MICROBIAL BIOTECHNOLOGY

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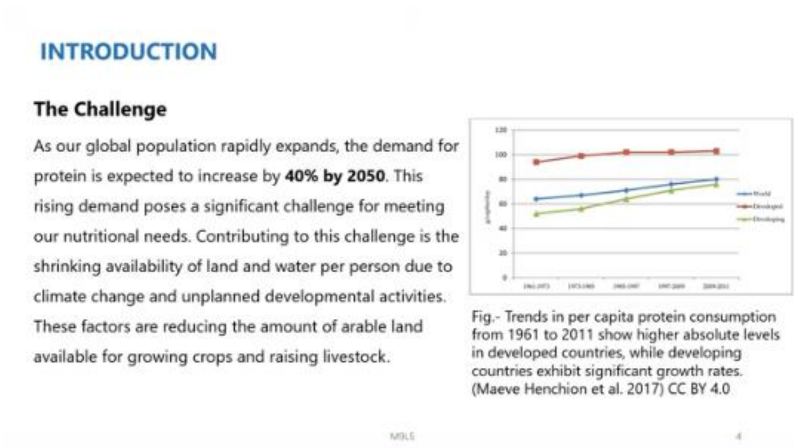
Lecture-33
Lec 33: Single Cell Protein production

Hello friends, welcome back to my course on microbial biotechnology. We are in module 9, discussing food production involving microorganisms and their products. So today, we are going to learn about single-cell protein production, which we shortly call SCP. This has broadly three sections. The first section deals with the introduction and overview of single-cell protein.

The second section deals with SCP production and the key factors. The third section deals with the applications, impact, and future of SCP. So in the first section, we are going to learn about the introduction, importance of SCP, historical development of SCP, sources of SCP, merits and demerits of single-cell protein, and SCP from different microorganisms. So let us learn about the challenge we are really trying to address in this particular lecture. We know that our global population is increasing rapidly, and this has resulted in an increased demand for protein.

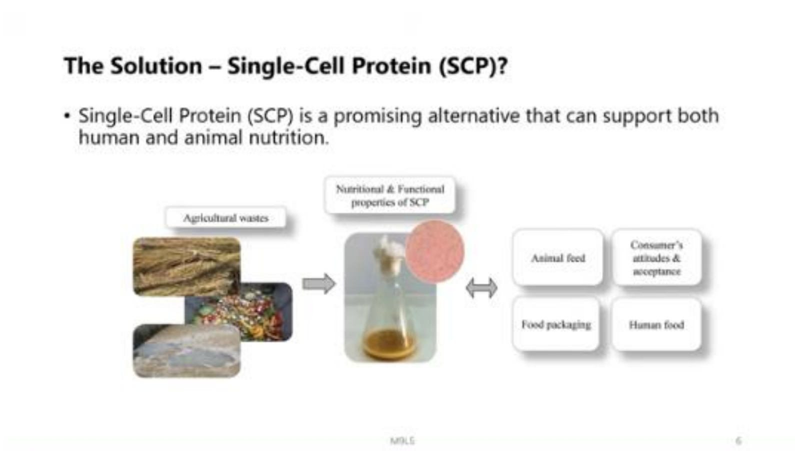
This is expected to rise by around 40% by the year 2050. This rising demand poses a significant challenge for meeting our nutritional needs. A 40% increase in protein means we need 40% additional resources to produce—maybe even more resources—in terms of land, water, and other inputs. Contributing to this challenge is the shrinking availability of land and water per person due to climate change and unplanned developmental activities, and we know that urbanization is also rapidly increasing, thereby reducing cultivable land. These factors are reducing the amount of arable land available for growing crops and raising livestock, which actually provides us protein.

Now, this is the trend in per capita protein consumption from 1961 to 2011. So here, we see the blue color represents the global average. And then you see the green one represents the consumption pattern in the developing world, and the red one is the consumption pattern in the developed world. In both of these different economies, consumption is actually increasing every year or every decade. And it is likely to grow and reach an additional 40% by 2050, as we have already discussed.



So, producing this has become a big challenge because our natural resources are depleting, and waste generation is increasing. Together, these issues complicate the process of producing enough protein to satisfy the growing demand. In this context, ensuring nutritional security becomes a pressing concern as we seek solutions to address these interconnected challenges effectively. One idea could be whether we can take this waste, which is currently a silage, and use it as a resource in our scheme of things for protein production. Here, you can see children playing in a garbage dump in Africa, representing the growing urban waste problem. Even in rural areas, waste is becoming very omnipresent, particularly non-degradable plastic waste floating around in the environment.

So, what is the solution to this protein demand? Increased protein demand against a backdrop of decreasing natural resources like land, water, and other inputs. Single-cell protein is a promising alternative that can support both human and animal nutrition in such a scenario. We can actually use agricultural waste to produce microorganisms, which can then generate single-cell proteins for animal feed and human food—subject to consumer attitudes, acceptance, packaging, and processing. So, we can view these disadvantages as advantages by meeting the nutritional demand for proteins through agricultural waste.



So, we have efficient microbial single cell producers and production systems which can utilize solid and liquid agricultural waste and convert them into resources. Let us have a overview of microbial process applications highlighting state-of-the-art methods and the role of genetic engineering in the current and future enhancements. So, here we have the traditional fermentation and then the basically state-of-the-art it has been used historically and for long and currently in processes like alcohol production, food preservation, taste and texture development. Then from the engineering and future developments perspective, active preservation of the traditional techniques, historical research into understanding early fermentation techniques in ancient cultures is very, very relevant. Now we have methods by which we have enhanced these traditional fermentation where we have gone for strain selection and then bred or engineered to improve the taste, texture and health benefits

and for these we use omics tools to select and breed optimal strains for processes. Engineered strains producing additional vitamins then proof us taste order in fermenting strains. Then in the case of animal feed, biomass are used in feed as nutritional supplement which is a primary food source or as nutraceutical and or therapeutic platform. Here, strange solutions were engineered to match needs of specific animals, agricultural methods and aims. Engineered into a therapeutic platform, biomass grows on waste substrate to optimize sustainability.

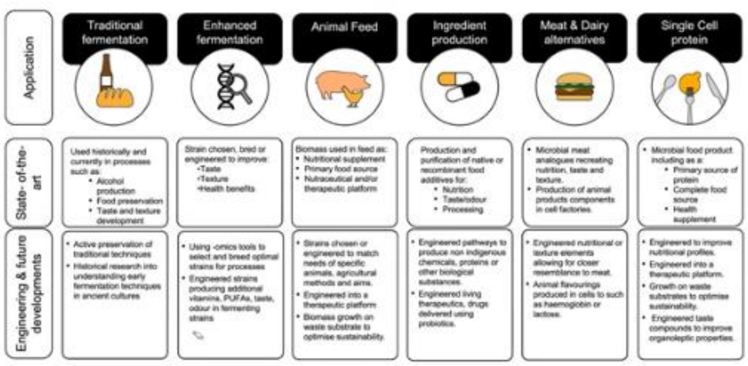


Fig - Overview of microbial process applications, highlighting state-of-the-art methods and the role of genetic engineering in current and future enhancements (Graham and Ledesma-Amaro 2023) CC BY 4.0

We also have a focus on ingredient production where production and purification of native or recombinant food additives for nutrition, test order and processing purposes. Here engineered pathways to produce non-indigenous chemicals, proteins or other biological substances are undertaken. Engineered living therapeutics drugs delivered using probiotics are also one of the focus. Then we have meat as well as the dairy alternatives. Microbial meat analogs recreating nutrition, taste and texture is one of the goals of these.

The production of animal products components in cell factories is another. Here we go for engineered nutritional or texture elements allowing for closer resemblance to meat and then animal flavorings produced in cells to such as hemoglobin or lactose. Then the current topic of our discussion single cell protein. Here microbial product is included as a primary source of protein and this is a complete food source and is used as a health supplement. Basically, we go for engineered approaches to improve the nutritional profiles, then engineered into a therapeutic platform, growth and waste substrates to optimize sustainability, and then engineered test compounds to improve organoleptic properties.

So, in this scenario, all of them are actually important. The traditional fermentation will continue, the enhanced fermentation as well as other approaches like the meat and dairy alternatives. But then single cell protein is also emerging as an important particle in mitigating the nutritional demands of protein. Now, what is this single cell proteins?

We can simply define it as something which refers to a biomass of dried cells. produced from microorganisms which may be algae, yeast, bacteria and fungi and these are non-toxic. SCP is also known as a bioprotein, microbial protein or biomass. SCP serves as a protein rich supplement or ingredient for both human and animal diets. It is a valuable alternative to traditional protein sources and apart from its use as human food, animal feed, it is finding wide applications as bioplastics and in enzyme production and wastewater treatment.

Let us look into the protein supply chain: traditional versus single protein production. So we divide these into different discussion points, like raw material and growth and cultivation, how we harvest and process, what the quality control and safety standards are in place, how you distribute and store, and regarding the aspects of retail and customers. So in the traditional protein production process, we go for livestock breeding or feed production, then we go for rearing, and then harvesting and processing is done by slaughtering and butchering. Then we go for quality, antibiotics, and hormones, which need to be monitored, and then we go for cold storage distribution. And retailing is done mostly through supermarkets, butcher shops, restaurants, etc.

