

## Single cell protein (SCP): Microbial bioconversion strategies for sustainable protein security and circular bioeconomy

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The growing global demand for sustainable protein sources has contributed to an increased interest in microbial single cell protein (SCP) as an alternative to traditional plant and animal-based proteins. The term SCP refers to protein-rich biomass obtained from non-pathogenic microorganisms such as bacteria, yeast, filamentous fungus, and microalgae. Because of their rapid growth rates, excellent substrate conversion efficiency, and resistance to climatic and seasonal changes, these microorganisms represent ideal biological platforms for protein production. The physiological and metabolic characteristics influencing biomass yield, protein composition, and nucleic acid content are highlighted in this paper, which provides an overview of the advancements in microbial strain diversity. The ability of diverse microorganisms to use renewable and low-cost substrates such as agro-industrial residues, lignocellulosic biomass, waste effluents, methanol, and gaseous carbon sources is highlighted, allowing for sustainable single-cell protein (SCP) production via integrated bioconversion processes. Recent improvements in fermentation technologies, such as batch, fed-batch, and continuous cultivation systems, are evaluated in terms of downstream processing, substrate management, oxygen transfer efficiency, and overall process optimization. Important factors such as high nucleic acid concentration, digestibility of cell walls, cost of harvesting, and safety concerns are also evaluated. Important factors are also investigated, including increased nucleic acid levels, the digestibility of microbial cell walls, harvesting expenses, and safety concerns. In order to increase nutritional quality and economic viability, options for nucleic acid reduction, cell disruption, strain improvement, and genetic manipulation are addressed. Overall, microbial SCP synthesis is a biologically sound and industrially scalable strategy for addressing the global protein deficit as well as contributing to the principles of waste valorization and circular bioeconomy. Continued advancements in microbial physiology, metabolic engineering, and bioprocess optimization are necessary to improve cost-effectiveness, product safety, and large-scale implementation.

**Keywords:** Circular bioeconomy, global protein security, microbial bioconversion, single-cell protein, waste valorization.

### INTRODUCTION

The rapid expansion of the world's population, along with the increasing demand for high-quality protein, has raised concerns regarding long-term protein supply<sup>1</sup>. Conventional protein sources, such as meat, fish, and plant-derived proteins, require significant land, water, and energy inputs, which may limit their ability to meet growing

global nutritional demands<sup>2</sup>. Current statistics suggest that by 2050, the demand for animal-derived protein will reach 1,250 million tons, making sustainable alternatives a global priority. Consequently, the single-cell protein (SCP) market is likely to increase significantly, with its valuation rising from USD 13.1 billion in 2022 to approximately USD 74.6 billion by 2032<sup>3,4</sup>. Driven by these economic

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and environmental pressures scientific research has focused on alternate and sustainable protein sources that can complement or partially replace conventional protein supplies<sup>5</sup>.

Single-cell Protein (SCP) refers to the protein-rich microbial biomass derived from microorganisms such as bacteria, yeast, filamentous fungi, and microalgae<sup>6</sup>. The term was first introduced by Carroll L. Wilson in 1966 to describe protein obtained from microbial cells cultivated for nutritional purposes<sup>7</sup>. In recent years, SCP has emerged as a significant solution in the “microbial food revolution,” providing a reliable biological substrate for protein production irrespective of seasonal and climatic fluctuations. Microorganisms used for SCP production possess several advantages, including rapid growth rates, high protein yield, efficient substrate conversion, and the ability to utilize a wide variety of substrates. These characteristics make microbial biomass a promising alternative source of protein for both human consumption and animal feed<sup>3,6,8</sup>.

For many years, researchers have been investigating the use of microbial biomass as a protein supplement. During World War I, Germany largely substituted imported protein sources with yeast-derived protein, which was one of the first industrial uses of yeast biomass as an animal feed supplement<sup>7,9</sup>. The commercial potential of microbial protein production was later shown by products like “Pruteen,” which was made from bacterial biomass. Recent advances in precision fermentation and metabolic engineering have significantly boosted the safety and economic sustainability of these processes, allowing for the large-scale industrial implementation of SCP<sup>7,10</sup>.

There are several microorganisms used for SCP production include bacteria (*Aeromonas*, *Achromobacter*, *Bacillus*, *Pseudomonas*, *Cellulomonas*, and *Alcaligenes*), algae (*Chlorella*, *Spirulina*, and *Chondrus*), filamentous fungi (*Trichoderma*, *Fusarium*, *Aspergillus*, and *Rhizopus*), and yeasts (*Candida*, *Saccharomyces*, and others)<sup>11–15</sup>. These microorganisms can utilize diverse substrates such as agricultural residues, industrial by-products, lignocellulosic biomass, and various organic wastes. Bioconversion of low-value substrates into high-value protein coordinates perfectly with the principles of the sustainable bioeconomy and waste valorisation, effectively completing the nutrition loop while minimizing environmental pollution<sup>16,17</sup>.

