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Utilization of Food Waste for Single-Cell Protein: A Cutting-Edge Technological Approach for Sustainable Protein Generation in Agricultural and Food Industry Applications

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Abstract

The swiftly growing population and climate change present a significant danger to our existing food systems. Additionally, the extensive reliance on animal-derived and plant-derived protein presents disadvantages, as these food sources demand significant land and water, are subject to seasonal fluctuations, incur high costs, and lead to environmental pollution. Single-cell proteins (SCPs) are attracting significant research attention because of their impressive characteristics, including protein content comparable to other sources; minimal land and water requirements; reduced carbon footprint; and a brief production timeframe. This examination investigates the utilization of food waste as an eco-friendly feedstock for the progression of SCP processes. It examines SCP research that utilizes food waste as a substrate, along with the biocatalysts (bacteria, fungi, yeast, and microalgae) employed. The operational setpoint conditions that regulate SCP yields and SCP fermentation pathways are also clarified. This assessment also illustrates the application of the biorefinery concept in literature to enhance the economic viability of "waste-to-protein" innovations, resulting in the creation of multiproduct value chains. A brief part that examines the Researcher SCP situation is included as well. The technical and financial challenges encountered by second-generation SCP processes are examined, along with future outlooks. Consequently, SCP technologies may significantly contribute to fast-tracking a "sustainable protein market" and addressing the worldwide hunger emergency.

Keywords

Biorefinery concept, SCP-Producing Microbes, Single-Cell Protein Production, Single-cell proteins (SCPs), Solid-State Fermentation.

LITERATURE SURVEY

Rapid population increase, climate changes, water scarcity, and decreased agricultural land availability have led to a rise in sustainable protein-rich food sources. Data from the Food and Agriculture Organization (FAO) of the United Nations indicates that over 1 billion individuals globally experience malnutrition; if this issue is not effectively tackled, this figure is projected to double in the coming ten years. In sub-Saharan Africa, the issue of malnutrition is concerning, with figures increasing from 5.5 to 30 million in the past ten years. A study also confirmed that global food supplies need to expand by 70% to 100% to alleviate global hunger and malnutrition, particularly in developing countries where food insecurity is still a significant issue. The significant dependence on animal-based proteins poses various issues, including (i) their role in greenhouse gas emissions; (ii) the need for extensive water and land for raising and sustaining cattle/livestock; (iii) elevated costs of animal feed; and (iv) the depletion of half of the world's fish stocks as a result of overfishing and human activities, all of which intensify the challenge of food insecurity. Research indicates that worldwide meat (a source rich in protein) consumption is projected to reach approximately 300 million tons annually by 2050. This indicates that additional alternative protein sources will be crucial to satisfy the continuously rising demands. Consequently, contemporary food production methods that prioritize sustainability are garnering significant interest from diverse stakeholders, such as scientists, food industry leaders, and government officials. These eco-friendly methods are viewed as effective answers to several of the urgent problems mentioned earlier [1], [2], [3], [4], [5], [6], [7], [8], [9], [10].

An alternative strategy suggested in the past twenty years is the utilization of plant-derived proteins. Despite offering numerous socioeconomic advantages, they demand significant amounts of arable land and water, along with chemical fertilizers, which will become unsustainable as the global need for protein continues to rise. To address this constraint, the scientific community needs to create strong and scalable technologies that are socially, economically, and environmentally significant, especially those integrated within the circular bio/economy framework. Recent developments in bioprocessing and synthetic biology have broadened the application of microorganisms, and these areas are now utilized to meet global nutritional demands, including the production of second-generation microbial protein, which entails converting a range of biowastes into protein-dense biomass known as SCP. The scientific community is promoting the biorefinery concept to support circular bio/economy practices by facilitating the creation of

multi-product chains (SCP and other biobased products) via a network of microbial-driven processes that achieves "zero waste" compared to traditional waste management strategies. Moreover, the conversion of bio-waste's, especially food waste, into high-value products like single-cell proteins (SCPs) has been suggested as an innovative and eco-friendly technology that might economically generate protein-rich feeds, potentially acting as protein additives for both human and animal nutrition. SCPs possess many exceptional qualities that enable them to act as an alternative protein source due to their high protein levels (30–70%) and low fat; utilizing biowastes lowers operational costs; recovering SCPs is fairly straightforward; and they require minimal water and have a small carbon footprint. With society's increasing health awareness, a transition towards healthier protein sources like SCPs is essential, as this supports a healthy lifestyle and/or minimizes health issues in consumers, while also tackling concerns such as animal welfare and environmental impacts. Figure 1 illustrates a schematic diagram of the SCP process derived from food waste.

Importance of Research and Its Contribution to Existing Knowledge

In recent decades, a substantial scientific understanding of the SCP process has emerged due to its previously mentioned health, environmental, and economic advantages. There is currently a focus on utilizing 2G feedstocks, like food waste, to render this technology cost-effective and reduce environmental pollution through circular bioeconomy approaches. The results achieved from the "waste-to-protein" technologies could result in numerous technological advancements along with a range of economic prospects. Additionally, this will be crucial in promoting the United Nations' Sustainable Development Goals (SDGs), particularly in providing healthy nutritious foods (SDG 2) such as SCPs through innovative sustainable technologies, especially those SCP methods that utilize food waste as the primary substrate. Furthermore, the essential operational setpoint parameters influencing the recovery of SCPs are examined [10].

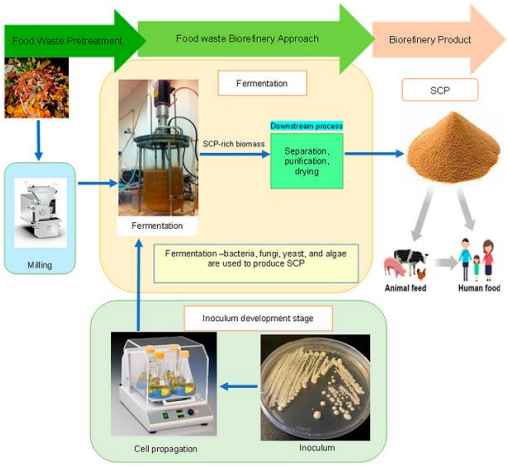


Figure 1. A conceptualized illustration of an SCP process from food waste

A brief segment that addresses the Researcher SCP landscape (market dimensions, SCP start-ups/enterprises, etc.) is also provided. This paper illustrate

(i) Methods to generate economic value from biowastes;
(ii) Ways to implement the circular bio/economy concept through the SCP process; and
(iii) Strategies for environmental remediation using accessible feedstocks like food waste.

