



Review Article

Influencing Factors on Yeasts for Sustainable Single Cell Protein Production: A Review

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SUMMARY

Yeast Single-Cell Proteins (SCP) production is a bioavailable product using various inexpensive industrial byproducts and agriculture wastes substrate have significant potential as an alternative to the soy meal, and fish meal protein used for livestock and aquaculture feeds. The current effort confers factors strategies for protein-enriched yeast biomass production 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. The review article presents Sources of Single Cell Protein, Method of Production of Single Cell Protein and influencing factors on Yeast for sustainable single cell protein production.

Keywords: Cultivation conditions, Fermentation, Single cell protein, Waste Substrate and Yeasts

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INTRODUCTION

In current years, worldwide concerns surrounding food security and sustainability have become progressively prominent (1). With projections indicating a potential worldwide population increase to 9.3 billion by 2050, a pressing need arises to address the challenges posed by rising 50% -80% food demands (2, 3). Compounded by issues such as undernourishment affecting millions

worldwide and the alarming food wastage rate, the quest for sustainable protein sources has garnered significant attention (4). The United Nations Food and Agriculture Organization (FAO) emphasizes the critical role of proteins as essential macromolecules in cellular structures and metabolic functions (5). Compared to the other majority ingredients in human diets, fats and carbohydrate, protein is most expensive.

Amongst alternative proteins, plant-based solutions, insects, lab-grown meat, and SCP have been studied. In response to the pressing challenges of providing accessible protein at low cost and environmental impact, researchers have focused on single cell protein production.

Single-cell protein is derived from cells of certain strains of microorganisms such as yeasts, fungi, algae, and bacteria, which are grown on various carbon sources for synthesis (6). They can be used as a protein supplement for animal feed (7), e.g., by replacing fishmeal in the fodder of aquatic species, particularly in aquaculture, and chicken rearing. As research in this field develops, the potential for SCP to revolutionize the protein industry and ease pressure on conventional food production systems becomes increasingly evident.

Single-cell proteins have emerged as a promising solution to address the growing demand for protein sustainably (8), by allowing low-cost production with minimized demand for resources. They can be cultivated using a variety of substrates, including agricultural waste and industrial by-products, thus reducing pressure on traditional agricultural practices and mitigating issues related to land and water usage (9). With ongoing advancements in biotechnology and fermentation processes, the commercial viability and scalability of SCP production are progressively improving, paving the way for their integration into mainstream food systems and contributing to global food security efforts (10)

Sources of Single Cell Protein: Microorganisms Used for SCP

Given the accelerated growth of the global alternative protein market, microbial

proteins produced through fermentation have gathered attention because of their advantages, including minimal resource consumption, high production efficiency, environmental sustainability and comprehensive nutrition. The most used microorganisms for microbial protein production include yeast, filamentous fungi, algae, and bacteria.

A list of the microorganisms used to produce Single Cell Protein is as follows:

- **Fungi:** *Aspergillus fumigatus*, *Aspergillus niger* and *Rhizopus cyclopean*
- **Yeast:** *Saccharomyces cerevisiae*, *Candida tropicalis* and *Candida utilis*
- **Algae:** *Spirulina (spa)*, *Chlorella pyrenoidosa* and *Chondrus crispus*
- **Bacteria:** *Pseudomonas fluorescens*, *Lactobacillus* and *Bacillus megaterium*

Single-cell proteins, derived from microorganisms with high-protein content, show attractive features as a nutrient supplement, providing a balanced amino acid spectrum, low-fat content, and a higher protein-to-carbohydrate ratio than forages as presented in table 1 (11). Table 1 shows the typical composition of the “classic” microorganisms useful for SCP production: fungi, algae, yeast (which belong to the fungus kingdom), and bacteria (12). First-generation SCP principally involves the utilization of microorganisms, such as bacteria, yeast, and fungi, to convert various organic substrates, such as sugar, into biomass, similar to first-generation biofuels. These microorganisms are cultivated under controlled conditions, optimizing their growth and protein production. On the other hand, the second generation of SCP

explores innovative approaches, including nonfood feedstocks such as agricultural residues, into protein-rich biomass (13), as well as deploying genetically modified

microorganisms and novel bioreactor designs, to improve protein yields and reduce production costs (14)

. Table 1. Here are the average compositions of the different microorganisms present in the % dry weight of Single-cell protein (11).