From a nutritional perspective, SCP is considered a valuable protein source due to its high protein content and balanced amino acid composition. However, certain limitations such as high nucleic acid content, digestibility issues related to microbial cell walls, and potential safety concerns must be addressed to ensure its safe use as food or feed. For human consumption, the nucleic acid concentration of single-cell protein should be kept to less than 2% of dry biomass. Excessive nucleic acid intake can lead to increased uric acid levels, which may contribute

to health conditions such as “gout” or “kidney stone” formation<sup>18</sup>.

Therefore, Recent research has concentrated on innovative technological approaches, like as enzymatic treatments and strain improvement tactics, to assure the safety and excellent nutritional quality of SCP products for world-wide consumption<sup>19,20</sup>.

In addition to nutritional benefits, SCP production offers several technological and environmental advantages. Microorganisms exhibit rapid growth and high biomass productivity, enabling efficient protein production within a relatively short time. Furthermore, microbial cultivation is largely independent of climatic conditions and can be carried out throughout the year using controlled fermentation processes. The ability of microorganisms to utilize inexpensive and renewable substrates further enhances the economic and environmental viability of SCP production.<sup>3,6</sup>

## MICROBIAL SOURCES FOR SCP PRODUCTION

Comparative biochemical profiles of SCP micro-organisms The choice of a microbiological source for Single Cell Protein (SCP) production is determined by a balance of biomass productivity, nutritional density, and safety criteria,<sup>6,14</sup> as summarized in Table 1. Although all four microbial groups—fungi, yeast, bacteria, and algae—are effective sources of protein, their biochemical compositions differ greatly depending on their physiological and metabolic traits<sup>21</sup>. Bacteria are the most protein-dense source, making them ideal for high-yield industrial applications. However, their small cell size and low density hinder harvesting processes<sup>22</sup>. In contrast, microalgae have a superior lipid profile and generally lower nucleic acid (NA) contents compared with other groups. This decreased NA level is crucial for human consumption, as high ingestion is connected to metabolic disorders. Fungal and yeast sources are in the middle; although they provide slightly less protein than bacteria, they have a greater ash content and, because of their bigger cellular structures, are typically simpler to collect from fermentation environments<sup>22–24</sup>.

## Advantages and disadvantages of microbial group used for SCP

Compared to bacterial cells, yeasts have the advantages of having a larger size (making them easy to harvest from the growth medium), lower NA content, high lysine content, and the ability to grow at an acidic pH<sup>14</sup>. Conversely, a chief advantage is their familiarity, appropriateness and acceptability owing to the long history of their use in traditional fermentations<sup>14</sup>. Main drawbacks include lower growth rates, lower protein content (45% to 65%), and lower methionine content compared to bacterial cells. Furthermore, the outer mannoprotein layer and high NA concentration (6–12%) remain major characteristics

**Table 1.** Comparison of different microorganisms for nutritional value (% dry weight)

Nutritional value	Algae	Fungi	Yeast	Bacteria	References
Protein	40-60	30-45	45-55	50-60	25,26
Lipids	7-20	2-8	2-6	1-3	27,28
Nucleic Acid	3-8	7-10	6-12	8-12	29
Ash content	8-10	9-14	5-10	3-7	30

that limit the nutritional value of yeast for human consumption<sup>31,32</sup>.

On the other hand, the filamentous fungi have advantages in ease of harvesting, but have their limitations in growth rates, protein content, and acceptability. Algae have disadvantages of having cellulosic cell walls which are not digested by human beings. Secondly, they also concentrate heavy metals. In the case of algae, it has to be stressed that, owing; additionally, they can concentrate heavy metals. While algae cultivated in ponds can yield 20 tons (dry weight) of protein per acre per year, it is important to emphasize that the overall goal is to increase the total amount of algal biomass rather than isolate and use a single protein source due to technical and economic constraints<sup>33</sup>. Therefore, the term 'Single Cell Protein' is not pretty correct, since the microalgal matter is indisputably more than just a protein.

In contrast, bacteria are characterized by higher protein content (50 to 80%), and a faster growth rate. Nevertheless, they also have serious disadvantages: they are small and have low density which complicates collecting them from the fermented medium and makes such a harvest quite expensive<sup>34</sup>. Moreover, they are rich in NA than in fungi and yeast and require extra processing. One of the greatest problems on the social level is the fact that people still believe that all forms of bacteria are pathogenic; therefore, a large-scale educational course is needed to eliminate this myth and emphasize the positive side of the use of bacterial protein by the population<sup>35</sup>.

Till date, worldwide several state-of-the art technologies have been employed for the mass production and processing of photo-autotrophic algae. Annual world production of all microalgae species is estimated to about 15,000 tons/year<sup>36,37</sup>. Algal biomass as sun dried product or in compressed form as tablets is the predominant form of product in micro-algal industry<sup>38</sup>. More than 75% of the annual micro-algal biomass production is used for the manufacture of powdered biomass, tablets, pastilles or capsules. This biomass is harvested from natural or waste waters, artificial ponds or using photobioreactors (PBR) and consequently separated from growth media followed by drying process<sup>39</sup>. Two main species cultivated for this purpose are unicellular green alga, *Chlorella* sp. and filamentous blue-green algae also known as Cyanobacterium, the *Spirulina*<sup>40</sup>. While production of single cell protein from the numerous microorganisms- predominantly from bacteria and fungi

has received substantial consideration, only a few studies have dealt with the possibility of using single cell protein from<sup>41,42</sup>.