In recent decades, a substantial amount of scientific knowledge has accumulated regarding the SCP process, owing to its previously mentioned health, environmental, and economic advantages. The results derived from the "waste-to-protein" technologies may yield several technological advancements along with numerous economic prospects. Furthermore, this will be crucial in advancing the United Nations' Sustainable Development Goals (SDGs), particularly in the provision of healthy nutritious foods (SDG 2) such as SCPs through innovative sustainable technologies, especially those SCP methods that utilize food waste as the primary substrate.

ILLUMINATING SCP

From a food technology perspective, SCP denotes the biomass high in protein generated via fermentation methods. SCPs are generated through a diverse range of microorganisms, such as bacteria, yeasts, fungi, and microalgae.

The concept of SCP originated in the 1960s from MIT researchers, and it gained recognition through prominent commercial products like Quorn™ (made by Marlow Foods), MarmiteR (created by Unilever), Spirulina (manufactured by Cyanotech Corporation), etc., derived from fungus (Fusarium venenatum), yeast (Saccharomyces cerevisiae), and microalgae, respectively. SCPs serve as an alternative and cost-effective protein source for animal feed, including aquaculture and livestock [11]. Notably, a significant number of microbes possess a high protein concentration [12], [13] [14], [15], [16].

For example, algal species (such as Arthrospira platensis, Chlorella vulgaris, Dunaliella salina, Galdieria sulphuraria, and Tetraselmis chui) are known to produce 40–60% of SCPs [17], [18], whereas edible fungi can yield 30–70% of SCPs. Nonetheless, SCP sourced from bacteria and fungi has its drawbacks, as these microbial cell factories generate a significant RNA content (15–16% on a dry basis), which is harmful to humans. This issue is typically tackled by applying heat treatment to the protein-rich biomass (e.g., 60–70 ◦C for 30 min), although the financial impact of this downstream process requires investigation. Recent progress in recombinant DNA technology has enabled researchers to simultaneously generate SCP alongside other valuable substances using genetically modified strains, thereby establishing new industrial value chains.

ACCESSIBILITY OF FOOD WASTE AS A SUSTAINABLE RAW MATERIAL FOR SCP GENERATION

A considerable focus is placed on utilizing second-generation feedstocks to prevent the "Food vs. Fuel" dilemma, and there is also a shift in the production of SCP. Researchers in science are utilizing inexpensive and non-edible substrates to ensure sustainability in the process and enhance industrial competitiveness, all while alleviating the environmental impacts resulting from food waste [19], [20] [11], [17], [18].

Food waste is by far the most common and easily accessible feedstock that can be utilized for the achievement of sustainable and economically viable SCP technology. The Food Waste Index from the United Nations Environment Program estimates that approximately 1 billion tons of food wastes are generated worldwide annually, with industrialized countries accounting for a significant portion. Historically, these countries depended significantly on conventional and unsustainable methods for food waste treatment, such as landfilling and incineration, but strict waste disposal regulations are driving them to adopt environmentally friendly food waste management strategies—these technologies embody the principles of a circular bioeconomy, with developed regions like the EU leading in this research field.

Research conducted by the Council for Scientific and Industrial Research (CSIR) indicated that around 10.3 million tons of food waste is generated annually in South Africa, and this figure is expected to rise in the upcoming years due to the previously mentioned factors. The Department of Environmental Affairs in World recently implemented stringent environmental regulations targeting the total elimination of organic waste disposal by 2027, indicating that local businesses and researchers must react swiftly and innovate new biowaste valorization technologies to tackle this societal challenge. This study by Dahiya et al. indicated that utilizing food waste in microbial biorefinery technologies could result in significant advancements in biobased economies, as this approach may facilitate the creation of various market-oriented products like biomaterials, biofuels, biochemicals, bio-additives, and biofertilizers, including SCP, through a "cascade" and/or "one-pot" fermentation method.

In terms of its nutritional makeup, food waste serves as an excellent source of SCP due to its lower lignin levels in comparison to agro-wastes, which necessitate complex and frequently costly biomass fractionation techniques to obtain C5 and C6 fermentable sugars.

NUTRITIONAL MAKEUP OF FOOD WASTE AND ITS APPLICABILITY FOR SCP PRODUCTION

Numerous research efforts have been conducted in microbial processing to determine the nutritional makeup of food waste from industrial, agricultural, commercial, and municipal sources. Consequently, this paper expands on current scientific information to emphasize the importance of food waste as a sustainable resource that can enhance SCP processes. Research conducted by Zhang et al. demonstrated that food waste originating from agricultural, industrial, food retail, and municipal sectors serves as an ideal substrate for cultivating various microbial cell factories; it features a moisture content of 74–90%, a volatile solids to total solids ratio (VS/TS) of 80–97%, and a carbon to nitrogen ratio (C/N) of 14.7–36.4. In other reports, it was indicated that food waste is highly suitable for numerous biochemical processes due to its abundant supply of micro-nutrients (such as iron, magnesium, manganese, copper, zinc, vitamin B6, vitamin C, vitamin K, selenium, etc.) and macronutrients (including fiber, minerals like iron and potassium, and vitamins such as vitamin B9, vitamin E, vitamin A, vitamin K, riboflavin, etc.) that serve as stimulatory agents for microbial consortia. These findings show minor differences since additional factors also influence food components (e.g., geographic location, climate, food waste processing, storage conditions, etc.); nonetheless, the majority of studies align with these results, as indicated by similar research performed in other areas like Asia, Europe, Africa, and North and South America. From a techno-economic perspective, fruit and vegetable waste is considered appropriate for SCP because of its substantial amounts of monomeric sugars (glucose and sucrose), minimal lignin content, biodegradable characteristics, ability to be utilized by various microorganisms, and high availability. Research indicates that fruit by-products like pineapple peel, orange peel, potato peel, banana peel, and carrot pulp enhance the SCP yields because of their substantial sugar content, which varies between 54.17 and 83%. Conversely, there is doubt about utilizing agro-food waste like corn cobs, rice straw, bean husks, and bagasse, since they primarily contain cellulose (35–50%), hemicellulose (25–30%), and lignin (25–30%), requiring extensive fractionation steps prior to application in SCP production processes; therefore, these materials are not cost-effective. Recent research addressing the utilization of food waste as a substrate in SCP revealed encouraging outcomes. For instance, the highest protein levels ranged from 27 to 36% when producing SCP with bread waste as a carbon source and utilizing the fungus Rhizopus delemar [21], [22], [23], [24], [25].