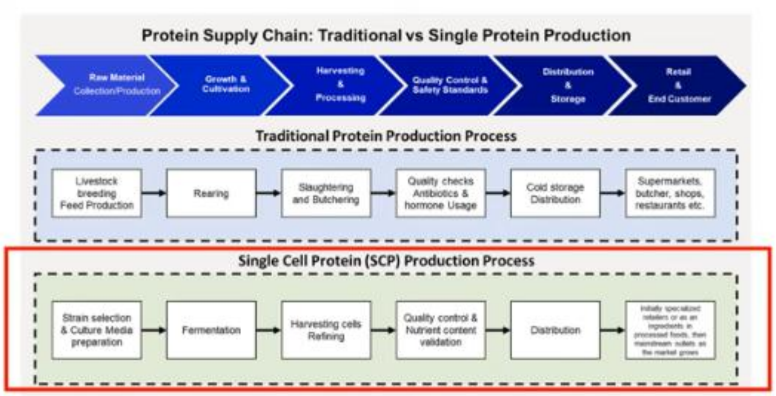


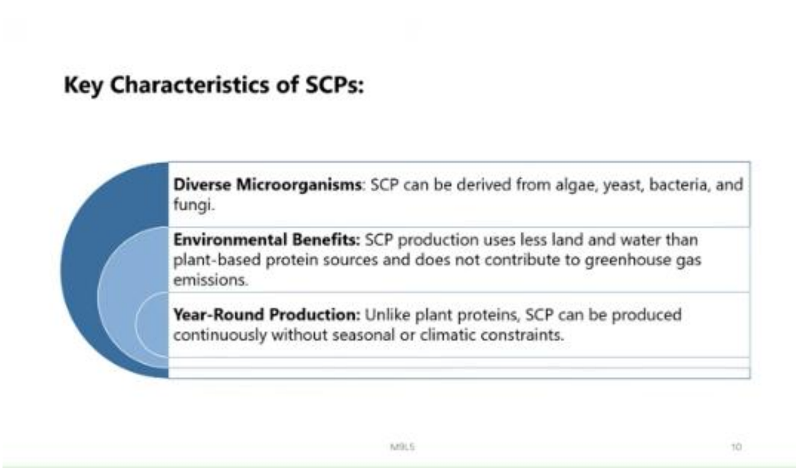
Fig - A simplified protein supply chain: traditional vs single cell protein (SCP) (Marttin Paulraj Gundupalli et al. 2024) CC BY 4.0

In the case of single-cell protein production, the process is entirely different. We do not have livestock here; we have microbes. So, you go for strain selection and culture media preparation instead of feed production. Then, instead of rearing, we go for fermentation, and there is no slaughtering or butchering here. We just go for harvesting cells and, of course, refining them.

Then, of course, quality control and nutrient content validation are important here. And then we may require cold-chain distribution. We may not require it in certain cases. We can simply go for distribution. Initially, these are specialized retailers or used as an ingredient in processed food.

Then mainstreaming outlets as the market would be growing. So, these are the two differences between the traditional versus single cell protein production. What are the key characteristics of single cell proteins? They are produced by diverse microorganisms like algae, yeast, bacteria and fungi. Single cell protein production comes with a baggage of rather advantage points of environmental benefits,

because it uses less land and water than plant-based protein sources and does not contribute to greenhouse gas emissions. Rather, some of the greenhouse gas emissions gases can actually be converted into proteins and that can be one of the steps in climate change mitigation. Then we have the advantage of year-round production in case of animals there may be challenges or in the case of crop based proteins it may not be year-round but since we are growing these under artificial conditions and control conditions we can go for year-round productions. So there is no any climatic constraints so and we get a continuous supply of single cell proteins. So, now, to some extent, you know what could be the importance of SCP.

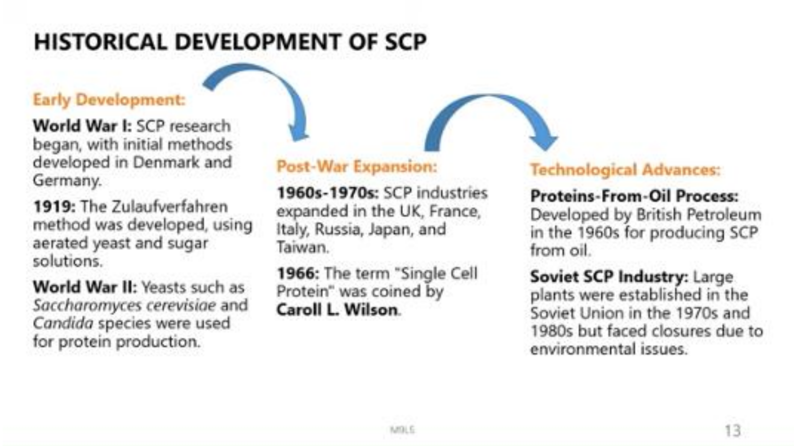


For example, it can be a tool for climate change mitigation. It can help us in converting agricultural waste into wealth. But let us try to learn them in a more structured way. Number one, it addresses the protein demand or the gap in that protein demand because we know that there is a rising consumption globally of protein and it is projected to increase from 40 kg per capita in 2013 to around 51.5 kg in 2050. And in this context, the CPU offers a cost-effective and nutritionally rich alternative to meet this demand.

From a nutritional point of view, SCP can have many advantages, but it varies depending on the microorganism and the substrates used, including amino acids, proteins, carbohydrates, and lipids. SCP provides essential macro- and micronutrients, including proteins, lipids, carbohydrates, vitamins, and minerals. From a production standpoint, considering efficiency and nutritional composition, SCP's protein content ranges from 30 to 70 percent. It can be enriched through genetic engineering to match the amino acid profile of traditional proteins like fish or soy protein. Additionally, it is environmentally sustainable.

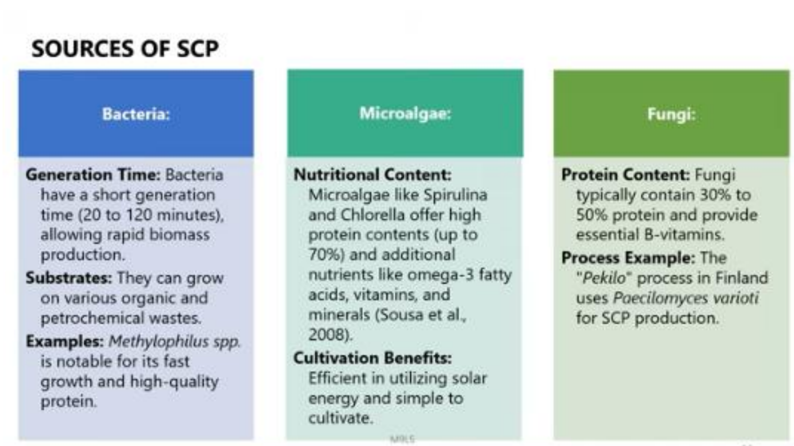
SCP can be produced from readily available and inexpensive raw materials, such as carbon sources (e.g., methanol, ethanol), renewable sources (e.g., molasses), waste substances (agricultural and industrial), and even greenhouse gases (e.g., methane). Let us briefly discuss the history of SCP development. SCP was known as early as World War I, when research began with initial methods developed in Denmark and Germany. In 1919, the Julep Warfarin method was developed using aerated yeast and sugar solutions. During World War II, yeasts such as Saccharomyces cerevisiae and Candida species were used for protein production.

After World War II, from the 1960s to the 1970s, SCP industries expanded in the UK, France, Italy, Russia, Japan, and Taiwan. In 1966, the term 'single-cell protein' was coined by Carol L. Winson. Various technological advances have taken place over time. For example, British Petroleum developed proteins from oil in the 1960s for SCP production. The Soviet Union established large plants in the 1970s and 1980s, but they faced closure due to environmental issues.



What are the sources of single-cell proteins? We can obtain it from different microbes like bacteria, microalgae, as well as fungi. The generation time in bacteria is very short, 20 to 120 minutes, allowing rapid biomass production. The substrates the bacteria use are various organic and petrochemical wastes, and some examples are Methylophilus species, which are notable for their fast growth and high-quality protein. Microalgae like Spirulina and Chlorella offer high protein content, up to 70%, and are very commercially successful.

And additional nutrients like omega-3 fatty acids, vitamins, and minerals. Then, from the cultivation point of view, they are efficient in utilizing solar energy and are simple to cultivate. In the case of fungi, they have 30 to 50% protein and provide essential B vitamins. And the Pekilo process in Finland uses Bacillomyces variotae for SCP production. So, microalgae we can see here around 60 to 70% protein, and these are mostly phototrophic producers of omega-3 fatty acids, and they have certain disadvantages, of course, like economic limitations of scale-up, digestibility, and a need for cell wall disruption to release the nutrients.



Some of the examples are Tetraselmis suecica, then Isochrysis galbana, etc. In the case of yeast and fungi, we have roughly around: yeast has 38 to 52% protein, and fungi have 0.23 to 15% protein. And they use different substrates and can produce vitamins and micronutrients. Some of the potential disadvantages are the improvement of protein content and essential amino acid profile, as well as the possible presence of toxins. Saccharomyces cerevisiae and Kluyveromyces marxianus are some of the yeasts used for SCP production.