The algal proteins are of very high quality and analogous to the conventional vegetable proteins. Conversely, owing to the high production costs as well as some technical difficulties, the cultivation of algae specifically as protein product is still in evaluation process<sup>43</sup>. The cellulosic cell wall of algal biomass, which represents approximately ten percent of the algal dry matter, poses a barrier to digestion in humans and other non-ruminants<sup>44,45</sup>. Henceforth, treatment processes are important to disrupt cell wall to make the protein and other constituents accessible to digestive enzymes. Several authors have studied the effect of various post harvesting treatments on digestibility of various algal species<sup>46</sup>. As shown in Table 2, various species of yeast, algae, fungi, and bacteria are used as SCP and produced at the commercial scale. These microorganisms are grown on various carbon sources for SCP production<sup>47</sup>. Microalgae, along with other supplements have been consumed as an important food product for the larval stages of fish, shellfish and other animals. The yeast cells have been considered as a better substitute owing to their small particle size, high protein content and comparatively less production costs<sup>48</sup>. However, poor digestibility may be a constraint in the use of this SCP as a food source in seed production of aquacultural organisms, since yeast has a complex and thick cell envelope. The external mannoprotein layer of the yeast cell wall is probably the major barrier to digestion, several methods have been developed to improve the digestibility of SCP products: mechanical disruption, autolysis and enzymatic treatment<sup>49</sup>.

For maintenance of a stock collection, preservation by drying or freeze-drying for long periods was reported to be effective for yeast or algae<sup>50</sup>. It has been agreed that the criteria used to evaluate SCP production are growth yield, total protein (39% -73%) and nucleic acid contents (1% -11%)<sup>40</sup>. Marine yeasts are better candidates for marifeed production due to their easy cultivation in fermenters, high cell density and high content of essential amino acids<sup>51</sup>. The substrates used for SCP production by yeasts so far include sorghum hydrolysate, sulfate waste liquor, pawn-shell wastes, dairy wastes, methanol, molasses, starch and plant originated liquid waste<sup>6</sup>. Several fungi such as *Fusarium oxysporum* f. *lini*<sup>7</sup> and *Chaetomium cellulolyticum*<sup>52</sup>; algae like *Chlorella*

**Table 2.** Microorganisms used as SCP and their growth substrate

S. No.	Microorganisms used as SCP	Substrate for microbes	References
A.	<b>Yeast</b>		
	<i>Saccharomyces cerevisiae</i>	Pentose, Maltose, Lactose	48
	<i>Candida novellas</i>	n-Alkanes	54
	<i>Amoco torula</i>	Ethanol	50
	<i>Candida intermedia</i>	Lactose	55
	<i>Candida utilis</i>	Glucose	46,56
	<i>Candida tropicalis</i>	Glucose, Maltose	49
B.	<b>Bacteria</b>		
	<i>Pseudomonas fluorescens</i>	Non protein nitrogen compounds, Uric acid	57
	<i>Aeromonas hydrophila</i>	Lactose	41
	<i>Lactobacillus sp.</i>	Amylose, Maltose, Glucose	39
	<i>Bacillus megaterium</i>	Non protein nitrogen compounds	47
	<i>Achromobacter delvacvate</i>	N-Alkanes	89
	<i>Methylomonas methylotrophus</i>	Methanol	37
	<i>Methylomonas clara</i>	Methanol	38
	<i>Cellulomonas sp.</i>	Hemicellulose, Cellulose	58
	<i>Thermomonospora fusca</i>	Hemicellulose, Cellulose	33
	<i>Rhodopseudomonas capsulata</i>	Glucose	40
	<i>Flavobacterium sp.</i>	Hemicellulose, Cellulose	42
C.	<b>Algae</b>		
	<i>Chlorella sorokiniana</i>	CO <sub>2</sub> through photosynthesis	43,58
	<i>Scenedesmus sp.</i>	CO <sub>2</sub> through photosynthesis	58
	<i>Chlorella pyrenoidosa</i>	CO <sub>2</sub> through photosynthesis	44
	<i>Porphyridium sp.</i>	CO <sub>2</sub> through photosynthesis	38
	<i>Spirulina sp.</i>	CO <sub>2</sub> through photosynthesis	40
	<i>Chondrus crispus</i>	CO <sub>2</sub> through photosynthesis	39
D.	<b>Fungi</b>		
	<i>Rhizopus chinensis</i>	Maltose, Glucose	46
	<i>Trichoderma alba</i>	Pentose, Cellulose	33
	<i>Aspergillus fumigatus</i>	Glucose, Maltose	41
	<i>Aspergillus oryzae</i>	Hemicellulose, Cellulose	49
	<i>Trichoderma viride</i>	Pentose, Cellulose	47
	<i>Cephalosporium eichhorniae</i>	Hemicellulose, Cellulose	50
	<i>Aspergillus niger</i>	Hemicellulose, Cellulose	49
	<i>Chaetomium cellulolyticum</i>	Hemicellulose, Cellulose	33
	<i>Penicillium cyclopium</i>	Lactose, Galactose, Glucose	55

and *Spirulina*; yeasts such as *Candida lipolytica* and *Saccharomyces cerevisiae*<sup>53</sup>; and phototrophic bacteria like *Rhodospirillum sp.*<sup>20</sup>. have been explored for Single Cell Protein (SCP) production.