Composition	Fungi	Algae	Yeast	Bacteria
Protein	30-45	40-60	45-55	50-65
Fat	2-8	7-20	2-6	1-3
Ash	9-14	8-10	5-10	3-7
Nucleic Acid	7-10	3-8	6-12	8-12

Production of SCP from Yeast

Yeast was one of the earliest microorganisms to be used by humans, with historical evidence indicating its use in the production of fermented beverages such as wine and bread (15). Yeast demonstrates robust growth crossways a broad pH range (2.5–8.5), temperature range (2–45°C) and several advantages including their larger size (i.e., 5–10 µm) which facilitates separation as well as high percentages of vitamins (group B) and most yeast species contain relatively low quantities of nucleic acids, ranging from 5 to 8%.. Yeast typically requires oxygen for optimal growth and its fermentation process can be efficiently scaled up for industrial applications (16). The foremost advantages of their familiarity and suitability are that they have been employed for conventional fermentation for a long time. Additionally, their ability to thrive in acidic environments and their size facilitate harvesting (17). The protein content of yeast biomass is typically analogous to or higher than that of meat

and soybeans and exceeds that of milk protein. Moreover, the standardized protein efficiency ratio for casein shows that SCP yeast is analogous to or more efficacious than traditional protein sources (18). This is advantageous for reducing uric acid accumulation in humans. The long-standing use of yeast in food and beverage applications has contributed to its widespread use. Moreover, yeast can use a range of agricultural industrial by-products, including sugarcane bagasse and potato residues, enhancing the environmental sustainability of yeast production (16).

Saccharomyces cerevisiae, commonly known as baker's yeast, is a unicellular eukaryote that has been utilized for thousands of years in baking, brewing, and winemaking. This yeast is distinguished by its relatively simple genetic structure, containing approximately 6000 genes across 16 chromosomes. Notably, it was the first eukaryotic organism to have its entire genome sequenced, making it a

valuable model organism in scientific research (16).

S. cerevisiae reproduces through both asexual budding and sexual reproduction, allowing for genetic diversity. Researchers favor this yeast for its nonpathogenic nature, rapid growth rate, and the ability to investigate fundamental cellular processes, including cell division, gene expression, and protein interactions. Techniques such as auxotrophic markers and the two-hybrid system enhance its utility in genetic and biochemical studies (16).

Saccharomyces cerevisiae (*S. cerevisiae*) is the most commonly used yeast species in industrial models for biotechnological research. It is generally recognized as safe (GRAS) and has applications in the food, feed, and pharmaceutical industries (18; 19). For example, Marmite®, a Unilever PLC product, has been consumed globally for over a century. Derived from brewing by-products, specifically *S. cerevisiae* extract, Marmite® contains 34% protein of dry cell weight (DCW) and various B vitamins, making it an appropriate dietary supplement for individuals with increased vitamin B requirements (20).

Another commonly used yeast strain in industrial biotechnology is *Yarrowia lipolytica* (*Y. lipolytica*), which has a protein content ranging from 30 to 50% DCW and a lipid content exceeding 40%. *Y. lipolytica* has been designated as Generally Recognized as Safe (GRAS) by the Food and Drug Administration (FDA) and is regarded as a valuable protein source due to its high essential amino acid content. Since 2010, the dried and heat-killed biomass of *Y. lipolytica* cultivated in biofuel waste has been approved as a feed additive (21; 22). In 2019, the European Food Safety Authority (EFSA) approved

the use of dried and inactivated *Y. lipolytica* cells as a novel food ingredient, extending its application as a dietary supplement for individuals aged three and above (23). In 2020, EFSA included selenium-rich *Y. lipolytica* biomass in the list of authorized novel foods owing to its high selenium protein content (24). Furthermore, the protein biomass of this yeast represents a valuable source of B-complex vitamins, including vitamin B12 (25). Furthermore, *S. cerevisiae* has a protein content between 30 and 55% DCW, high substrate acceptance and the concentration of essential amino acids (i.e. lysine, tryptophan, threonine, methionine and cysteine) is suitable when used as feed additives, rendering it an attractive feed ingredient that can partially replace products, such as fish meal and soybean meal (16).