A SUMMARY OF MICROBES THAT PRODUCE SCP

As indicated in Table 1, bacteria, microalgae, oleaginous fungi, and filamentous yeasts are powerful sources of inoculums that are mostly used in the production of SCP. This section discusses these biocatalysts.

Bacteria

The literature has extensively documented the use of bacteria in SCP; these inoculum sources are common and capable of assimilating a wide variety of carbon sources, including food waste. Because of their capacity to produce high protein yields (50–80%), as previously mentioned,

microorganisms are used in SCP at both the lab and pilot scales. It has been demonstrated that methane-oxidizing bacteria, such as Methylomonas sp. and Methylococcus sp., may create up to 56% and 70% of SCP, respectively. Because these bacteria can synthesise a wide range of new-value-added chemicals using the "closed-loop" biorefinery-based method, the production of methane-based bacterial SCP fits well within the framework of a circular economy. Additionally, using mixed-bacterial cultures creates a synergistic interaction that offers several advantages during SCP fermentation since certain microbial species help convert substrates while others increase the yields of SCP formation. Novel valorisation routes that target SCP and other value-added compounds like biopolymers, biofertilizers, biosurfactants, biolipids, biofuels, etc. have been investigated in the literature to achieve biorefinery-based strategies that also result in "zero waste" in order to maximise value chains from food waste. The use of bacteria has been questioned, nevertheless, because these inocula are less dense and smaller than yeast and fungus, making recovery challenging [26], [27], [28], [29].

Fungi

Because of their metabolic resilience, high SCP yields, high density, and cost-effective downstream procedure, fungi have been widely used in SCP manufacturing research. A variety of micronutrients, including vitamin B12, riboflavin, phosphorus, zinc, and manganese, as well as low levels of cholesterol, are present in fungus-derived SCP, which has proven health advantages. Moreover, it is well known that fungi secrete substantial amounts of hydrolytic enzymes, which are crucial for the biodegradation of substrates, including organic wastes. Because Fusarium venenatum, Aspergillus niger, and Trichoderma sp. may provide SCP yields ranging from 30 to 70% and have modest nucleic acid yields (7–10%) that need processing, research on their application is garnering a lot of scientific and industrial attention. Large companies like Marlow Foods, Unilever, and Vitam Hefe-produkt GmbH, respectively, create commercial SCP brands like QuornR, MarmiteR, and Vitamin-RR as a result of the success of fungal mycoprotein. Furthermore, the use of food waste in fungal-derived SCP processes is beneficial because significant progress has been made in the development of fungal strains that produce enzymes (such as Trichoderma, Aspergillus, Rhizopus, Neurospora, etc.); these strains are used for the hydrolysis, saccharification, and fermentation of various biowastes, in contrast to the modern biomass fractionation techniques that require the use of expensive and hazardous chemicals. The "closed loop" fungal strategies phenomena, which addresses sustainability and the circular bio/economy while facilitating the synthesis of a variety of biobased products based on the one-pot fermentation technique, was discovered as a result of these scientific breakthroughs. By developing new avenues for circular possibilities, fungal research is also utilised to solve the problems brought on by agro-food industry sidestreams. The "zero-waste" notion has emerged as a result of the large-scale manufacture of SCPs using wastewater from the fruit, cheese, potato, wheat, and brewing sectors [30], [31], [32].

Microalgae

Since microalgal research is currently utilised in SCP production investigations, it has helped scientists make several scientific advances. Because some species may produce high protein content (60–70%) together with other vital elements (omega-3, omega-6, vitamin-A, -B, -C, -E, minerals, etc.), microalgae are ideally suited for human and animal nutrition. Nowadays, a lot of food formulations use microalgae, which has improved their value chains. These supplements are offered for sale as dry powders or capsules. Spirulina (Arthrospira platensis), a product marketed as a protein-rich supplement, is the most well-known microalgal-derived SCP product. Because of its high protein content (up to 70%) when grown in non-limited nitrogen conditions, it is one of the most widely grown microalgae. Additionally, it is abundant in other beneficial substances including phycobiliproteins, carotenoids, and chlorophylls, all of which are used in the food business. Additionally, it has been demonstrated that spirulina strengthens the immune system and lowers the likelihood of contracting diseases including cancer, degenerative chronic disease, and cardiovascular disease [32], [33], [34], [35], [36].

The US Food and Drug Administration (US FDA) has approved spirulina as safe for human consumption because to its nutritional profile and health benefits. Senedesmus and Chlorella are two more algae that make SCP. These algae species were co-cultured during a bench-scale investigation on the anaerobic digestion of food waste, and they are also helpful in other applications including wastewater treatment and waste stabilization.

According to a research, the addition of two algae species led to a notable generation of SCP (76.35%) in addition to a high biomethane yield (639.8 mL/gVSadded).