Table 2 provides a comprehensive summary of the variety of microorganisms used for Single Cell Protein production, arranged according to their respective categories and the particular carbon sources or substrates needed for their growth.

### Production processes and substrates

All over the globe, largescale production and development of single cell protein (SCP) processes has added meaningfully to the progression of today micro-biotechnology. Research and development of SCP processes has involved work in the fields of biotechnology, phycology, mycology, microbiology, biochemistry, genetics, chemical and process engineering, food and feed technology, environment and agriculture, animal and poultry nutrition, bionetwork, toxicology, medicine, economics and

veterinary sciences<sup>20,97</sup>. Recent developments highlight how the “microbial food revolution,” which uses metabolic engineering and precision fermentation to customize nutritional profiles for particular dietary requirements, is fundamental to the future of SCP<sup>59</sup>.

In developing the SCP procedures, methods new procedural elucidations for other related technologies in waste water handling, alcohol production, enzyme expertise and nutritive and dietetic science also improves<sup>60</sup>. Future of ‘single cell protein’ will be profoundly dependent on sinking the production costs and enhancing the quality by using novel methods of downstream processing, fermentation, and develop upgradation in the producer organisms as a result of the usual applied genetics techniques collectively with recombinant (RDT) DNA technology<sup>3,61</sup>. Additionally, in order to maximize metabolic flux and biomass yield, the integration of artificial intelligence (AI) and real-time biosensors in bioreactors is now being assessed<sup>62-64</sup>. The SC proteins have many applications in animal nutrition such as: fattening of calves, pigs, poultry, goat and fish breeding. Other applications are in the products area such as: fragrance carriers, vitamin carrier, emulsifying aids and also to enhance the nutritive value of baked products<sup>65</sup>. Moreover, the SCP are also used in soups, in ready to serve foods, in the diet recipes and also in technical field such as: paper and cardboard processing, leather and polyvinyl processing and also as foam stabilizers<sup>66</sup>.

Large scale production of the single cell protein takes place in fermentation process<sup>3,34</sup>. This is achieved by the selected potential microbial strains which are multiplied on appropriate raw materials in the specialized cultivation process which directed to the growth of culture and cell mass followed by the separation processes<sup>3</sup>. The process development commences with the microbial screening, in which the suitable SCP production strains are selected or attained from samples of plants, water, soil, air or from inorganic or biological materials and are consequently boosted by the process’s selection, alteration, or other genetic approaches. After that procedural cultivation conditions for optimized microbial strains are done and all the metabolic pathways and cell structures will be governed<sup>34</sup>.

Moreover, the process engineering and gadget technology acclimatize the technical functioning of the process in order to make the manufacture ready for use on the large technical scale. Here comes the most important and vital factor, that is economic and marketing factors (such as man power, space, energy, cost, marketing cost) come into role. Most importantly, safety demands and the environmental protection is also considered in the production of single cell protein in relation both to the product and to the process. Lastly, safety and the protection of innovation throw up legal and controlled aspects, namely operating licenses, product authorizations for particular applications and the legal protection of new process and strains of microorganisms<sup>67</sup>.

## Raw materials and wastevalorization

The classical raw materials for SCP production are substances which chiefly contain the mono and disaccharides, since almost all potential microbes for SCP can digest simple sugars such as glucose, other hexose and pentose sugars and commonly available disaccharides<sup>68</sup>. These microbes’ metabolic adaptability enables them to channel these sugars through primary pathways such as glycolysis and the pentose phosphate pathway to produce adenosine triphosphate (ATP) and carbon skeletons required for fast biomass synthesis<sup>69</sup>. However, in substrates generated from lignocellulosic hydrolysates, preferential glucose uptake frequently results in carbon catabolite repression<sup>69</sup>, which might impede the simultaneous utilization of mixed sugars. To overcome this, modern bioengineering tactics emphasize the development of co-utilization strains capable of efficiently processing both hexose and pentose sugars at the same time, hence boosting protein output and guaranteeing that the carbon source is fully utilized<sup>70</sup>. These raw materials also are consumed in other industrial branches with high price level, which puts the cost economy aspect of microbial biomass production in doubt<sup>71</sup>. To overcome these issues, research has shifted to a “waste-to-protein” paradigm, focusing on the valorization of low-cost agro-industrial residues to boost economic viability. The choice of substrates that are normally available and abundant has governed the design and SCP processes strategies. Most prevalent and frequently used SCP substrates for production have been those where the energy and carbon sources are obtained. Additionally, the shift from second-generation to third-generation substrates, including gaseous carbon (CO<sub>2</sub> and methane), is becoming more popular since it reduces the “FOOD VS. FEED” competition and aligns with the objectives of a circular economy<sup>48</sup>.