Methylotrophic yeasts include *Pichia pastoris* (*P. pastoris*), *Ogataea polymorpha*, and *Candida boidinii*. These yeasts use methanol as a carbon source via the xylulose monophosphate (XuMP) pathway (26). *P. pastoris* is a prominent industrial methanol yeast that has obtained GRAS certification from the FDA and is extensively used for the production of recombinant proteins (27; 28). Methanolic yeast exhibits a high protein content and is rich in essential amino acids and vitamins. This process effectively converts methanol into high-value SCP, reducing carbon emissions and promoting sustainable development. However, the intricate methanol metabolic pathway and toxicity of intracellular formaldehyde contribute to low methanol utilization efficiency in natural methanol-producing yeasts. Using a combination of elevated fermentation temperatures and adaptive evolution, Meng et al. enhanced the methanol utilization efficiency of *P. pastoris* to 43% (29).

Advantage of Yeast in SCP Production

- Harvesting is easy due to their larger size than bacteria (larger than bacteria)
- Malic acid percentage is higher
- Lysine content is higher
- It has the ability to grow and maintain at acidic pH
- It has been traditionally used since a long time ago

Disadvantages of Yeast in SCP Production

- It has a slower growth rate than bacterial cells
- It contains lesser protein content (45–65%) as compared to bacteria
- Its methionine content is lower than bacteria. Hence, addition of methionine in the final product is required to increase its value thus adding another step during processing (30).

Filamentous fungus

Filamentous fungi are a rich source of nutrients, comprising approximately 45% protein, 25% fiber, 13% fat, and 10% carbohydrates in dry cell weight (DCW), along with a variety of vitamins and minerals (31). Compared with the standards set forth by the Food and Agriculture Organization of the United Nations (FAO), the amino acid profile of filamentous fungi is advantageous. These organisms typically exhibit elevated levels of threonine and lysine while displaying relatively lower methionine content (32). One of the distinctive advantages of filamentous fungi as microbial protein producers is their capacity to flourish on a diverse array of carbon sources, including fruit by-products, spent grains from beer

production, and agricultural residues (32). This versatility not only augments their efficiency in protein production but also offers a sustainable result for the management of waste and by-products from food production and processing. Filamentous fungi are therefore of value not only for their nutritional output but also for their role in the promotion of circular economy principles through the conversion of waste materials into high-quality proteins. The optimal temperature range for fungal growth is 20–30 °C, with a pH range of 5.5–8.0 being conducive to growth. Oxygen is a vital component of fungal metabolism and growth. The most commonly used filamentous fungi for protein production include *Fusarium venenatum*, *Aspergillus oryzae*, and *Paecilomyces varioti*. For example, fungal proteins derived from *F. venenatum* are employed as suitable substitutes for chicken breast tissue in chicken nuggets, whereas *A. oryzae* contributes to burger patty production (33, 34). *F. venenatum* is extensively used for the production of fungal protein. For example, the Marlow Foods SCP product Quorn™ contains approximately 50% protein as determined by DCW (20). This product is principally used in the production of sausage patties and ready to-eat burgers. Furthermore, fungal proteins are a source of B vitamins and beneficial secondary metabolites, including β -carotene and ergosterol. The growth of fungi is slower than that of bacteria, predominantly for large edible fungi. Most fungi comprise nucleic acids at concentrations between 7 and 10% (35). Nevertheless, during the cultivation process, toxins produced by some fungi represent a considerable concern when using fungi as strains to produce microbial proteins (36).

Table 2 Advantages and Disadvantages of Single-Cell Protein (SCP) Production (31).