Recent progress in genetic engineering and molecular biology has enabled scientists to identify microalgal strains with strong metabolic properties, particularly those strains that can enhance the creation of diverse value-added products including proteins, antioxidants, biopolymers, carotenoids, fatty acids, carbohydrates, and more, through microalgal biorefinery-based approaches. These newly created technologies are essential for achieving circular biobased economies. In these methods, the main extraction techniques are utilized to obtain the protein, succeeded by secondary extraction techniques aimed at collecting other valuable products.

Aurantiochytrium sp. is a genus widely utilized in food waste valorization approaches, as it generates a significant amount of polyunsaturated fatty acids (PUFAs) and has demonstrated favorable yields of protein and carbohydrates when implemented in a biorefinery context [37].

Yeast

Yeast species are utilized in SCP research due to their high nutritional yields and strong metabolic robustness. They can incorporate various carbon sources such as food waste, making them an attractive inoculum source. To adopt the idea of "waste-to-protein" via sustainable innovations, yeasts like Saccharomyces cerevisiae, Candida utilis, Cryptococcus curvatus, Kodamaea ohmeri, Debaryomyces prosopidis, Yarrowia lipolytica, Lipomyces starkeyi, Rhodotorula glutinis, Rhodosporodium toruloides, etc., are extensively utilized in SCP production because of their significant protein yields (30–70%); additionally, they can thrive on food waste substrates [38].

Like fungal biorefineries, ongoing studies are investigating yeast-based biorefinery approaches

(i) For different biowaste conversion methods utilizing yeast strains;
(ii) To minimize the operational expenses linked to upstream and downstream processes; and
(iii) To facilitate the upcycling of industrial byproducts to lower their disposal expenses yeast cells do not require lysis unlike algae.

Additionally, yeast species show impressive adaptability, and their development can be improved using various strategies. This encompasses the meticulous choice of yeast strains, identification of appropriate substrates, evaluation of byproducts produced during whole-cell biomass creation, and the enhancement of biomass processing methods to achieve a functional, final prototype.

Solid-State Fermentation

Solid-state Fermentation has its roots in ancient history, utilized for various purposes. This fermentation method has garnered growing global interest because of its multiple advantages, namely, it requires minimal energy and water, generates minimal wastewater, and aids in environmental rehabilitation. This technology is additionally utilized in

MODES OF FERMENTATION USED IN SCP PRODUCTION

Submerged Fermentation

Submerged fermentation involves utilizing a liquid medium in which the processed substrate is placed in a liquid nutrient solution, facilitating enzymatic and/or microbial hydrolysis throughout the SCP procedure. The fermentation process takes place in a bioreactor ideal for growing SCP-producing microorganisms, functioning under batch, semi-batch, or continuous mode to yield protein-rich biomass. Furthermore, the bioreactors function under ideal pH, temperature, aeration, and fermentation parameters. The obtained SCP is retrieved via filtration or centrifugation, and then the biomass is dried [39], [40].

Semi-Solid Fermentation

Semi-solid fermentation has demonstrated impressive outcomes in fermentation technology and bioprocess engineering; it produces high yields, demands less energy, and utilizes minimal water. Nonetheless, some researchers have countered its application in SCP, suggesting that it could increase operational expenses due to the necessity for sophisticated biomass fractionation techniques and/or the employment of genetically altered microorganisms to break down the substrate. Additionally, other important measures like effective sterilization, appropriate conditions for the upstream process, and product recovery must be taken into account when implementing this fermentation method [41], [42].

Table 1. A summary of SCP production research derived from food waste [43] [44] [45] [46] [47] [48] [49]

Food Waste	Inoculum	Fermentation Type	Protein (%)
Corn Stover	*Rhodococcus opacus*	Submerged	52.7
Olive fruit waste	*Candida lipolytica*	Submerged	69
Fruit waste	*Saccharomyces cerevisiae*	Submerged	79.14
Molasses	*Kluyveromyces marxianus*	Submerged	50.5
Food waste	*Saccharomyces cerevisiae*	Solid-state	38.43
Soy molasses	*Candida tropicalis*	Submerged	56.41
Cheese whey	*Trichoderma harzianum*	Submerged	34.21
Tomato, mango, orange, apple, and banana	*Trichoderma reesei*	Solid-state	14.06–78.17
Paddy straw, wheat straw, sugarcane, and maize straw	*Bacillus licheniformis*	Solid-state	24.56
Soybean wastewater	*Rhodobacter sphaeroides Z08*	Submerged	52
Fruit waste	*Saccharomyces cerevisiae*	Solid-state	48.32
Food waste	*Yarrowia lipolytica*	Submerged	38.8
Agri-industrial wastewaters	Purple phototrophic bacteria, algae	Submerged	60
Tofu waste	*Chlorella* sp.	Semi-solid-state	52.32
Tempeh	*Chlorella* sp.	Semi-solid-state	52
Cheese whey	*Chlorella* sp.	Semi-solid-state	15.43
Food waste	*Saccharomyces cerevisiae, Candida utilis, Yarrowia lipolytica*	Solid-state	25.14
Waste bread	*Neurospora intermedia*	Submerged, solid-state	27–33
Potato protein liquor	*Neurospora intermedia, Rhizopus oryzae, R. oligosporus, R. delemar, Aspergillus oryzae,*	Submerged	53
Stale bread and brewers' spent grain	*Neurospora intermedia, Rhizopusoryzae*	Solid-state	21.1
Bread residues	*Neurospora intermedia, Aspergillus oryzae*	Submerged	45

SCP, and scientists have noted favorable SCP results. It is typical in research involving fungal species. like Aspergillus niger, Candida spp., Trichoderma spp., and Saccharomyces cerevisiae, since these Inocula generally generate biomass that is high in protein content [50], [51], [52], [53].

FACTORS INFLUENCING THE YIELDS OF SCP

Alongside the carbon source, the SCP technology is regulated by multiple physicochemical factors.