There are many companies which are producing SCP including BP (UK), Liquichimica (Italy) and Kanegafuichi (Japan) appeared on the global scene. In the USA less than 15 percent of the SCP manufacturing plants were said to depend on source of hydrocarbons as the supply of energy and carbons for the microbes.<sup>21</sup> There are other impending substrates for single cell protein such as sugarcane bagasse, citrus wastes, sulphite waste liquor, molasses, animal manure, whey, starch, sewage, etc.<sup>7</sup>.

## SCP production from industrial and agricultural wastes

Amount of some industrial and agricultural wastes employed for SCP production can be very high and might influence to a vital level of watercourses pollution. Consequently, the consumption of such raw materials in SCP processes and production serves two functions as reduction in pollution and creation of edible protein. From agriculture and forestry sources, the cellulose forms the most copious renewable resource on this planet as a potential source

for SCP production<sup>29</sup>. The cellulose has been emerged as a striking substrate for SCP production but in nature it is typically associated with other substrates such as lignin, hemicellulose, starch, etc., which forms a complex form. Consequently, if cellulose is to be employed as a substrate, then it must be pretreated with chemicals (acid hydrolysis) or with effective enzymes (cellulases) to separate cellulose as a fermentable sugar<sup>72</sup>. Recent advances in pre-treatment, such as the use of ionic solutions and customized enzymatic concoctions, have greatly enhanced the yield of fermentable sugars from complicated lignocellulosic matrices<sup>73</sup>. For the optimum utilization of substrate lignocellulose, a pretreatment is also generally usually necessary. Many pre-treatment methods have been developed and reported which differ from acid or alkali treatment, exposure to steam or even x-ray radiation<sup>74</sup>. As of right now, the cultivation of mushrooms is the only economical use of lignocellulosic wastes. Also, our well known cultivated and commonly consumed mushroom *Agaricus bisporus*, which excretes lignocellulolytic enzymes and are cultivated for food mainly in Asia, Africa and other countries<sup>75</sup>. There are some other edible fungi which are of great economic importance and are also cultivated on larger an industrial scale. The common examples of such important ones include *Volvariella* sp., *Lentinus edodes* and *Pleurotus* sp.<sup>76</sup>. Single Cell Protein (SCP) can be produced from paper waste by breaking down its high cellulose content (60–70% sugar) into fermentable sugars, which microorganisms like *Candida* sp. or *Saccharomyces* sp. convert into high-protein biomass<sup>77</sup>. This process requires pre-treatment (physical/chemical/enzymatic) to turn waste into substrate, typically yielding high protein for feed<sup>78</sup>. In one process, wood can also be prepared in a medium containing calcium sulfite with excess free sulfur dioxide. Therefore, Lignin is converted to lignosulfonates and hemicellulose which is further hydrolyzed to monosaccharides and may be further broken down to furfurals or other simpler compounds. Number of free sugars in the spent liquor varies with the type of process chosen, as numerous cellulose fibers may be obtained with different degrees of degradation<sup>79</sup>. Spent sulfite liquor has been used as a substrate for fermentations since 1909 in Sweden and later in many other parts of the world<sup>80</sup>. The first organism to be used was *Saccharomyces cerevisiae*, although this organism is unable to metabolize pentose sugar which are found in considerable amounts in this waste product<sup>81</sup>. Later, other organisms better suited for the assimilation of all the sugar monomers were chosen, namely *Candida tropicalis* and *Candida utilis*<sup>21</sup>. Yeast produced from sulfite liquor has been used for feeding at war periods, but lost favor in peace time<sup>56</sup>. However, experiences of baker's yeast produced from sulfite liquor exist in Finland by Peliko process<sup>82</sup>. The protein content of the fungus *Paecilomyces variotii* exceeds 55% (w/w) and has been officially approved as a food in Finland<sup>3</sup>.

### Fermentation technologies (batch, fed-batch, and continuous)

The fermentation process requires a pure culture of the chosen organism that is in the correct physiological state, sterilization of the growth medium which is used for the organism, a production fermenter which is the equipment used for drawing the culture medium in the steady state, cell separation, collection of cell free supernatant, product purification and effluent treatment<sup>3,34</sup>. A fermenter is the instrument, which is set up to carry out the process of fermentation mainly the mass culture of plant or animal cells<sup>67</sup>. Fermenters can vary in size from laboratory experimental models of one- or two-liters capacity, to industrial models of several hundred liters capacity. A bioreactor is different from a fermenter as it used for the mass culture of microorganisms. The chemical compounds synthesized by these cultured cells such as therapeutic agents can be extracted easily from the cell biomass. Modern bioreactors are progressively combining advanced sensors for real-time metabolic flux measurement, allowing for exact regulation of nutrient feed rates to minimize the accumulation of inhibitory metabolites<sup>59</sup>.