And then in the case of fungi, we have Aspergillus oryzae and Yarrowia lipolytica, which produce SCP. Bacteria have around 50 to 80% protein content. It has a high protein content, and growth on C1 substrate is one of its characteristics. However, they have certain disadvantages like palatability issues, high nucleic acid content, and toxin production. Some examples are Methylobacterium extorquens, Methylococcus capsulatus, etc.

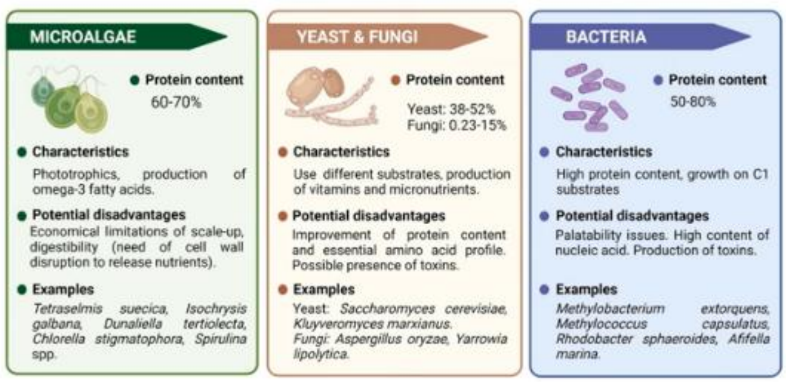
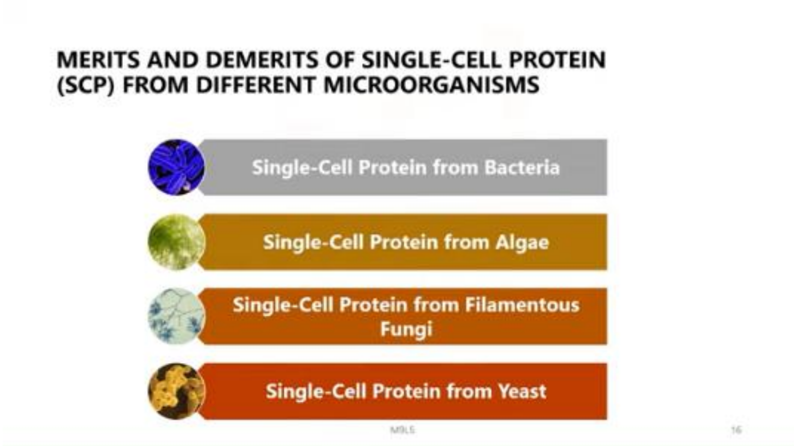


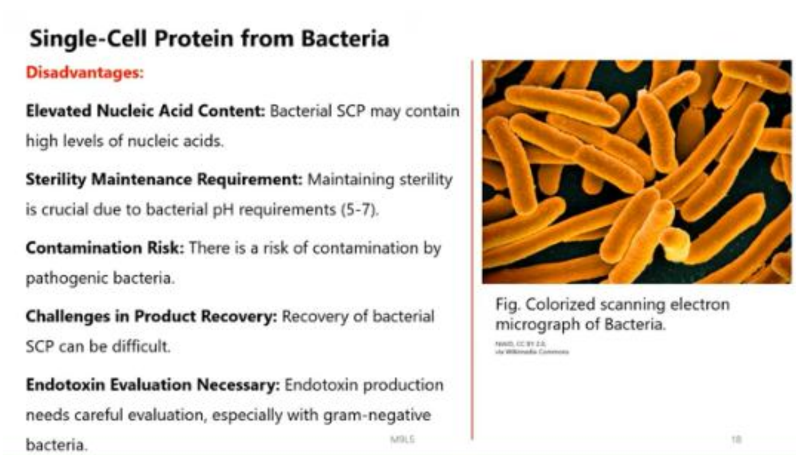
Figure- Comparison of SCP characteristics produced by various microbes (Pereira et al., 2022) CC BY 4.0

What are the merits and demerits of single-cell protein from the different microorganisms we just discussed? So we'll discuss these disadvantages and advantages with respect to each of these organisms one by one. In this picture, you can see a colorized scanning electron micrograph of bacteria, and some advantages of SCP from bacteria are the diverse bacterial species available for SCP production. These diverse species utilize diverse substrates, giving us many options by combining these bacterial species with available substrates. Certain bacteria like Methylophilus and Methylotrophus are used on a commercial scale.



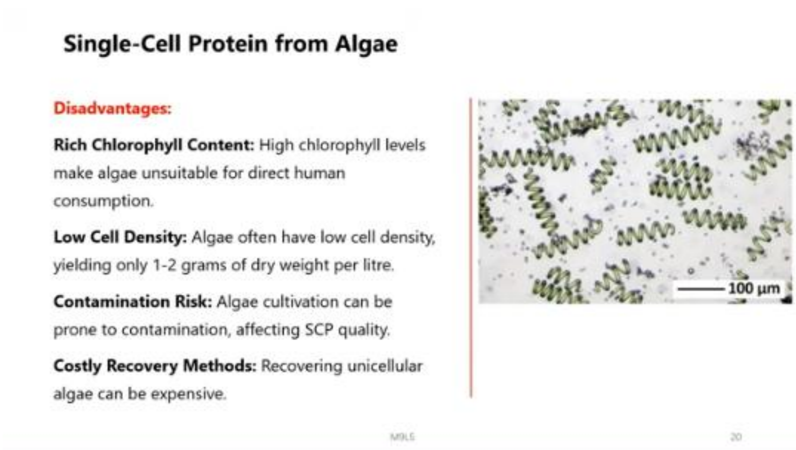
There is no problem with scaling up, and we know bacteria have very high SCP content, up to 80% or more crude protein, with a good amino acid profile, though they may be low in sulfur-containing amino acids. However, they have certain disadvantages, like elevated nucleic acid content. Bacterial SCP may contain high nucleic acid levels, and maintaining sterility is crucial due to bacterial contamination risks. There is also a risk of contamination by pathogenic bacteria. Additionally, recovering bacterial SCP can be challenging.

Then, there is always the risk of endotoxin production. So, it needs careful evaluation, especially with gram-negative bacteria. Let us now look into the advantages of having single-cell proteins from algae. It has a very high content—around 60% crude protein. It is suitable for animal feed, and algae grow using carbon dioxide and sunlight, which are low-cost resources.



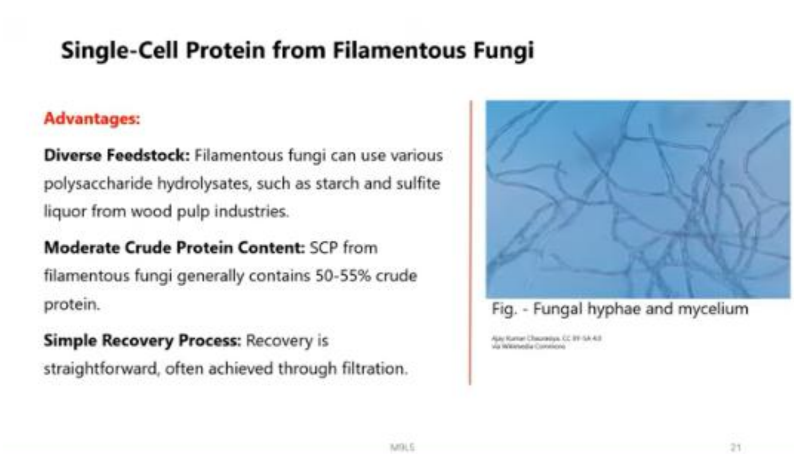
So, it basically uses cost-effective substrates. However, there are certain disadvantages. For example, it has rich chlorophyll, which is unsuitable for direct human consumption. Then, there is low cell density.

Algae often have low cell density, yielding only one to two grams of dry weight per liter. And there is a contamination risk. Algae cultivation can be prone to contamination, affecting the SCP quality. And the recovery methods for getting unicellular algae can be very expensive. So here, you can see a right-handed spirulina.



Then, what are the advantages of using filamentous fungi for producing single-cell proteins? So, it can be raised on diverse feedstock. Filamentous fungi can use various polysaccharide hydrolysates, such as starch and sulfite liquor from wood pulp industries. And it has moderate crude protein content. SCP from filamentous fungi generally contains 50 to 55% crude protein.

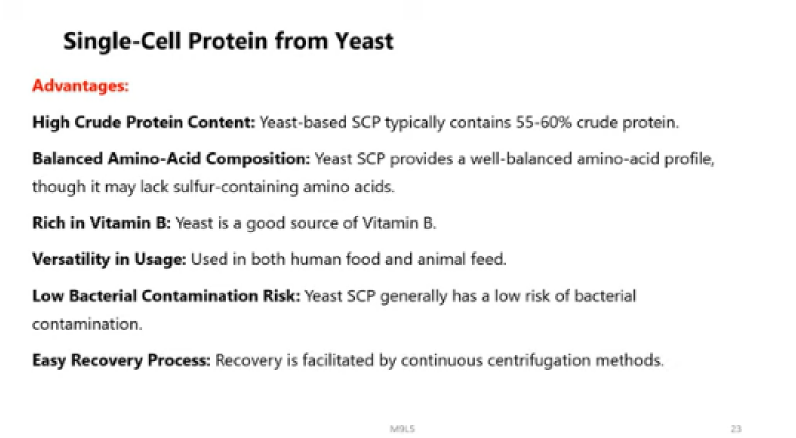
Recovery is straightforward, often achieved through filtration. And here, you can see a fungal hypha and mycelium. However, there are many disadvantages of obtaining single-cell proteins from filamentous fungi. For example, they grow very slowly compared to bacteria and yeast. And there is a higher risk of contamination by yeasts during production.