Factors like the pH of the fermentation environment, temperature, and nitrogen source, inoculum size, oxygen transfer, and so forth. These setpoint parameters are typically analyzed, one by one, in scientific research to gain deeper understanding of their specific functions in Pathways for SCP production and SCP yields in the downstream processing stage. For this reason the individualistic influence of the setpoint factors that determine SCP productivity is addressed in this section. Among the parameters recorded in the literature, the temperature continues one of the most examined setpoint variables in the literature, as it influences the (i) development and function of microorganisms, (ii) raw material usage, and (iii) metabolic pathways aimed at SCP.

A temperature span of 25–30 ◦C is frequently utilized in SCP manufacturing. Nonetheless, various fermentation research studies indicated ideal temperatures of 35 °C and 37 °C. It is crucial to comprehend the impact of temperature regarding the development of inocula, as bacteria, fungi, and microalgae exhibit different optimal temperature ranges. For instance, it was shown that Candida utilis increases the SCP yield when the temperature is raised from 25◦C to 35◦C [54], [55], [56], [57], [58], [59], [60], [61].

In the meantime, research utilizing different microbial species like Saccharomyces cerevisiae and Bacillus subtilis exhibited optimal temperatures of 30 °C and 37 °C, correspondingly. Alternate SCP creators like Fusarium venenatum thrives effectively within a temperature range of 28–30 ◦C. pH represents another crucial setpoint variable that should not be ignored in SCP research, because It influences enzymatic pathways, the growth of inhibitors, and rates of hydrolysis.

Conflicting values have been documented in the literature concerning the ideal pH for SCP. fermentations. Similar to the carbon source (food waste), the nitrogen source must be utilized within an optimal range to achieve favorable outputs. Somda et al. examined the impact of adding various supplements nitrogen sources (peptone, ammonium sulfate, ammonium nitrate, and yeast extract) concerning SCP generation utilizing Candida utilis FJM12. The authors obtained significant protein-derived biomass after 72 hours using peptone (6.48 g/L) as a nitrogen source, succeeded by ammonium. sulfate (5.74 g/L) and ammonium nitrate (3.77 g/L). In every trial run, a 1% (w/v) nitrogen solution was utilized as an additional growth medium.

A research investigation studied the influence of aeration on SCP production employing Candida utilis noted 1 vvm as an ideal aeration rate. This was confirmed by a study that employed various yeast strains; the maximum SCP yield was obtained at 1 vvm with K. lactis, S. cerevisiae, K. fragilis and K. marxians. Fermentations employing bacteria like Bacillus, E. coli, B. coagulans, B. stearothermophilus, and B. licheniformis were found to prosper in aerated conditions. of 0.5–1.5 vvm.

PRODUCTION OF SINGLE-CELL PROTEIN

Present Situation, Market Assessment, and Future Perspective Worldurgently requires sustainable protein production technologies, as its ever-increasing population and adverse climatic conditions adversely affect the availability of existing protein production sources (both animal- and plant-based proteins). While it is not yet on par with that of Western countries, research into single-cell protein/mycoprotein is gaining significant attention in South Africa, as food industries, scientists, and the government are dedicated to enhancing the nation's protein resources through environmentally friendly and sustainable methods. Furthermore, this technology will assist in tackling some of the issues arising from the heavy dependence on conventional protein sources (both animal and plant proteins) demand considerable land and water, are influenced by seasonal variations, and are expensive, as noted previously. Being a semi-arid and ecologically challenged nation, Worldmust secure its nutritional protein supplies. However, the majority of SCP products available for human consumption and livestock farming are sourced from global companies located in Europe, North America, and Asia. At present, Unilever continues to be the leading food company in SCP production—MarmiteR is the top yeast product in South Africa, commonly utilized as a protein spread [62], [63].

Recent studies on eating habits indicate that many Researchers are growing more health-aware, creating a demand for alternative protein options. The nation holds the 22nd and 23rd positions globally for vegan and vegetarian lifestyles, respectively. Worldaccounts for approximately 57% of the entire plant protein market in Africa, which is estimated to be valued at around USD 400 million. It is important to note that the protein sources mentioned above are primarily utilized in animal feed; some of them are imported to guarantee an adequate supply. In 2021, the animal feed sector in Worldproduced approximately ZAR 50 billion in revenue and supports over 17,000 jobs. Although it has thrived, the Animal Feed Manufacturers Association (AFMA) of World has stated that the industry's long-term profitability and success will depend on the availability of affordable and sustainable feedstocks.

From an R&D perspective, numerous SCP-related research initiatives have been conducted by the country's top academic institutions like the University of Cape Town, Stellenbosch University, University of Free State, and the University of Pretoria, along with the leading science councils in the nation, including the Council for Scientific and Industrial Research.

ECONOMIC ANALYSES

In addition to the ecological advantages of converting food waste into SCPs via sustainable innovations, researchers and industries seek biobased technologies that yield favorable economic results, enabling these methods to act as substitutes for fossil-based technologies, as supported by the United Nations' SDGs. Consequently, numerous studies have been conducted in recent years to gain a better understanding of the economic effects of creating SCP technologies based on 2G feedstocks like food waste. To accomplish this, scientists utilize a scientific method called techno-economic analysis (TEA) to assess the economic effectiveness of SCP processes. According to the research by Aggelopoulos et al., utilizing food waste as raw materials for producing SCPs is economically feasible, covering 35–55% of the production expenses. Moreover, utilizing low-lignin (biodegradable) feedstocks like food waste will lower operational expenses, given that considerable investment is necessary for acquiring costly commercial enzymes and biomass fractionation reactors. Recent studies indicate that synthesizing SCP through anaerobic digestion is more profitable because it generates both biogas and biodigestates, unlike conventional methods that concentrate only on biogas as the primary biobased product. Additional TEA studies have demonstrated the economic viability of establishing biorefineries focused on multiproduct value chains (SCP, natural fibers, and methane) by utilizing agro-industrial effluents. A recent study conducted by Vlaeminck et al. showed that third-generation biorefineries utilizing industrial off-gases could represent another economically viable method for biobased SCP innovations, as this approach resulted in cost reductions from 4.15 to 2.78 USD/kg [64], [65], [66].