The design engineering and operational parameters of both fermenters and bioreactors are identical. Fermenters and bioreactors are also equipped with an aerator, which supplies oxygen to aerobic processes also a stirrer is used to keep the concentration of the medium the same. A thermostat is used to regulate temperature and a pH detector and some other control devices, which keep all the different parameters needed for growth constant<sup>59</sup>. For the producing and harvesting of microbial proteins cost is a major problem<sup>61</sup>. Such a production even in high rate causes dilute solutions usually less than 5% solids. There are many methods available for concentrating the solutions like filtration, precipitation, centrifugation and the use of semi-permeable membranes. The equipment used for these methods of de-watering is expensive and so would not be suitable for small scale productions and operations. The removal of the amount of water that is necessary to make the material stable for mass storage is not economically viable. Single cell proteins need to be dried to 10% moisture or they can be condensed and denatured to prevent spoilage. The physiological features of microbial organisms recommend the control of the carbon source concentrations, as a limiting substrate, as well as an adequate supply of oxygen for the maintenance of balanced growth under an oxidative metabolic pattern<sup>83</sup>. Maintaining the volumetric oxygen transfer rate ( $K_L A$ ) in large-scale systems is a major limiting element; as biomass density rises, the biological oxygen demand frequently exceeds the system's capacity, potentially triggering anaerobic fermentation and lowering protein yield<sup>83,84</sup>.

However, since microbial growth is a time dependent process, it exerts continuous modifications on all process

parameters which influence physiology, but most dramatically, over substrate concentration. Therefore, an adequate technology which maintains appropriate growth conditions for a prolonged period of time must be implemented specifically for the purpose of obtaining high yield and productivity values<sup>85</sup>. Batch fermentations are clearly inadequate for the purpose of biomass production, since the conditions in the reaction medium change with time<sup>86</sup>. Fed-batch fermentations are better suited for the purpose of biomass production, since they involve the control of the carbon source supply through feeding rates. Continuous cultivation systems are presently being prioritized for global industrial adoption because they provide steady-state kinetics and better volumetric productivity, lowering downtime and operational costs. However, the shift to continuous mode presents significant practical challenges, particularly with regard to the strain's long-term genetic stability and the increased risk of system-wide contamination over extended runs<sup>10,89</sup>. Furthermore, as the biomass concentration increases in these high-density systems, the oxygen demand of the culture reaches a level which cannot be met in engineering or economic terms due to the energy costs associated with agitation and the constraints of oxygen solubility in water. The preferred industrial approach to balancing productivity with operational control is still fed-batch cultivation, which is crucial for applications like baker's yeast production because it allows for the dynamic regulation of nutrient feeding and oxygen supply in direct response to increasing cell density, minimizing substrate inhibition and maintaining optimal metabolic flux (Yap *et al.*, 2022)<sup>83</sup>.

## DOWNSTREAM PROCESSING AND FOOD SAFETY

### Processing of SCP food

The effective use of microbial protein for human food requires:

Liberation of cell proteins by destruction of indigestible cell walls<sup>49,88</sup>

Reduction of nucleic acid content<sup>89,90</sup>

### Methods of cell wall disruption

The use of microorganisms for refined SCP requires not only an adequate amount of the specific organism but also an efficient means of disrupting the cell wall (Baldwin and Robinson, 1994)<sup>91</sup>. The diversity of microbial structures contributes to the complexity of cell wall disruption; such as the rigid glucan-chitin network in yeast and the refractory cellulosic barriers in algae demand specialized techniques to enhance protein biological bioavailability<sup>92</sup>. Mechanical integration of cell wall can be carried out either by crushing, crumbling, grinding, pressure homogenization or ultra-sonication<sup>45</sup>. Various enzymes or combination of enzymes can be used to digest and disrupt cell wall, either

partially or completely<sup>49</sup>. Enzymatic hydrolysis of cell wall is attractive in terms of its delicacy and specificity for only the cell wall structure. Enzymatic lysis, unlike severe chemical treatments, reduces the possibility of protein denaturation, but it remains an economically hard choice due to the high cost of commercial enzymes and extended incubation durations<sup>13</sup>. It may be used as an alternative to the mechanical disruption, especially for materials that can be inactivated during the mechanical process and it can be performed by endogenous or exogenous enzyme from other microorganisms. However, extensive enzymatic lysis of cells is a very slow process compared to mechanical disruptions. It is possible to use two or more methods for cell disruptions. Combined mechanical and enzymatic degradation of yeast cell wall was tested by Kumar *et al.*<sup>13</sup>. In case of yeast cells, they first can be mechanically broken and then incubated with a lytic enzyme<sup>93</sup>. This resulted in the release of a substantial amount of protein mostly from organelles and cell walls. According to recent developments, dual disruption strategies, like initial bead milling followed by targeted enzymatic treatment, can achieve up to 90% protein release while considerably reducing total energy consumption<sup>49</sup>. Baldwin and Robinson, 1992<sup>91</sup> reported enhanced disruption of *Candida utilis* by enzymatic pretreatment and high-pressure homogenization. Other methods employed for yeast cell breakage include: autolysis followed by enzymatic or alkali treatments<sup>94</sup>, NaCl induced autolysis at different temperature, chemical disintegration using detergent such as sodium dodecyl sulfate or Triton X-100, acid or solvent<sup>95</sup>. The digestibility of yeast and microalgae can be greatly increased by drying at high temperature under certain conditions. However, the heat treatment needed to increase the digestibility of the cells also affects the protein quality and other valuable cell components<sup>45</sup>.