And it has elevated nucleic acid content, which can impact SCP purity. And potential mycotoxin production requires evaluation for product safety. Let us look into the single-cell protein production from yeast. It has advantages, like its high crude protein content, typically around 50 to 60% crude protein. Then, it has a balanced amino acid composition.

Yeast SCP provides a well-balanced amino acid profile, though it may lack sulfur-containing amino acids. Yeast SCP is rich in vitamin B, and it is used in both human food and animal feed. There is a low bacterial contamination risk. Yeast SCP generally has a low risk of bacterial contamination. It's an easy recovery process.

Recovery is facilitated by continuous centrifugation methods. However, it has many disadvantages, such as a slower growth rate and elevated nucleic acid content. Due to a deficiency in sulfur-containing amino acids, SCP may lack these essential amino acids in many instances. These are yeast cells in Gram staining of Candida growth on SDA. Now, let us see this figure, which shows in A the components of microorganisms beneficial for nutrition, including proteins, fiber, and bioactive compounds.



So, we have high protein content in most microorganisms. They grow on waste substances and produce vitamins in many microorganisms. Nutritional chemicals, such as phytochemicals and antioxidants, are also produced, and there is high fiber in mycelium and yeast strains, which include fiber such as beta-glucan. So now, if you compare the levels of fiber, protein, and micronutrients in the recipe of various microorganisms used in food applications, you can see in figure B with these spots, which are proportional. So, in bacteria, the fiber is very low. Protein is quite good. Micronutrients are also satisfactory.

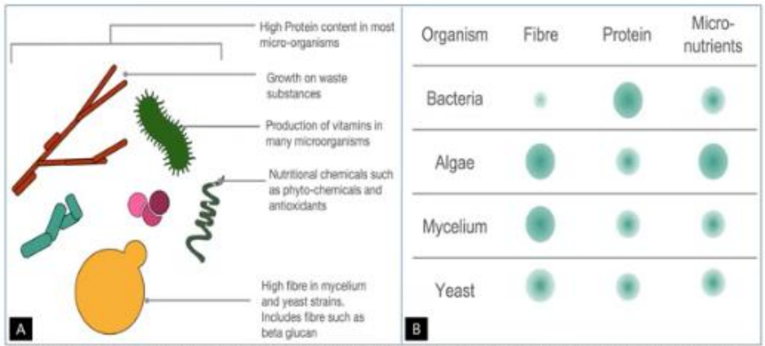
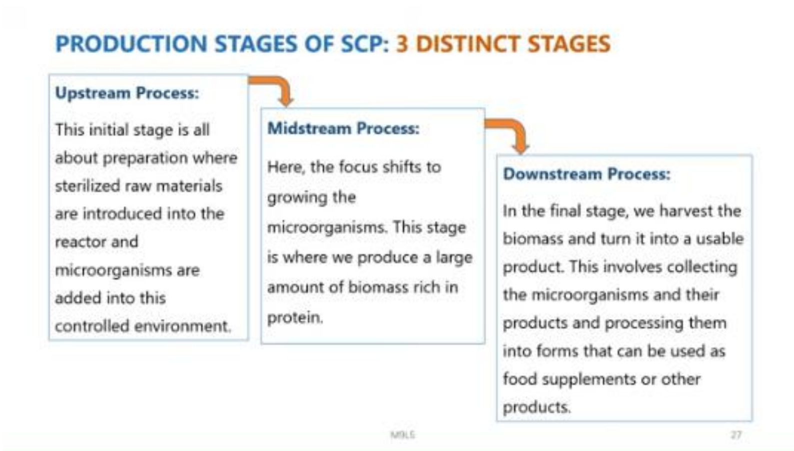


Fig. **A:** Components of microorganisms beneficial for nutrition, including proteins, fiber, and bioactive compounds.
B: Comparative levels of fiber, protein, and micronutrients in SCP of various microorganisms used in food applications.
(Graham and Ledesma-Amaro 2023) CC BY 4.0

But algae fiber is very high, protein is comparatively lesser, so in the case of mycelium and yeast when compared to bacteria. But algae provides a lot of micronutrients amongst all these various microorganisms, and so algae, mycelium, and yeast actually provide a lot of fiber. So, this is a pictorial representation of the different components produced by the different microorganisms. Let us now move to section 2, where we will discuss the key factors and production process of single-cell proteins. So, here we will go through the production stages, optimization of SCP production, and key considerations for selecting microorganisms for SCP production.

The critical properties of SCP, then the production process, factors affecting SCP production, substrate requirements for SCP production, and SCP production using agricultural waste and by-product streams. So, SCP production can be divided into three distinct categories. Like the upstream process, which is the initial stage and a preparative stage where sterilized raw materials are introduced into the reactor, and microorganisms are added to this sterilized raw material kept in a controlled environment. In the midstream process, the focus shifts to growing these microorganisms on this substrate. This stage is where we produce a large amount of biomass rich in protein.

And in the downstream process, which is the final stage, we harvest the biomass and turn it into a usable product. This involves collecting the microorganisms and their products and processing them into forms that can be used as food supplements or other products. So, how do you optimize this entire SCP production? It begins with the selection of the microorganism which we need to choose for optimal growth.



Productivity and substrate utilization. Then, we optimize the culture conditions, such as temperature, pH, and nutrient levels. Before introducing the substrate, there is a pretreatment step to enhance nutrient availability. Next, we proceed with nutrient supplementation to ensure a balanced nutrient supply. The selection of the fermentation process is crucial and must be based on the microorganism and substrate characteristics. Strain improvement is essential, utilizing techniques like mutation breeding and genetic engineering to enhance traits.

Here, we can observe the optimal single-cell protein production process. This integrates circular economy approaches. We have two types of waste that can serve as substrates: agricultural and food waste, and urban waste. These require sterilization, and we cultivate various organisms at a laboratory scale, such as microalgae, yeast, fungi, and bacteria. The producers of SCP are acquired from collections or isolated from natural habitats.

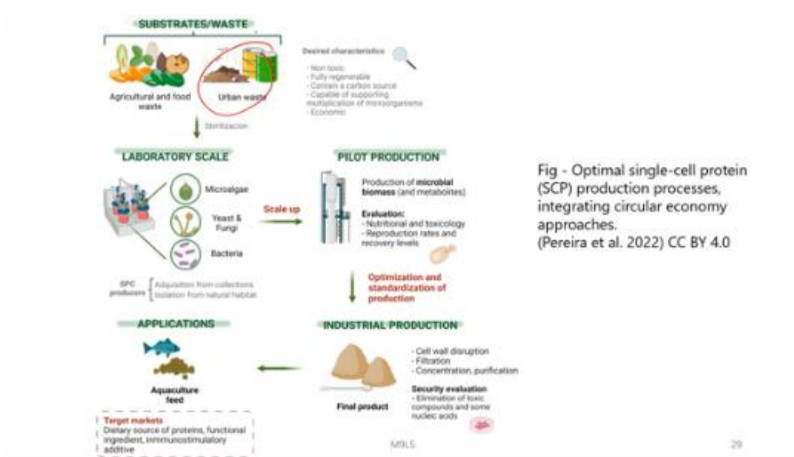


Fig - Optimal single-cell protein (SCP) production processes, integrating circular economy approaches.
(Pereira et al. 2022) CC BY 4.0

Acquisition from collections may include various government or private repositories. For substrate selection, we must ensure the substrate is non-toxic. It should be fully renewable, contain a carbon source, support microbial multiplication, and be economically viable. After optimizing SCP production at the laboratory scale, we scale up to pilot production, generating microbial biomass and metabolites while evaluating nutritional, toxicological, reproductive, and recovery aspects. Following optimization and standardization, we proceed to industrial-scale production.

So, here we go for cell wall destruction, filtration, concentration, purification, and then, of course, elimination of toxic compounds and some nucleic acids to enhance the protein percentage. And we get the final product. And this can be used as animal feed or as human food. So, this is the optimal single-cell protein production process, starting from the laboratory scale to the pilot scale to the industrial or commercial scale. So, one of the important things in SCP production is the selection of microorganisms.

So, when selecting microorganisms for single-cell protein production from methane, for example, several key priorities are critical to ensure that the final product is safe, nutritious, and commercially viable. Microorganisms used in SCP production from methane should be safe, produce high-quality and digestible protein, grow rapidly, adapt to various environmental conditions, and efficiently utilize methane and nitrogen sources. These properties ensure that the SCP is not only effective as a protein source but also economically viable and safe for its intended applications. What are the advantages and disadvantages of single-cell protein production?

Advantages and Disadvantages of Single-Cell Protein (SCP) production.

Advantages
Reduced Land and Resource Use
Lower Water Usage
Lower Greenhouse Gas Emissions
Reduced Pollution
Year-Round and Efficient Production
Reduced Overfishing
Nutrient Recycling
Less Land Degradation
Resource Efficiency
Reduced Biodiversity Loss
Food Security

Disadvantages
High Energy Consumption
Resource Competition
Biomass Separation
Dependency on Raw Materials
Potential for Limited Amino Acid Profile
Scale-Up Challenges
Waste Generation
External Cooling System required
Capital Intensive

(Marttin Paulraj Gundupalli et al. 2024)

So, we already know that SCP is actually a solution to the challenges of scarcity of land, water resources, and other resources. So, the advantage is that it requires very little land and fewer resources. And then low water usage, lower greenhouse gas emissions, and less pollution. Year-round and efficient production, reduced overfishing, and nutritional cycling. Because we are producing the protein in artificial conditions and we are not harvesting fish either in rivers, wetlands, or oceans.