FINDINGS AND RECOMMENDATIONS FOR UPCOMING STUDIES

This examination investigated the practicality of utilizing food waste as a sustainable resource for the development of SCP processes. Additionally, it addressed the SCP research that utilizes food waste as a substrate along with the inocula (bacteria, fungi, yeast, and microalgae) that are applied.

The conditions of operational setpoints influencing SCP yields and SCP fermentation pathways were also examined. This article highlighted how the biorefinery concept is utilized in literature to enhance the economics of "waste-to-protein" innovations, which results in the development of multiproduct value chains; additionally, South Africa's initiatives aimed at achieving alternative protein sources were examined [14], [20], [51], [52], [53], [54], [44], [45], [67], [68], [69].

- The majority of SCP research remains at bench-scale, indicating that the real process intricacies are not completely grasped in the existing literature. Consequently, scientific researchers need to tackle this technological shortfall by performing additional pilot-scale demonstrations; this will facilitate the wide-scale deployment of SCP technologies, as shown by the emergence of SCP-oriented companies investigating the utilization of various organic wastes as key feedstocks for their upstream processes. Advancements in "waste-to-protein" systems are designed for the creation of multiproduct value chains, in which SCPs are generated alongside other biobased products like biopolymers, organic acids, biofertilizers, enzymes, and more, via a network of biorefinery routes. A few of these investigations have achieved pilot-scale.
- The introduction of innovative scientific tools in bioprocessing, microbial ecology, and synthetic biology allows scientists to leverage these advanced methods to improve the efficiency of biocatalysts by focusing on the biochemical pathways that produce SCP. These modified strains should ideally tolerate chemical inhibitors, shorten fermentation times, be suitable for biorefinery processes, control metabolite growth, and improve SCP yields.
- While this is extensively documented in the literature, additional toxicology and safety screening tests are necessary to confirm that SCPs are free from contaminants, excessive RNA levels, heavy metals, or pathogens, as these elements are harmful to human and animal consumption. This means that SCPs need to undergo intense downstream procedures. In that context, multiple essential measures are taken to retrieve the SCP.
- To achieve industrialization for second-generation protein-based technologies, it is essential for researchers across various scientific fields (such as molecular biology, microbial ecology, fermentation technology, chemical engineering, material sciences, etc.) to form collaborative R&D partnerships to successfully tackle the technological obstacles hindering the scalability of these processes. Crucially, it is essential to involve industries in these partnerships to advance this technology to higher TRL levels (TRL $\geq$ 6). A more effective strategy for food processing industries could involve establishing "cascade biorefinery" systems that allow the utilization of their byproducts for SCP generation, thus broadening their product range. This method has been implemented by major biomass processing companies.

Figure 2 summarizes several bottlenecks along with the commercial prospects for SCP technologies.

- A significant obstacle for second-generation SCPs is their complete acceptance as a feed and food supplement, stemming from regulatory structures pertaining to safety and quality assurance.

For products derived from SCP, the quality of the feedstock is essential since it can influence the nutritional content and safety of the final SCP product. Consequently, stringent regulations govern the production of alternative foods for animal and human consumption in Western countries. The Novel Global trends indicate that SCP products will gain significant momentum in Asia, Europe, and North America; this growth is also backed by the

agricultural sector, which is experiencing heightened demand for alternative animal feeds.

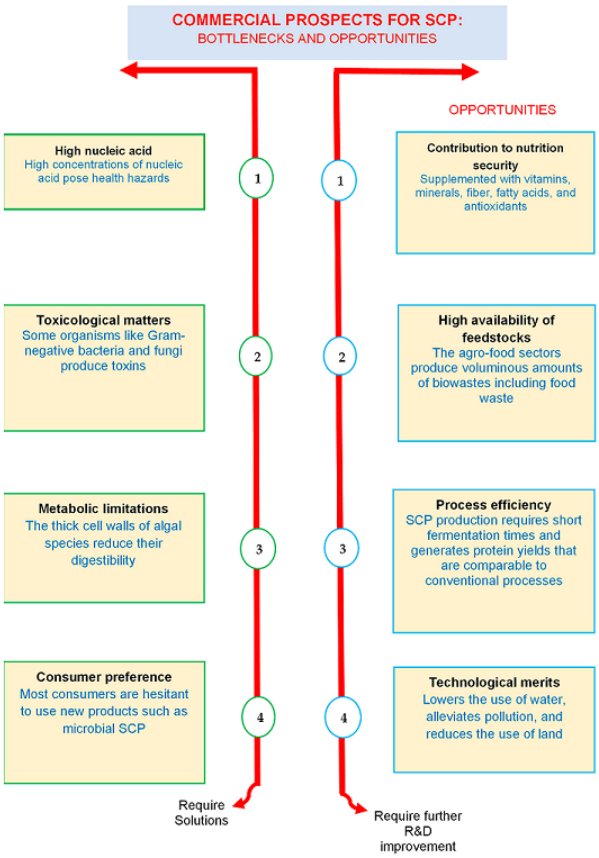


Figure 2. Obstacles and prospects for business SCP

OUR RESEARCH AND DISCUSSION

Selection of the Best Substrate for the SCP Production

Fermentation substrates were prepared from fruit wastes such as orange peel, sugarcane bagasse, watermelon skin, pine apple skin and banana peel for utilising them as sole carbon source for cultivating *Saccharomyces cerevisiae* (Figure 3A and Figure 3B). After inoculation and incubation, cell density was monitored on daily basis for five days after which fermentation process was terminated. All the batch culture experiments were carried out in triplicates for concordance.

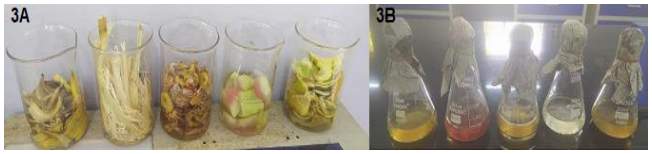


Figure 3A. Chemical pretreatment of fruit wastes for substrate preparation; 3B Fruit waste extracts as fermentation substrate

The suitability of the substrate on SCP production was determined based on the weight of dry mass and its protein content (**Table 2**). Among other fruit waste extracts, sugarcane bagasse extract yielded a significantly higher biomass (11.7 ± 0.8 g/L) while lesser biomass was observed with water melon (5.3 ± 0.6 g/L) and pine apple skin (7.8 ± 1.9g/L) media.