## METHODS FOR CELL DISRUPTION<sup>96</sup>

### Non-mechanical methods

Chemical treatment: acid, base, solvent, detergent

Enzyme analysis: lytic enzymes, phage infection, autolysis

Physical treatment: freeze-thaw, osmotic shock, heating and drying

### Mechanical methods

High pressure homogenization

Wet milling

Sonification

Pressure extrusion: French press, freeze pressing

Decompression (pressure chamber)

treatment with grinding particles

### Strategies for nucleic acid reduction

There are several methods which have been proposed to reduce NA levels in single-cell protein, commonly involving chemical and enzymatic treatments<sup>18,97</sup>. Each method has its own disadvantages both in terms of cost and potential nutritional concerns<sup>29</sup>. During 1977, the extraction of NA by acidified alcohol, salt, acid and alkalis was suggested, while the alkaline extraction method at raised temperature was implemented as early as 1970 to achieve protein produce with lower NA levels<sup>97</sup>. Conversely, alkaline hydrolysis of NA at higher temperatures (specifically above 80°C) leads to the formation of potentially toxic compounds such as lysinoalanine (LAL)<sup>98</sup>. LAL is a non-protein amino acid produced by the nucleophilic addition of the lysine epsilon-amino group to the dehydroalanine residue<sup>99</sup>.

This uncommon amino acid is involved in the cross-linking of alkaline proteins, which has been revealed to decrease digestion and encourage renal changes, such as nephrocytomegaly in rats<sup>100</sup>. It has also been implicated in skin allergy in some persons consuming treated protein<sup>101</sup>. The chemical modification of yeast nucleoproteins using anhydrides has also been utilized to lessen the NA levels<sup>102</sup>. Furthermore, yeast contains substantial endogenous ribonuclease enzyme activity can hydrolyze RNA. However, the use of endogenous nucleases in experiments necessitates fine-tuning the kinetics; under ideal circumstances, when ribonuclease is at its peak activity, endogenous proteases are frequently co-stimulated, resulting in proteolytic breakdown and a notable decrease in total protein production<sup>18,29</sup>. To address these intrinsic constraints, exogenous nuclease enzymes have been used, including pancreatic ribonuclease (RNase A) and fungal ribonuclease from *Aspergillus candidus* strain M16<sup>25</sup>.

Bacterial or pancreatic nucleases have also been also studied for NA removal from the yeast cells<sup>20</sup>. The hydrolysis of NA has also been achieved by employing the immobilized enzymes<sup>103</sup>. Immobilized enzymes are increasingly being used for Na hydrolysis in recent downstream technique<sup>20,62</sup>. This technique increases credibility and economic sustainability by allowing for enzyme reuse and continual processing, ensuring that Na levels remain below the 2% threshold required for safe human consumption while maintaining protein integrity<sup>104</sup>.

### ECONOMIC CONSIDERATIONS AND MARKET TRENDS/ TECHNO-ECONOMIC ANALYSIS AND GLOBAL MARKET DYNAMICS

For SCP production large-scale fermenters are required to manage high biomass production, high oxygen transfer rates and high respiration rates. These factors increase metabolic

heat production, necessitating the implementation of an efficient colling system. In continuous operation for SCP production, the economics of this production must be strongly taken into account. The Economics factors that should be taken into account during this fermentation period are: Investment, Energy, Operating costs, Waste, Safety and the Global market<sup>7,105</sup>.

### Substrate costs

The substrate costs are the largest single operational expenditure factor. Simplifying the manufacture and purification of raw material can significantly reduce costs. The “waste-to-protein” paradigm, which uses agro-industrial leftovers to reduce raw material prices by 30–50% when compared to refined sugar sources, is the focus of current developments<sup>106</sup>. Factors involved in the raw materials costs include site location, production scale, process capacity of the plant and substrate yield<sup>72</sup>.

### Utilities

The energy for compressing air, cooling, sterilizing and drying forms the next most important cost factor. Sites with cheaply available thermal, electrical, fossil or process derived energy are to be preferred. Modern biorefineries increasingly incorporate renewable energy and heat recovery systems to alleviate the high energy needs of aeration and thermal processing<sup>107</sup>.

### Capital load

Capital expenditure (CAPEX) is determined by a variety of factors, including equipment pricing, plant size, and operational conditions. According to the concept of economies of scale, increasing plant size can lower the cost per unit of protein produced; but it also demands a relatively high initial investment, which could discourage smaller enterprises from entering the market. Smaller facilities can remain viable only if the production process is significantly simplified. Furthermore, even while enhanced fermentation productivity might boost production, it frequently comes with higher energy consumption, therefore it's important to find the ideal balance in operating circumstances.

### Product-specific variables

The absolute value of the product is governed by the amount of product relative to costs and the final quality. Because protein purity has a significant impact on the marketability of SCP, enhanced downstream processing (dsp) is necessary to improve the biomass for high-value human-grade applications, which commands a higher price over animal feed. The upgrading of the product may consist of purification and separation of the microbial biomass<sup>108,109</sup>.