And because we are using agricultural waste or some urban waste, we are recycling the nutrients. And because we are using less land, there is less land degradation, and this results in resource efficiency, reduced biodiversity loss, and ensures food security. However, there are certain disadvantages because, although we may not be using the land and other resources, we require a lot of energy. So, there is high energy consumption, and sometimes there is resource competition. Biomass separation is a challenge—separating the protein content from the total cell mass.

Then there is a dependency on raw materials at all levels. Even the waste can be a limiting factor sometimes, as it is used as a substrate. Potential limited amino acid profiles and scale-up challenges exist. There are also challenges of new types of waste generation, and external cooling systems are required, which is very capital-intensive. So, what are the critical properties of SCP?

Number one is the absence of pathogenicity and toxicity. Microorganisms for SCP production must be non-pathogenic, ensuring they don't cause disease or health issues, especially if used as animal feed or human food. The microorganisms must not produce harmful metabolites or toxins. So, the absence of toxicity is very important. And the substrates should also not be toxic in large amounts or accumulate in the final product.

The protein content and quality. The microorganism should produce a high protein content relative to its biomass. Measured as a percentage of its dry weight to ensure SCP is a valuable nutritional source. The quality of the protein is also important and is determined by the amino acid profile. The SCP should offer a balanced profile of essential amino acids, ideally matching or surpassing traditional protein sources like soy or meat to meet human and animal nutritional needs.

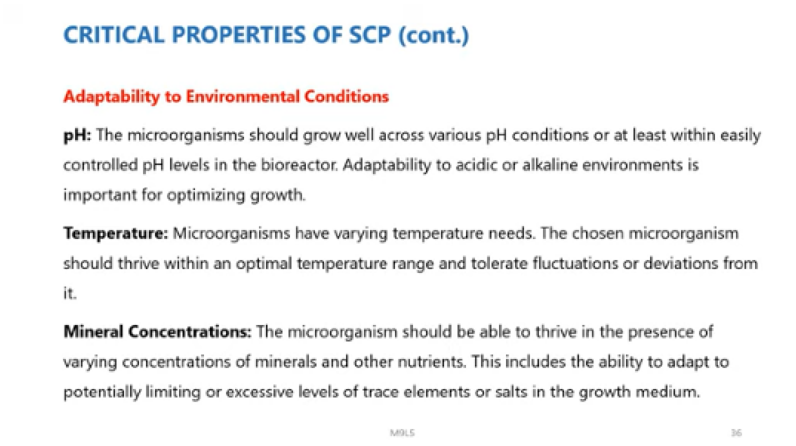
Digestibility and organoleptic qualities of the SCP are very important. It should be easily digestible by the target organisms, whether animals or humans. This involves not only the protein content but also how well it can be broken down and utilized by the digestive system of these organisms. Organoleptic qualities for SCP used in food applications. Organoleptic qualities such as taste, odor, and texture are important.

The SCP should be palatable and not impart any off-flavors or undesirable textures that might affect its acceptability. The next important property is the growth rate. The microorganisms should have a high growth rate, which translates to efficient production of SCP. Fast-growing microorganisms can reduce production time and costs, making the process more economically viable.

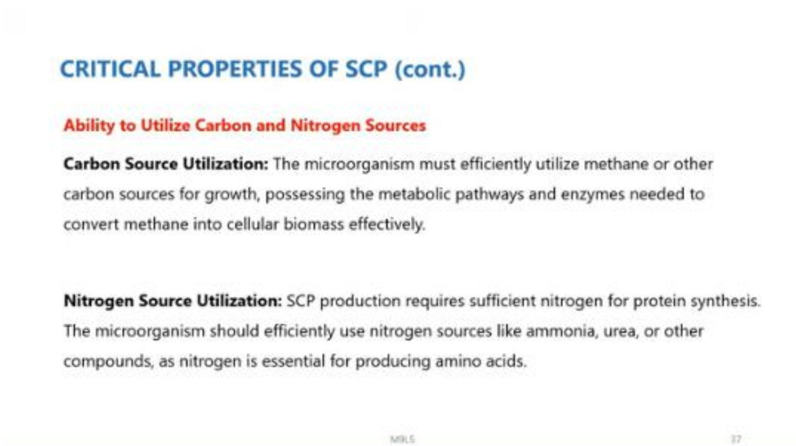
In addition to the growth rate, the productivity of SCP production is a crucial factor. High productivity ensures that the bioprocess is cost-effective and efficient. Another important property is adaptability to environmental conditions. For example, pH. The microorganisms should grow well across various pH conditions or at least within easily controlled pH levels in the bioreactor.

Adaptability to acidic or alkaline environments is important for optimizing growth. The next important parameter is temperature. Microorganisms have varying temperature needs. The selected microorganisms should thrive within an optimal temperature range and tolerate fluctuations or deviations from it. Another important factor is the concentration of minerals.

The microorganisms should be able to thrive in the presence of varying concentrations of minerals and other nutrients. This includes the ability to adapt to potentially limiting or excessive levels of trace elements or salts in the growth medium. Another important property is the ability to utilize carbon and nitrogen sources. For example, the microorganism must efficiently utilize methane or other carbon sources for growth, possessing the metabolic pathways and enzymes needed to convert methane into cellular biomass effectively. SCP production requires sufficient nitrogen for protein production or synthesis.



The microorganisms should efficiently use nitrogen sources like ammonia, urea, or other compounds, as nitrogen is essential for producing amino acids. Let us now discuss the general production process, some of which are already known to you from earlier discussions. Substrate and strain selection is very important, as we already know, but the criteria include selecting fast-growing microorganisms that yield high protein content and have suitable growth characteristics. Substrate preparation includes pre-treatment methods like grinding, sterilization, and hydrolysis. Fermentation technology is also very important.



We may opt for submerged fermentation, which utilizes a liquid growth medium, often continuous, with biomass requiring filtration or centrifugation, or we may choose semi-solid fermentation. This involves solid or semi-solid substrates with lower capital and operational costs, or we can use solid-state fermentation, which employs solid substrates incubated to produce SCP. Downstream processing includes harvesting techniques such as centrifugation, filtration, or sedimentation, and cell disruption methods like homogenization or chemical lysis to release the single-cell protein, followed by purification to remove impurities using filtration, chromatography, or precipitation, and finally drying methods like freeze-drying or spray-drying. This is the schematic for the commercial single-cell protein production process. We begin with strain selection and strain development.

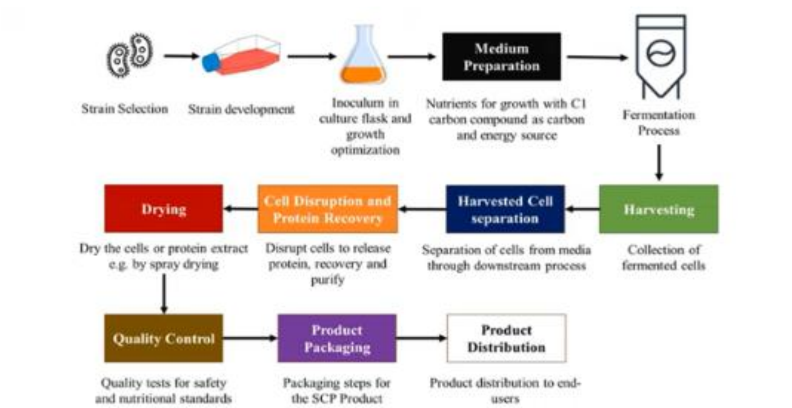


Fig – Schematic for commercial single cell protein (SCP) production process.
(Marttin Paulraj Gundupalli al. 2024) CC BY 4.0

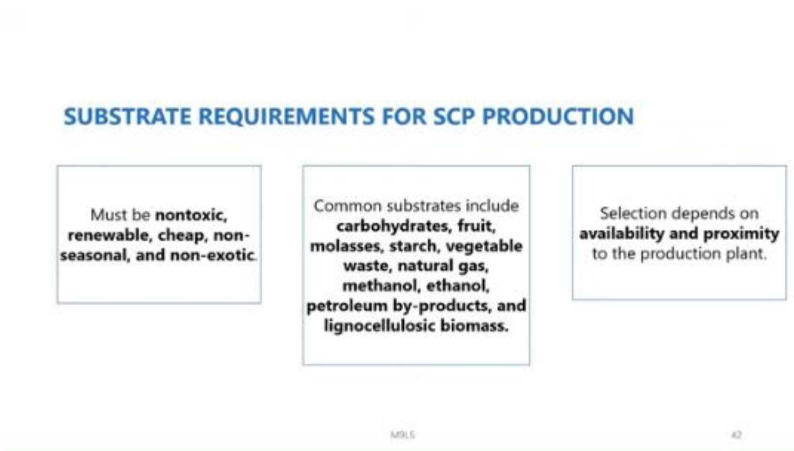
Then we proceed to inoculum in a class culture flask and growth optimization. Next, medium preparation involves nutrients for growth with C1 carbon as the carbon and energy source, and we proceed to fermentation. After fermentation is complete, we harvest the cells produced by this process. Then we separate the cells from the media through downstream processing, which includes cell disruption and protein recovery, followed by drying the cells or protein extract via spray-drying. Finally, we assess various quality parameters in the quality control step.

