n/a	6.94	7.5	0.77	5.48	7.74	3.58	1.9	n/a	[33]
W. anomalus	1.41	n/a	0.96	0.24	0.87	1.36	0.84	0.35	0.87	[57]
	3.06	1.89	1.95	0.33	1.84	2.90	1.63	1.12	2.57	[28]
Soybean meal	3.41	1.95	2.61	0.72	2.59	4.13	2.71	1.34	3.86	[80]
Corn protein isolate	1.0	1.8	2.1	1.1	1.7	8.8	3.4	1.1	1.7	[36]
Rice protein isolate	1.9	2.3	2.8	2.0	2.0	5.8	3.7	1.5	5.4	[36]
Fish meal	6.79	3.97	3.93	2.50	3.35	6.25	3.26	1.97	5.23	[81]

Note: n/a – not analysed

In general, yeasts have lower methionine content than fishmeal; however, some studies show that good results can be achieved under optimal fermentation process conditions. In studies by Gao *et al.* [19] and Umesh *et al.* [32], methionine content in yeast species *C. tropicalis* and *S. cerevisiae* was reported to be 2.27 and 3.2 g per 100 g of protein. Among the most commonly used crops, only rice protein comes close to the value of the essential amino acid in fishmeal. Among plant proteins, rice also has a high content of 5.4 % arginine and 3.7 % phenylalanine, and corn has a higher content of 8.8 % leucine [36]. Among plant proteins, rice also has a high content of 5.4 % arginine and 3.7% phenylalanine, and corn has a higher content of 8.8 % leucine. In turn, soybean meal has a higher content of lysine and isoleucine but in a smaller amount compared to fish meal and yeast protein [19], [24], [30], [31], [33], [80], [81]. Therefore, protein-rich yeast biomass appears to be a sustainable alternative to fishmeal and plant origin proteins.

4. CONCLUSIONS

The research results reviewed in this study show that yeast culture conditions such as temperature, pH, and time of cultivation have a huge impact on the protein content of yeast biomass. The most preferred pH and temperature of the medium for SCP production by yeasts are pH 5.0–5.5 and 28–30 °C. Time of cultivation is an important criterion for harvesting protein–rich biomass since in the later stages of fermentation, when the maximum biomass is reached, the protein content in it already decreases. This feature must be considered and investigated for each selected strain growing on a specific medium composition. Properly selected carbon to nitrogen ratio also has a strong influence on protein content, since the metabolic pathways of yeasts are directly related to the available amount of carbon and nitrogen. According to literature, the optimal C:N ratio for protein production is 5:1–8:1. The addition of trace elements also has a positive effect on biomass growth and can affect the amino acid profile. At the beginning of the fermentation process, an important parameter is

the size of the inoculum, which can affect the growth rate of the biomass. In conclusion, it should be noted that optimally selected cultivation conditions and skilfully designed culture medium composition allows the production of high protein content with a well–balanced amino acid profile.

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