## ROLE OF SCP IN GLOBAL PROTEIN SECURITY AND CIRCULAR BIOECONOMY

### Role in global protein security

The growing global population, urbanization, and changing dietary preferences have significantly increased demand for protein-rich foods and feeds<sup>110</sup>. Traditional protein sources like fishmeal and soybeans are becoming scarcer due to their significant demands for arable land, freshwater, and energy<sup>107</sup>. Microbial Single Cell Protein (SCP) is a sustainable alternative that generates high-quality biomass rich in vital amino acids, vitamins, and lipids in a short period of time. From a techno-economic standpoint, microbial protein synthesis offers unrivaled scalability; unlike traditional agriculture, it takes up to 99% less land and 90% less water, establishing Bacteria, yeast, fungi, and microalgae may grow quickly on inexpensive substrates, allowing for year-round production that is unaffected by climatic or seasonal changes<sup>94</sup>. It serves as a climate-resilient solution to the estimated 1,250 million-tonne protein deficiency by 2050<sup>56</sup>. As a result, SCP has the potential to minimize reliance on traditional agricultural and marine resources, thereby contributing significantly to global protein security over time.

### Contribution to the circular bioeconomy

The circular bioeconomy paradigm encourages resource efficiency, waste reduction, and biological recycling of materials. SCP production closely adheres to these principles by converting low-value byproducts into high-value protein-rich biomass<sup>107</sup>. Agro-industrial effluents, lignocellulosic wastes, and gaseous emissions are recycled as nutrient supplies in this “waste-to-worth” transformation, which replaces the linear “take-make-dispose” model with a closed-loop system of resource recovery. Microbial systems exhibit exceptional metabolic flexibility, using a variety of substrates from gaseous carbon sources like CO<sub>2</sub> and methane to food processing waste, molasses, whey, and glycerol<sup>111–113</sup>. SCP manufacturing effectively closes nutrient loops and increases overall resource efficiency by utilizing these resources as feedstock rather than waste<sup>114</sup>. Therefore, SCP acts as a crucial biotechnological link between systems for producing sustainable food and waste generation.

### Sustainable waste valorization

Industrial and agricultural wastes frequently generate major environmental issues, such as water contamination and greenhouse gas emissions. These wastes can be transformed into protein-rich biomass using optimal microbial fermentation, lowering environmental impact while increasing economic value. In addition to protein production, these microbial systems are effective bio-remediation tools, substantially reducing the chemical

oxygen demand (cod) and biological oxygen demand (bod) of industrial effluent while turning potential contaminants into nutritionally useful products<sup>114</sup>.

SCP production specifically contributes to:

**Organic waste reduction:** Mitigating the accumulation of hazardous organic matter<sup>114</sup>.

**Landfill minimization:** Reducing the volume of waste destined for traditional disposal sites<sup>107</sup>.

**Emission mitigation:** Lowering the greenhouse gas footprint of waste decomposition<sup>94</sup>.

**Nutritional conversion:** Transforming environmental pollutants into safe, high-quality protein<sup>112</sup>.

This dual benefit of environmental remediation and protein generation positions SCP as a core strategy in contemporary sustainable biotechnology.

## FUTURE PROSPECTUS AND CHALLENGES

The expanding global food crisis has expedited the transition toward microbial protein synthesis, prompting research into scalable Single Cell Protein (SCP) technologies to provide long-term nutritional security. Although microorganisms cultivated on renewable substrates—such as molasses, methanol, CO<sub>2</sub>, and agro-industrial residues—represent a flexible biological platform for animal feed and human food, their commercial viability is still largely constrained by cost effectiveness and the difficulty of competing with well-established commodities like fishmeal and soybean meal. The next frontier in SCP production is the integration of emerging genetic technologies, like as metabolic engineering and crispr-cas9, to create “designer strains” with reduced endogenous nucleic acid content and tailored amino acid profiles in order to overcome these economic barriers<sup>8</sup>. Furthermore, the use of artificial intelligence (AI) and machine learning (ML) for real-time fermentation monitoring will be critical for predictive process management, assuring consistent biomass quality and reducing costly batch failures. To increase economic viability, future biorefineries must implement multi-product co-production techniques in which SCP is produced in association with high-value secondary metabolites, antibiotics, biopolymers, or biofuels to minimize operating expenditures (OPEX). Finally, in order for SCP to be successfully adopted worldwide, it must have a balanced essential amino acid profile, maintain nucleic acid levels below the 2% threshold through optimized downstream processing, and have appropriate organoleptic qualities, such as color, flavor, texture, and aroma, while being completely free of allergens or deadly toxins. By integrating microbial physiology with the circular bioeconomy principles, SCP production can evolve from a supplementary technology to a cornerstone of the global sustainable food system.

## CONCLUSIONS

Microbial Single Cell Protein (SCP) production is a physiologically viable and industrially scalable solution for solving the global protein deficiency while also supporting the key principles of waste valorization and the circular bioeconomy. This technology uses non-pathogenic microorganisms such as bacteria, yeast, filamentous fungi, and microalgae to produce a high-quality biomass rich in vital amino acids, vitamins, and lipids that is resistant to climatic and seasonal changes. The bioconversion of low-value agro-industrial residues into refined protein products successfully closes nutrient loops, transforming potentially harmful contaminants into nutritionally useful resources. In conclusion, the simultaneous benefits of environmental remediation—achieved through considerable reductions in organic waste and emissions—and the provision of sustainable nutrition establish SCP as an integral component of contemporary biotechnology and long-term global food security.

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