And then finally, once the quality is okay, we proceed with packaging. And then finally, the product distribution. What are the factors that affect HCP production? Microorganism selection is the first important thing, and the growth rate, tolerance, and substrate utilization are very important. Growth rate is essential for efficient production.

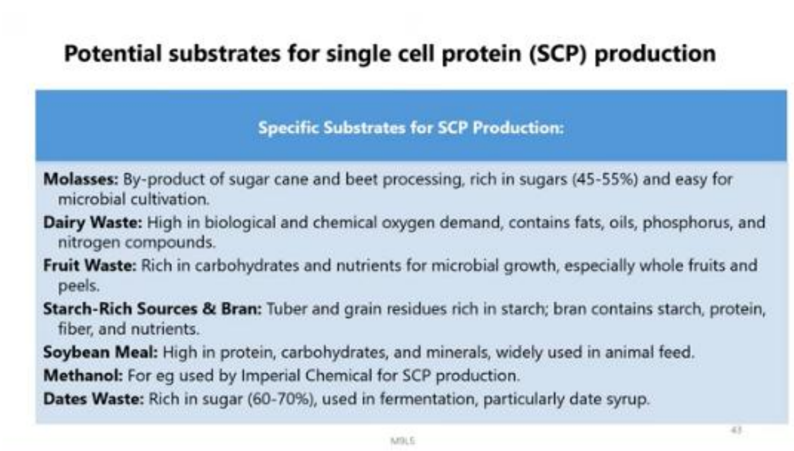
Tolerance includes resistance to temperature fluctuations, low or high pH, and other stress factors. The efficiency of low-cost substrates is very crucial. Safety and acceptability are very important. Health risks include potential allergic reactions and nucleic acid content in the SCP final product. Then, regulatory compliance is necessary for safe human or animal consumption.

Let us now look into the substrate requirements for SCP production. The substrate must be non-toxic, renewable, cheap, non-seasonal, and non-exotic. We try to utilize local waste resources. Common substrates include carbohydrates, fruit molasses, starch, vegetable waste, natural gas, methanol, ethanol, petroleum byproducts, and lignocellulosic biomass. Selection depends on availability and proximity to the production plant.

If they are very far away, the transportation cost will build up into the cost of the production plant. Let us now look into the potential substrates used for single-cell protein production, which may include molasses, which are the byproducts of sugarcane and beet processing. These are rich in sugar, 40% to 55%, and easy for microbial cultivation. Or we may go for dairy wastes, which are high in biological and chemical oxygen demand, contain fats, oils, phosphorus, and nitrogen compounds. Or we may go for using fruit waste, which is rich in carbohydrates and nutrients for microbial growth, especially whole fruits and peels.



Then we have starch-rich sources and bran, tuber and grain residues rich in starch. Then bran contains starch, protein, fiber, and nutrients. Then we may have soybean meal, which is high in protein, carbohydrates, and minerals, and these are widely used in animal feed. And we may use methanol, for example, which is used by Imperial Chemical for acid production. Then we may have waste from dates or plantation, palm plantation, which is rich in sugar, 60 to 70 percent, used in fermentation, particularly the date syrup.

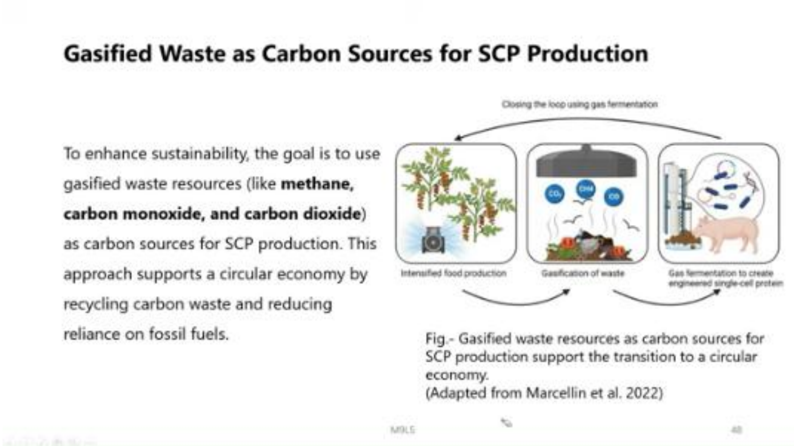


And we may use agricultural wastes for SCP production. This includes rice bran, pineapple peels, brewer's waste, and sodas. Agricultural waste is cheap, easily obtainable, and supports microbial growth. So, let us look into the SCP production using agricultural waste and byproduct streams. Agricultural and industrial waste are increasingly used for SCP production, ethanol, and biogas, preventing environmental harm and adding economic value.

Though costly initially, this approach is gaining importance due to future food supply concerns. Then we have industrial waste, which includes paper and pulp effluents, methanol, oil, latex waste, crude glycerol, waste cooking oil, fruit waste, leaf juice, and poultry and slaughterhouse waste. Here, we can use different microbes like Cloveromyces, Candida, Saccharomyces, Pichia, and so on. These are some of the rotten fruit and vegetable wastes that we can use for SCP production. This figure shows that renewable resources for SCV production include crop waste, straws, hulls, husks, and bran, as well as combined agricultural waste, glycerol (a byproduct of many industrial processes), fruit waste (peels, pulp, whole fruit), molasses, and dairy waste, which we have already discussed in the past few slides.

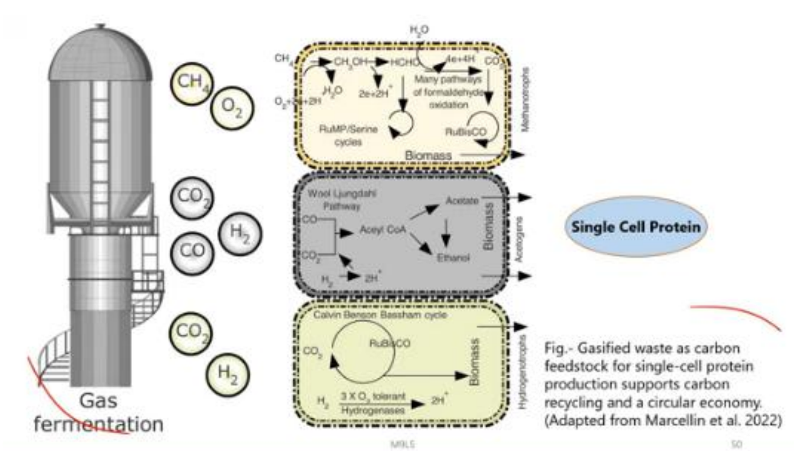
We can also use gasified waste as a carbon source for SCP production to enhance sustainability. The goal is to use gasified waste resources like methane, carbon monoxide, and carbon dioxide as carbon sources for SCP production. This approach supports a circular economy by recycling carbon waste and reducing reliance on fossil fuels. In this picture, we have gasified waste resources as carbon sources for SCV production, supporting a transition to a circular economy. Here, we go for the gasification of the waste.

Then we have intensified food production, which may produce many of these wastes due to food processing. These are all gasified. These are then fermented to create engineered single-cell proteins. We obtain single-cell proteins from the fermentation of these gasified wastes. These can be used as animal feed, resulting in enhanced animal growth, which can be a source of protein. Additionally, the waste from these animals can be reused as fertilizer in the plant system.



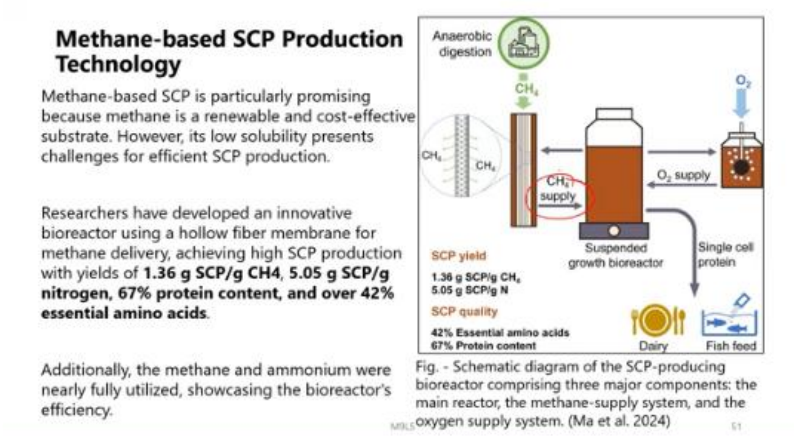
So, thereby this closes the loop. So, basically using agricultural waste products, gasifying them and using that gas for creating single cell protein by fermentation and using that as a food source for the animal. And the animal meat is used as the protein source, waste as an input back into the plant production system. So, now synthetic biology is advancing functional food production through precision fermentation with tools to engineer acetogens, methanotrophs and hydrogenotrophs. Many companies are leveraging these innovations to develop sustainable foods.

So, here in this figure, we have this gas fermentation with different gaseous products like methane, carbon dioxide, carbon monoxide, hydrogen and oxygen. And then this gaseous waste is used as carbon feedstock for single cell protein production, which supports the carbon recycling in a circular economy. And you can see the movement of these carbon to various metabolic pathways in various organisms, which may be methanotropes or which may be acetogens or which may be hydrogenotropes. So let's go for a little bit of discussion on the methane-based SCP production technology. This is a promising technology because methane is a renewable and cost-effective substrate.