Advantages	Disadvantages
Reduced Land and Resource Use	High Energy Consumption
Lower Water Usage	Resource Competition
Lower Greenhouse Gas Emissions	Capital Intensive
Reduced Pollution	Biomass Separation
Year-Round and Efficient Production	Dependency on Raw Materials
Reduced Overfishing	Potential for Limited Amino Acid Profile
Nutrient Recycling	Scale-Up Challenges
Less Land Degradation	Waste Generation
Resource Efficiency	External Cooling System required
Reduced Biodiversity Loss	–
Food Security	–

Method of Production of Single Cell Protein

The selection of yeast strain, fermentation medium composition, trace element supplementation, cultivation conditions, and harvested biomass treatment can all alter the final SCP composition (37, 38).

Selection of substrate and strain

The first step in the production process elects the appropriate microorganisms that produce a good amount of protein. The strains are collected from different sources such as water, air or soil, among others. Microorganisms are selected by different studies containing mutagenesis and other genetic methods, sometimes wild types can also be used (39).

- Then suitable substrates are chosen that are essential for the growth of selected microorganisms.

Fermentation

- A fermenter is an instrument, which is set up to carry out the process of fermentation mainly the mass culture of plant or animal cells.
- The inoculated medium is then placed in a fermenter where the microorganisms grow and multiply. The conditions inside the fermenter, such as temperature, pH, and oxygen levels, are carefully controlled to optimize the growth of the microorganisms.

Harvesting

- After fermentation, the microbial cells are harvested and separated from the growth medium.
- However, the harvesting and purification after production of SCP production remain a problem.

Post-harvest Treatment

- The harvested microbial cells are then dried to preserve them and reduce their volume, making them easier to store and transport.

SCP processing for Food

- The dried cells are then processed to remove impurities, improve their nutritional content, and enhance their flavor and texture.

According to the studies summarized in Table 1, yeasts of the genus *Candida* are most often used for protein production, which can accumulate approximately 39–79 % of protein in dry cell biomass.

Factors Affecting the Microorganisms Production of Single Cell Protein

The concentration of protein and amino acids profile in yeast cells is mainly genetically predetermined (3); however, to fully exploit the biotechnological potential of the selected yeast strain, it is necessary to optimize microbial fermentation process (40). The yield and productivity of SCP production are strongly dependent on culture medium composition (carbon, nitrogen, and trace elements, environmental conditions (pH, temperature, dissolved oxygen, aeration), time of cultivation and requirements for) and selected microorganisms (41; 42; 43).

Microorganisms can respond to environmental changes and the availability of nutrients and trace elements in the fermentation medium. This ability is essential for metabolism, growth, and reproduction of

microorganisms. Yeast metabolism is a set of complex enzymatic reactions to substrate components penetrating the cell wall membrane. Therefore, optimizing these factors is crucial for maximizing yield and productivity, while also considering the inoculum quality and choose fermentation time (44; 9).

Carbon Sources

The most commonly used materials to produce single cell protein include orange peels, sweet oranges, sugar cane, wheat straw, rice husk, sawdust, corn cobs, grape waste, coconut waste, mango waste, sugar cane pulp, milk etc. (45). As well as traditional materials such as date molasses, fruit and vegetable residues, and non-conventional carbon sources such as starch, alcohols, polyols, hydrocarbons, and fatty acids (45; 46). Lignocellulosic biomass such as hemicellulose and cellulose are a suitable substrate for good production (47). The use of the correct substrate is one of the most important factors for its direct effect on the production process. Organisms interact differently with each substrate, so the rate of consumption of nutrients varies according to the substrate used.

A number of agro-industrial waste and by-products have been used for the production of SCP and other metabolites, including glycerol (48), cheese whey (49), waste milk (50), different fruits peels (51), industrial waste cooking oil (47), salad oil manufacturing wastewater (52), potato processing wastewater (53; 54), olive mill wastewater (55) municipal solid waste (56). Production of single-cell protein by fermentation process has been revealed by many researchers and summarizes studies with the biomass and protein content using different substrates,

cultivation conditions, and yeast species
Table 3 below

Table 3. Different Substrates Used as a Carbon Source and SCP Content in Different Yeast Species (19, 31, 78).