Hence, ash, crude fiber, crude fat and protein contents of sugarcane bagasse extract were determined and tabulated in **Table 2**. The highest crude protein yield was shown by sugarcane bagasse (52.4 ± 0.4%), followed by orange peel (45.2 ± 0.7%) and banana peel (30.4 ± 0.6%) (**Figure 4**).

Table 2. Dry biomass and crude protein contents of SCP from different fruit waste medium

Substrate	Dry biomass (g/L)	Crude protein content (%)
Orangepeel	10.2±0.4	45.2±0.7
Sugarcanebagasse	11.7±0.8	52.4±0.4
Watermelonskin	5.3±0.6	11.7±0.2
Pineappleskin	7.8±1.9	16.5±0.3
Bananapeel	9.8±0.2	30.4±0.6

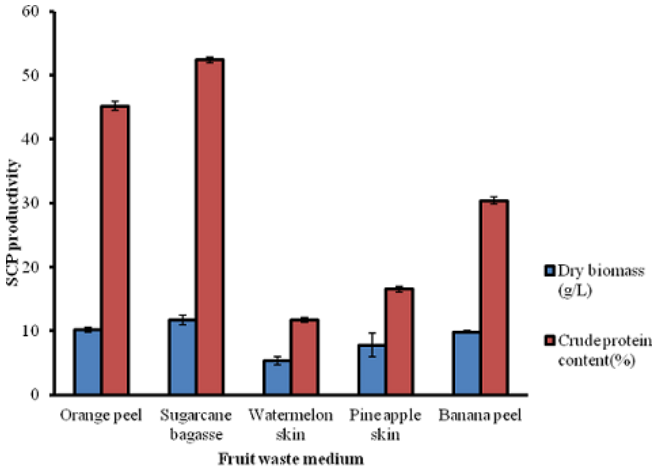


Figure 4. Dry biomass and crude protein contents of SCP from fruit waste medium

Biochemical Characterization of SCP

Table 3. Biochemical composition of sugarcane bagasse extract

S. No.	Biochemical constituent	Nutritional value (%)
1	Protein	0.14
2	Crude fat	0.75
3	Crudefibre	0.2
4	Ashcontent	0

Table 4. Biochemical composition of the yeast biomass

S.No.	Biochemical constituent	Nutritional value (%)
1	Protein	39.5
2	Crude fat	1.2
3	Carbohydrate	28.6
4	Fibre	9.1
5	Totalsolublesolids	30
6	Moisture content	10.9

Agro-wastes are rich in organic matter that can be used as a carbon and energy source by yeast in SCP production. Sugarcane bagasse extract contains high amount of crude fat (0.75%), followed by crude fibre (0.2%) and protein (0.14%) respectively. Ash content was found to be absent in the Sugarcane bagasse extract. After reducing the nucleic acid content in the produced biomass of *S. cerevisiae*, the biochemical composition such as protein, crude fat, carbohydrate, fibre, total soluble solids and moisture content of biomass were determined and presented in **Table 3**. The biochemical composition of the produced SCP includes: protein-39.5%, crude fat-1.2%, carbohydrate-28.6%, fibre-9.1%, total soluble solids-30% and moisture content-10.9%.

All these biochemical characteristics revealed that the produced biomass of *S. cerevisiae*could be used as single cell protein in dietary supplements.



Figure 5. Comparison of survival rate of Tilapia before and after treatment; A. Experimental fishes before treatment; B. Experimental fishes after treatment

Among the four diet formulations, formulation having 40% SCP revealed higher growth performance, survival rate and weight gain (by 2 fold), when compared with other formulations.

Table 5. Survival rate and growth performance of Tilapiafed with SCP formulations

SCP concentration (%)	Survival rate (%)		Length (cm)		Weight (gm)	
	Initial	Final	Initial	Final	Initial	Final
0	100	100	8.9±0.2	10.4±0.2	8.9±0.3	13.4±0.3
20	100	100	9.2±0.3	12.5±0.2	8.5±0.1	15.7±0.2
40	100	100	9.1±0.2	14.3±0.1	8.8±0.1	18.9±0.2
60	100	100	9.4±0.1	13.7±0.1	8.3±0.2	15.4±0.1

Table 4 and Figures 5 and 6 clearly indicated that the test diets showed significantly better growth in terms of length and weight gain over the control diet.

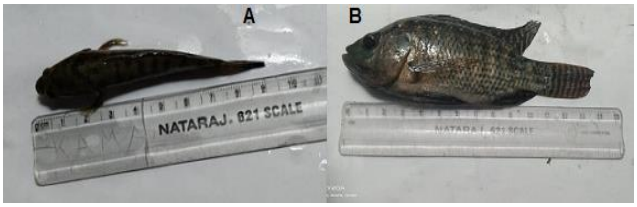


Figure 6. Comparison of length of Tilapia before and after treatment; A. Length of experimental fishes before treatment; B. Length of experimental fishes after treatment

Discussion

Suitability of Substrate for the SCP Production

Proteins, fats, carbohydrates, ash ingredients, water, and other elements such as phosphorus and potassium are rich in SCPs. Aside from the nutritional benefits of SCP, another benefit of SCP technology is constant production throughout the year. Water management strategy plays a vital role by using the waste material as the substrates. A small area of land is required, and SCP is made in less time. In this study, the fruit wastes were selected based on their bioavailability and affordability. Many workers in their investigation have used inorganic supplements for the mycelium growth on waste materials. Researcher studied the raw material used for the production of SCP. They utilized glucose (2%w/v) and $(NH_4)_2HPO_4$ (0.25%w/v) as nitrogen source supplement for the production of *Sachharomyces cerevisiae* biomass in papaya extract medium. Adoki, 2008 studied various factors influencing cell biomass production with *Candida species*. Several different fruit wastes have utilized as substrate by a number of researchers. Through heat and other pretreatments, these wastes were converted to fermentable sugar. The degree of mycelial biomass growth depends on the type of substrate used. Thus in the present investigation different types of fruit wastes were explored for biomass production (SCP) instead of dumping them. In the present study, based on the weight of drymass and protein content, sugarcane bagasse extract can be considered as the best substrate for the SCP production among the other fruit waste extracts. Higher reducing sugar content and protein content in sugarcane bagasse waste would favour the higher yield of SCP. Others obtained 34% of crude protein using papaya fruit as substrate for single cell protein production from this species.