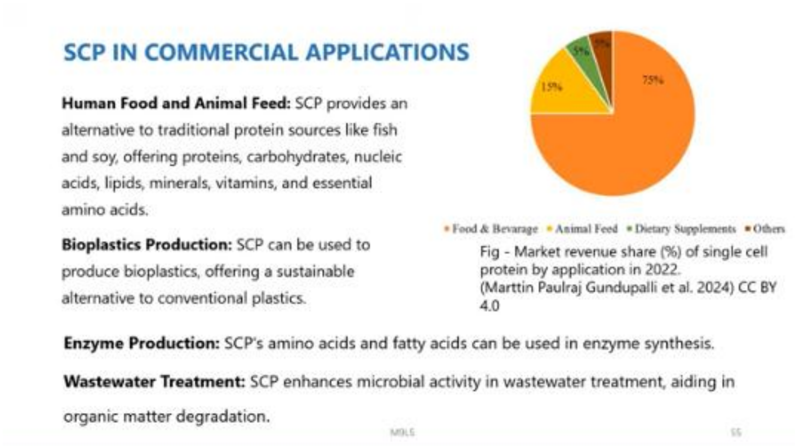
However, its low solubility presents challenges for efficient SCP production. Researchers have developed an innovative bioreactor using a hollow fiber membrane for methane delivery, achieving high SCP production with yields of 1.36 g SCP per g of methane or 5.5 g of SCP per g of nitrogen, 67% protein content and over 42% essential proteins. Additionally, the methane and ammonium were nearly fully utilized, showcasing the bioreactor's efficiency. So here, this is a schematic diagram of the SCB producing bioreactor that comprises three major components. The main reactor, the methane supply,

System and the oxygen supply system. So here is the suspended growth bioreactor where single-cell proteins are produced that can be used as animal feed or fish feed. So, here you can see some anaerobic digestion taking place, which produces methane, and this methane is used in this bioreactor. Let us now go to section three, where we'll discuss the applications, impact, and future of single-cell protein production. So, we'll discuss the commercial applications, functional properties, nutritional profile and safety, sensorial aspects and consumer perception, economic and environmental considerations, emerging technologies for SCP production, and future prospects.



So, let's begin with SCP in commercial applications. SCP provides an alternative to traditional protein sources like fish and soy, offering proteins, carbohydrates, nucleic acids, lipids, minerals, vitamins, and essential amino acids. It can also be used to produce bioplastics, offering a sustainable alternative to conventional plastics. Additionally, SCP's amino acids and fatty acids can be used in enzyme synthesis. SCP can enhance microbial activity in wastewater treatment, aiding in organic matter degradation.

So, this is the market revenue share of single-cell protein by application in 2022. Food and beverage is the major revenue generator, where 75% of the revenue comes from SCP utilization in the food and beverage sector. Followed by its use as animal feed (15%), dietary supplements (5%), and miscellaneous others (5%). So, these are some of the companies and their products produced using various organisms. You can see the country of origin or production, the protein content of the product, and the product name.



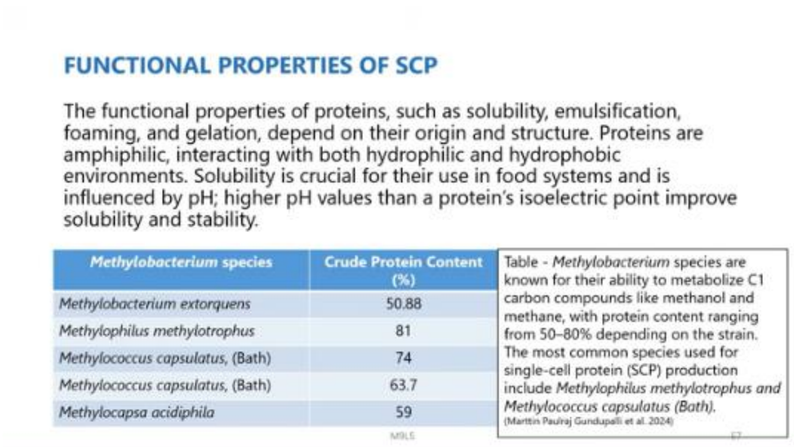
Here, for example, you have Prototitan, which is a trade name. It is produced using Clostridium-3 species, the company called biotech in USA and it has around 85% content, protein content and it is used, produced in a plant and it is used in salmon and shrimp feed. Then you have these cyanotic spirulina that is produced by using Arthospira platensis by the Cyanotec Corporation in USA has around 60% protein content and it is produced in deep ocean water and this is one of the most commercialized SCP products. Then we have linseed twig tree, produced using Saccharomyces cerevisiae by the company Lacephrae in USA, having a content of 55.7% protein.

Trade Name	Organism	Company	Country	Protein Content	Production	Product
			Microalgae			
ProTyton	*Clostridium spp.*	Biotech	USA	85%	Ethanol plant	salmon, shrimp feed
Cyanotech's spirulina	*Arthrospira platensis*	Cyanotech Corporation	USA	60%	Deep ocean water	One of the most commercialized products
			Yeast and Fungi			
Lynside® Nutri	*Saccharomyces cerevisiae*	LeSaffre	USA	55.7%	Extrusion	Dried inactive yeast
SylPro	*Candida utilis*	Arbiom	USA	>60%	Forestry by-products	Comparable to soy
			Bacteria			
UniProtein®	*Methylococcus capsulatus*	UniBio A/S	Denmark	70%	Natural gas	Particle size of 150–200 μm
ProFloc™	*Bacteria*	Nutrinsic	USA	60%	Wastewater from a local brewery	Fish meal in feeds

Table - Commercially available single-cell protein products. (Pereira et al. 2022)

It is produced by extrusion and the product is available as dried inactive yeast. Then we have the SealPro, produced with the help of Candida Utilis by the company R-Biome in USA, has a content of 60% protein. And it's basically a forestry byproduct, and the protein is comparable to soybean. Then we have Uniprotein, produced with the help of methylococcus capsulatus by Unibio AS in Denmark, and has 70% protein content,

content produced using natural gas and it has a particle size of around 150 to 200 micrometer and there is profloc produced using bacteria by a company called Nutrinsic in USA which has a 60 percent protein content produced from wastewater from a local brewery and it is used in fish meals in feeds. So, what are the functional properties of SCP? The functional properties of proteins such as solubility, emulsification, forming, and gelation depend on their origin and structure. Proteins are amphiphilic, interacting with both hydrophilic and hydrophobic environments. Solubility is crucial for their use in food systems and is influenced by pH.

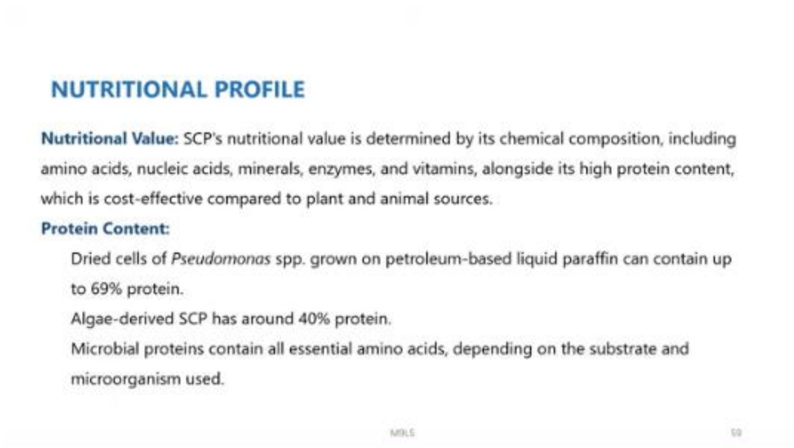


Methylobacterium species	Crude Protein Content (%)
Methylobacterium extorquens	50.88
Methylophilus methylotrophus	81
Methylococcus capsulatus, (Bath)	74
Methylococcus capsulatus, (Bath)	63.7
Methylocapsa acidiphila	59

Higher pH values than a protein's isoelectric point improve solubility and stability. So, this table shows Methylobacterium species, which are known for their ability to metabolize C1 carbon compounds like methanol and methane, with protein content ranging from 50% to 80% depending on the strain. The most common species used for single-cell protein production include Methylophilus, Methylotrophus, and Methylococcus capsulatus. And you can see these Methylotrophus species have a very high protein content—81%—followed by capsulatus at 74% and 63%, respectively. Proteins in emulsions stabilize the mixture, with SCP from kefir microorganisms showing emulsifying properties similar to defatted soy flour.

For foaming, proteins must stabilize foams effectively across various pH levels and concentrations. For example, protein... Power 5 from Saccharomyces cerevisiae demonstrates notable foam-stabilizing properties. Gels formed by proteins or high-molecular-weight substances create a network that holds liquid. SCP gels have been found to be stronger than those made from soy flour.

SCP's nutritional value is determined by its chemical composition, including amino acids, nucleic acids, minerals, enzymes, and vitamins, alongside its high protein content. It is cost-effective compared to plant and animal sources. Protein content, for example, dried cells of Pseudomonas species grown on petroleum-based liquid paraffin can contain up to 69% protein. Algae-derived SCP has around 40% protein. Microbial proteins contain all essential amino acids, depending on the substrates and microorganisms used.