Yeast Species	Carbons substrates	cultivation time, h	Cultivation Condition					SCP, %v	Ref.
			T, °C	pH	DCW g/L				
<i>Saccharomyces cerevisiae</i>	Glycerol	48	28	5.5	n/d	47.9	48		
<i>Candida utilis</i>	Mango residue	30	30	4.0	15.28	79.1	40		
	<i>Opuntia ficus indica</i> hydrolysate	50	35	5.0	12.2	14.0	64		
	Potato wastewater	48	28	5.0	5.65	48.9	60		
<i>Candida tropicalis</i>	Soy molasses	30	30	5.5	10.83	56.4	76		
	Sugarcane Bagasse	96	30	5.0	16.97	60.1	59		
	hydrolysate								
	Sugar beet pulp	10	30	4.5	16.21	47.8	72		
<i>Candida pararugosa</i>	Olive mill wastewater	96	30	n/d	21.68	39.4	71		
<i>Candida guillier mondii</i>	sugar beet pulp	10	10		4.5	15.5	49.2	70	
<i>Yarrowia lipolytica</i>	Glycerol	48	28	5.5	n/d	46.7	48		
	Waste cooking oil	120	28	n/d	57.37	12.6	47		
<i>Kluyveromyces marxianus</i>	<i>Opuntia ficus indica</i> Hydrolysate	50	40	5.0	11.1		10	54	
<i>Rhodotorula glutinis</i>	Potato Wastewater and 5% Glycerol	72	28	5.0	19.24	40.5	65		
<i>Pichia stipites</i>	Sugar beef pulp	10	30	4.5	19.54	45.6	70		
<i>Pichia kudriazevii</i>	Biogas substrate	12-15	37	7.0	7.36	32.7	56		
<i>Schwanniomyces etchellsii</i>	Oil mill Wastewater	96	30	n/d	15.11	35.9	55		
<i>Blastobotrys adeninivorans</i>	Biogas substrate	12-15			37	7.0	14.83	30.5	56
	spruce sugar	28	30	5.0	27.62	42.45	38		
	Hydrolysate								
<i>Wickerhamomyces Anamalus</i>	Biogas substrate	12-15	30	7.0	7.03	22.6	56		
	Spruce sugar	24	30	5.0	29.78	41.22	38		
	Hydrolysate								

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

Nitrogen Sources

The most important sources of nitrogen that are involved in protein synthesis are urea, nitrates, ammonia, ammonium salts

and organic nitrogen in different substrates such as potato and starch processing waste and cheese whey, are consumed by microorganisms (3; 57). Yeasts can utilize 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 (58),

The yield of protein production varies according to the nitrogen source used. In study (59) it was found that ammonium sulfate was better than ammonium nitrate as a nitrogen source for the growth of *C. utilis*. It was observed that the percentage of protein produced using *S. cerevisiae* was reduced by using organic nitrogen sources compared with inorganic sources. And when using *Candida* spp. to ammonium sulfate as a nitrogen source, it gave a high production of protein (60). (52) concluded in his study that the addition of C: N in a ratio of 1: 6 and 1: 8 to the growth medium of *Candida* spp. and *Rhodotorula* spp. it increased the amount of protein produced.

The best nitrogen source is tryptone and yeast extract. When tryptone was supplemented at a concentration of 0.8% w/v, the maximum yield of protein was obtained (61).