Researcher reported 35.5% protein production in the same species using biomass in papaya extract medium. Watermelon skin could not support higher growth of yeast biomass, may due to high water content of watermelon skin. Though orange peel also contained high concentration of carbohydrates but supported less biomass production. This may be due to the less mineral content in orange peels (3.55%) than that of in cucumber peels (6.96%). Furthermore orange peels had higher crude fat content especially limonene (antimicrobial) which makes hindrance in digestion process

of microbes thus depriving yeast cells from essential nutrients resulting in supported less biomass production. The findings on the biochemical composition of sugarcane bagasse used in this studyare comparable with those of other studies. They carried out proximate analysis to assess the potential of various wastes for cost effective biomass production using palmyrah natural toddy yeast. The yeast biomass production depends on multiple factors and the carbohydrates and mineral contents are the most important of all other factors. Also they analysed the biochemical composition of papaya, watermelon and pineapple peel and reported that it can be used as a potential substrate for the production of SCP due to its reasonable carbohydrate and mineral content.

Optimization of Fermentation Parameters

Yeasts are active in an extensive temperature range with an optimum temperature of 32°C–35°C. It is essential to maintain the pH when growing yeast cultures. Batch fermentation can be considered as a closed system. Initially, when the time is zero, the sterile nutrient solution in the fermenter is inoculated with yeasts and incubated to ferment. As a result of the metabolism of the cells, the composition of the culture medium generally changes. Under optimum physiological conditions, the inoculation of a sterile nutrient solution with yeasts can undergo four typical phases of growth, namely, the lag phase, log phase, stationary phase and death phase. But, when the substrate concentration of the medium and the environmental conditions are favourable, the microbes will growth at a rapid rate. In the present study, sugarcane bagasse at a concentrationof80% exhibited increased the protein and biomass of *Saccharomyces cerevisiae.* Similar observations have also been reported earlier by Jaganmohan*et al.,* (2013).

When the concentration of substrate was increased up to 10% there was an increase in protein content of the biomass. The initial pH affects the growth of yeast, the concentration of hydrogen ion change cell plasma membrane colloid charge consequently change the ion permeability of plasma membrane (a driving force for secondary transport). In this study pH ranging from 4 to 8 was studied for the detection of optimum pH for the growth of *S. cerevisiae* in the sugarcane bagasse medium. The maximum growth was noted at pH-6. Others also reported that the pH of 6 is ideal for SCP production from *Saccharomyces cerevisiae*. Researchers produced maximum biomass of *Candida utilis*at pH 6.0. Lower biomass and protein contents at pH 4.0 revealed that the metabolic activities of yeast had been inhibited at lower initial pH condition. Similar observation was recorded by other's report for SCP produced from okara-wheat grit substrates using *Rhizopusoligosporus* and *Aspergillusoryzae*.

Different incubation period from 2 to 10 days were studied for the detection of optimum incubation period for the growth of *S. cerevisiae* in the medium. The maximum growth was noted at day 6. Considering each media overall, the dried biomass increased with the increasing days suggesting that fermentation progressed gradually in those days. After which the biomass yields were decreased gradually, suggesting nutrient depletion in the growth media. Also reported that 4 days of incubation is needed to get optimum yield of *Candida utilis*, whereas Li *et al.,* (2009) optimized fermentation period of 69 hrs was best maximum cell biomass production of *Candida utilis*.

SUMMARY AND CONCLUSION

Among the selected fruit wastes (orange peel, sugarcane bagasse, watermelon skin, pine apple skin and banana peel), locally available sugarcane bagasse waste used in this studycan be suggested as a best substrate for the production of SCP using the yeast *Saccharomyces cerevisiae*. SCP produced using sugarcane bagasse as the substrate resulted in a high biomass concentrationof11.7±0.8g/L with the maximum protein yield of about 52.4±0.4%**.** Sugarcane bagasse extract contains high amount of crude fat (0.75%), followed by crude fibre (0.2%) and protein (0.14%) respectively. These biochemical characteristics of sugarcane bagasse extract made them highly suitable for production of yeasts SCP. Degree of SCP production depends on type of media used and on fermentation conditions. Hence, substrate concentration, pH of the growth media and incubation time were optimized for production of yeasts SCP as they have an effect on the liquid state fermentation process.

During batch culture experiments, sugarcane bagasse extract at aconcentrationof80%, with a pH range of 6 and incubation period of 6 days exhibited increased SCP production. The biochemical composition of the produced SCP includes: protein-39.5%, crude fat-1.2%, carbohydrate-28.6%, fibre-9.1%, total soluble solids-30% and moisture content-10.9%. In the *in vivo* evaluation experiment using Tilapia, fish meal formulation having 40% SCP revealed higher growth performance, survival rate and weight gain(by2 fold), when compared with other formulations. SCP production by yeast mainly depends on the cost of the substrate and the capital investment, including all associated costs such as collection, transportation, purification or separation, and sterilization. Since tremendous amounts of fruit wastes are available, the cost of the raw material will be low or negligible. Utilizing fruit waste as a substrate will also help to alleviate environmental pollution and will reduce the production cost of SCP. It could therefore be recommended that fish farmers and fish feed technologists make use of this under-utilized protein source. Further studies are required to ascertain yeastSCP substitution at higher levels than those used in this study.

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