Then we have the amino acid profile. Bacterial SCPs' amino acid profile resembles fish protein. Yeast-derived SCP is similar to soy protein. SCP is generally deficient in sulfur-containing amino acids, methionine and cysteine, but rich in lysine and other amino acids, necessitating supplementation. SCP is rich in vitamins, especially vitamin B12 and those produced by bacteria, as well as vitamin A from algae.

Common vitamins include riboflavin, thiamine, pyridoxine, niacin, and others. Microorganisms grow quickly, yielding sufficient biomass. Algae grow in 3-6 hours, bacteria in 30 minutes to 2 hours, and yeast in 40 minutes to 3 hours. SCP can be produced year-round, independent of environmental conditions, with low water requirements.

NUTRITIONAL PROFILE *(cont.)*

Amino Acid Profile:

Bacterial SCP's amino acid profile resembles fish protein.

Yeast-derived SCP is similar to soy protein.

SCP is generally deficient in sulfur-containing amino acids (methionine and cysteine) but rich in lysine and other amino acids, necessitating supplementation.

Vitamins:

SCP is rich in vitamins, especially Vitamin B12 (bacteria) and Vitamin A (algae).

Common vitamins include riboflavin, thiamine, pyridoxine, niacin, and others.

Biomass Yield:

Microorganisms grow quickly, yielding significant biomass (algae: 3-6 hours; bacteria: 30 minutes to 2 hours; yeast: 40 minutes to 3 hours).

SCP can be produced year-round, independent of environmental conditions, with low water requirements. (Sharif et al. 2021)

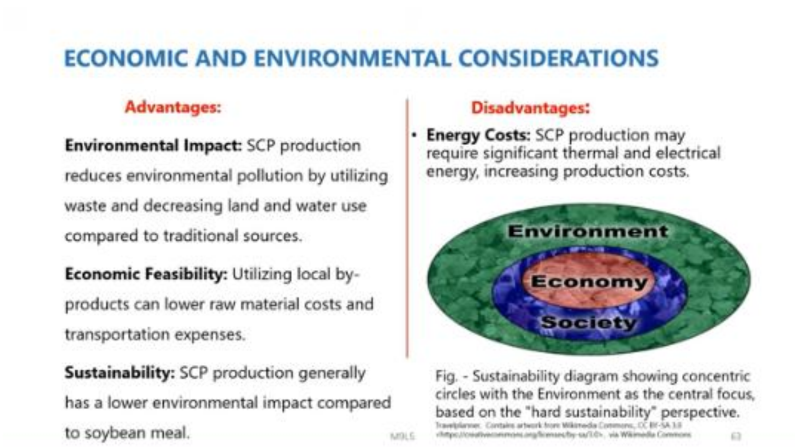
When it comes to nutritional safety, SCP is a nutritious protein source with essential amino acids produced by microorganisms, offering alternatives to fish meal and soybean meal. Saccharomyces cerevisiae, for example, grown on sugar-based substrates, provides a broad amino acid range but lacks threonine and tryptophan. Photohydrogenotrophic bacteria and algae like Chlorella vulgaris and Arthrospira platensis have amino acid profiles similar to soybean meal, making them suitable for animal feed. Microbial biomass from algae and bacteria recovers nutrients from wastewater and produces SCP. However, some SCP sources have high nucleic acid content, posing health risks, such as gout and kidney stones, for human consumption.

Nucleic acid levels must be below 2%. SCP also carries risks of allergies, toxicity, and contamination. Let us look into the sensory aspects and consumer perception. Interest in plant-based proteins, insect proteins, cultured meat, and single-cell proteins is increasing, with plant-based proteins being the most accepted. SCP ranks second in acceptance, followed by insect proteins and cultured meat, which are often rejected due to neophobia and perceived unnaturalness.

Cultural factors heavily influence the acceptance of alternatives. Research on SCP in food shows it can be added to bread, cereal bars, and pasta without affecting sensory properties. Bread with up to 4% SCP remains acceptable, while SCP-enriched cereal bars and pasta receive positive feedback. Arthrospira platensis in food is well-received for its nutritional value without altering taste acceptance. So, what are the economic and environmental considerations?

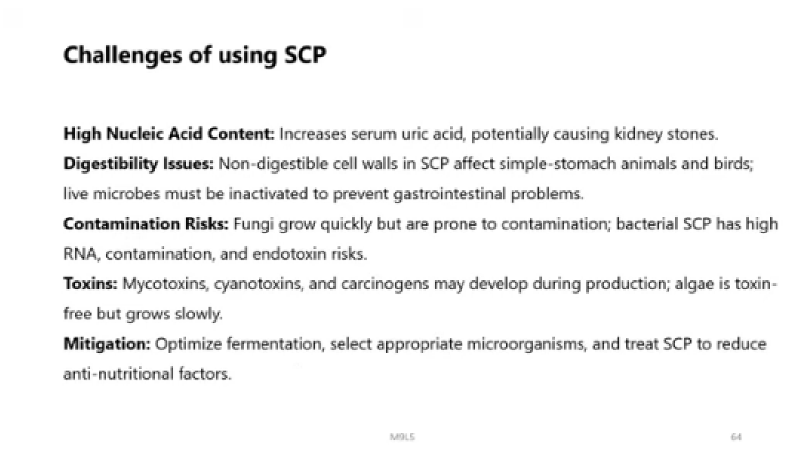
We can examine these from the advantages it offers and also the disadvantages that come along with it. For example, when it comes to environmental impact, SCP production reduces environmental pollution by utilizing waste and decreasing land and water use compared to traditional sources. From the point of view of economic feasibility, utilizing local byproducts can lower raw material costs and transportation expenses. SCP production generally has a lower environmental impact compared to soybean meal and is therefore sustainable. But it has certain disadvantages, like high energy costs; SCP production may require significant thermal and electrical energy, increasing production costs.

So, this figure shows sustainability with concentric cycles, where the environment is the central focus based on the hard sustainability perspective. So, given the greater number of advantages and fewer disadvantages like high energy costs, we can consider using alternative energy sources to make acid production more viable and sustainable. There are other challenges of using SCP apart from high energy costs, such as high nucleic acid content, which we have already discussed, increasing serum uric acid and potentially causing kidney stones. There can be digestibility issues, such as non-digestible cells in the SCP affecting simple-stomach animals and birds. Live microbes must be inactivated to prevent gastrointestinal problems.



There is always a risk of contamination. Fungi grow quickly but are prone to contamination. Bacterial acid has high RNA contamination and endotoxin risks. Certain risks from toxins like mycotoxins, cyanotoxins, and carcinogens may arise during production. Algae is toxin-free but grows slowly.

Mitigation strategies include optimizing fermentation, selecting appropriate microorganisms, and treating SCP to reduce anti-nutritional factors. Some emerging technologies for SCP production include innovative steps like genetic engineering, synthetic biology, or metabolic engineering, which optimize microbial metabolic pathways to enhance SCP production. Then we can consider phototrophic cultivation, which utilizes microalgae or cyanobacteria to convert sunlight and carbon dioxide into biomass, offering a sustainable approach with high growth rates and simultaneous production of valuable compounds. It also offers solutions like carbon dioxide sequestration, which is a challenge in the current climate change era. Some future prospects relate to sustainability and development, particularly focusing on the circular economy, where SCP production can contribute to sustainable development by revalorizing residues for aquaculture and other uses, thereby contributing to the circular economy.



Then SAP can help address undernutrition due to its high protein, vitamin, and lipid content. It can reduce reliance on traditional protein sources and utilize readily available raw materials, contributing to more sustainable food systems. So, here we can see a figure where we observe some of the obstacles and future developments in adopting microbial foods, particularly from the point of view of SCP. We consider the economic viability, which is an obstacle, as well as the high RNA content and consumer acceptance due to various issues, including palatability or digestibility concerns. And then here, we can use these as an alternative to animal meat. Then we may have enhanced flavor and odors, and it can provide improved nutrients and health properties.

And of course, the growth of home waste is a solution provider for us. And then we can also use this as food for difficult-to-reach areas, and it can be the ultimate nutrition food. So with this, we come to the end of this lecture. Thank you for your kind attention. Amen.

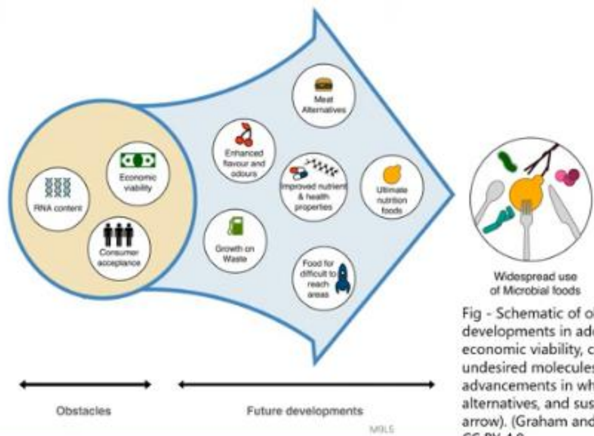


Fig - Schematic of obstacles and future developments in adopting microbial foods: economic viability, consumer acceptance, and undesired molecules (beige circle); future advancements in whole foods, animal product alternatives, and sustainable ingredients (blue arrow). (Graham and Ledesma-Amaro 2023)
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