Temperature and pH

Temperature is one of the most important factors affecting the growth of yeasts and thus affecting the production of monocytic protein. The optimum temperature for yeast growth ranges between 28 -35°C (62, 63). It was found that 30 °C is suitable for the growth of *S. cerevisiae* and the production of single cell protein, while 25°C was the optimum for the growth of *C. utilis* (64). The ideal pH value for yeast growth in Single Cell Production varies by yeast strain and culture conditions, but generally falls within the range of 4.0–7.0 (65, 40); within this range, the most

preferred pH of the medium for protein biosynthesis is 4.0–6.0, while others report better growth or protein accumulation at near neutral pH range. Recent research, observed optimization condition such as temperature of 32°C and pH of 7.3 underscore the potential of *R. marisflavi* NDS as a high-quality dietary protein supplement and provide a solid foundation for its industrial-scale production (66)

Several studies have used a pH between 3.5-7 to produce a single cell protein using *S. cerevisiae* and 3-6.2 with *Candida* spp. (67)

Supplementation of Trace Elements

Trace elements that play an important role in SCP production are essential minerals like iron, zinc, chromium and selenium in addition to the fermentation medium. Supplementation with these elements improved yeasts' requirements 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 (68). The most requested is phosphate, magnesium, calcium, potassium, zinc, and iron (69, 70;16, 71, 72). Yeast cells are starved for phosphate and Sulphur seizure in a quiescent state in which fermentation of glucose is suppressed: external glucose is not depleted (73). (74) 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 (74). This mechanism is explained in the work of.

(75), 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 (75). In another research work by (76) 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, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, K_2HPO_4 , and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ in the same amount had no notable effect (76). Also, in another study by (69) provides a more detailed overview of the effect of different salt concentrations on the growth of the yeast *C. utilis*. Mineral salts KH_2PO_4 , MgSO_4 , FeSO_4 , and KCl at 0.2, 0.07, 0.002, and 0.8 g/L concentrations significantly increased biomass production (69). The medium was optimized with 0.02 g/L selenium supplementation enriched *S. cerevisiae* and *C. utilis* biomass functional diversity in terms of protein and amino acid content (9). 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 (9).

Time of Cultivation

The cultivation time for Single Cell Protein varies but optimal periods typically range from 7 to 14 days for fungi and yeasts, depending on the specific organism, substrate and process. The cultivation time of yeasts is an important parameter for protein production, optimal production is achieved when the harvest time coincides with microorganism's peak growth or protein accumulation phase, which varies depending on the specific microbe and substrate use.

Results of research work described how the temperature of microorganism cultivation affects the kinetics of key molecular processes in the cell, thereby affecting the biomass specific growth rate (77). 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 ($\mu_{\text{max}} < 0.1 \text{ h}^{-1}$) the carbohydrates content is up to 50 % and proteins content is up to 40 % of the dry biomass, whereas at high growth rate ($\mu_{\text{max}} > 0.3 \text{ h}^{-1}$), the carbohydrates content linearly decreases to 15 % and proteins content increases up to 60 % of the dry biomass (77). This may explain the high values of the protein content in a short cultivation time as described in the studies of (70) and. (38), were 46–49 %

SCP in the biomass of *C. tropicalis*, *P. stipites*, *C. guilliermondii*, and 47–51 % in *C. jadinii*, *W. anomalus*, *B. adenivorans* were obtained after 10 and 12 h of cultivation, respectively.

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 influencing single-cell protein production (55, 40, 42), although research work describing the effect of different inoculum sizes on protein production are intermittent. The optimum inoculum size varies for different microorganisms (5), and depends on the total concentration of dissolved oxygen and nutrients in the fermentation medium (78, 55, 42). For example, in a study by (42), 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 (42). Similar results were obtained in 55), 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 (55). In another study by (40), 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 (40).

CONCLUSION AND RECOMMENDATION

In this review, the study shows that yeast culture conditions such as temperature, pH, and time of cultivation have enormous impact on the protein content of yeast biomass. For single cell protein production, yeast generally exhibit robust growth across a wide range of the medium conditions, with optimal ranges typically between pH and temperature are 4.0–6.0 and 28–35 °C. Time of cultivation is critical influencing factor for single cell protein production, directly affecting the maximum biomass yield and productivity. SCP yield generally increases with cultivation time until an optimal point, after which it may stabilize or slightly decrease. This aspect must be considered and examined 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

Thus, to optimize the protein production process, it is necessary to comprehensively consider the influence of different cultivation parameters, the exact optimal conditions can vary depending on the specific strain and the composition of the substrate/medium used for fermentation, but also the preferences and characteristics of each species of yeast.

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