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COST-EFFECTIVE WASTE SUBSTRATES FOR MICROBIAL SINGLE CELL PROTEINS PRODUCTION: A REVIEW

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Abstract

Single Cell Protein (SCP) is the term used for the whole biomass of cells of different microorganisms containing large quantities of proteins. It can be used as an alternative source of proteins for animal feed and human food. Several commercial industries produce SCP due to its applications in the food sector, from a variety of microorganisms such as yeasts, fungi and bacteria, usually recognized as safe (GRAS organisms). There are several microbial strains that have rapid growth rates and high accumulation of cellular proteins. They exploit and valorize renewable feedstocks from a variety of biological wastes. This review outlines the potential microbial strains for SCP production coupled with their cost-effective carbon sources from food, agriculture and industrial wastes. The nutrient potential of each SCP and its associated safety and future research challenges are also discussed in detail in this review.

Keywords: *Biological waste, Biomass, Microbial Protein, Renewable feedstock, Substrate*

INTRODUCTION

By definition, the SCP is the dead and dried biomass of microorganisms including bacteria, fungi and yeast, grown on commercial scale for food applications (1). There are several benefits of SCP such as using cost effective substrates, reasonable price with having optimal nutritional value for human food and animal feed purpose. SCP provides the essential amino acids that are in less number in animal and plant meals (methionine and lysine) and proven to be the replacement of costly plant-based protein sources (1). It has been predicted that the global population will increase to 9.3 billion by the year 2050, which may pose challenges regarding rising food demand. Hence, the global food and feed demand will increase due to this continuous growth in global population (2). The current consumption rates show that there will be an annual rise of 1250 million tons proteins to feed the population (3). Due to this reason, the researcher started looking for new protein rich foods. One of the milestone steps, is the objective of developing the SCP technology, which seems best alternative to conventional protein sources in order to solve the upcoming food security issue (4). Most of the developing countries of the world are facing a malnutrition and inefficiency in adequate protein production. Global population is growing rapidly, hence deficiency of nutrients and proteins in human food and as well as animals feed are seen. As the protein malnutrition prevails in developing nations so it would be convenient if proteins are produced from alternative microbial sources in order to fulfill the protein supplement of the regional population. There is an increase been seen in protein demand in animal feed for meat industry. The SCP technology would help the world in solving the protein dilemma for better future (5). There is a growing need of protein rich food alternatives in order to subside conventional protein sources. The SCP production is a crucial step to achieve this goal which is an alternative solution to the global protein demand. During late 1960s, the production of SCP had started. The term, single cell protein refers to the dead and dry cells of micro-organisms such as yeast, bacteria and fungi which grow on various carbon sources. The name “single cell protein” was suggested for the first time, by the M.I.T. Professor Carol Wilson. The SCP is dried or dead cells of microorganisms which function as a protein supplement in human foods or animal feeds (6). Various natural products have been tested for the microbial production of SCP such as waste of food and industrial products became trending for cultivation of microorganisms. Various raw materials have been considered as substrate (carbon and energy sources) for SCP production (6).

Agriculture is usually the main activity for food production, but the increasing human population has increased the food demand several folds (7). Dietary proteins are usually obtained from plant and animal sources, containing limited amount of essential amino acids (8). There is also massive shortage of conventional protein on global level (9). The plant-based proteins need enough water and land resources to meet global protein supply. As a result of this, efforts have been intensified to identify alternative proteins sources (9). Thus, an increase in human population forced researchers to look for alternative protein rich feed and foods sources (10). Therefore, alternative protein sources and approaches are needed in order to enhance sustainability and food security. In this scenario, the single cell protein production seems a promising sustainable solution, considered as an alternative protein rich source for human and animal purpose. They contain essential amino acids, nucleic acids, lipids, minerals and vitamins (11). SCPs are among best replacement to conventional protein sources (6). Recent studies have highlighted the scope of food and agricultural waste for SCP production (6). Thus, the use of such organic waste decreases environmental pollution while producing valuable bioprotein from microbial systems. There are number of associated benefits of SCPs such as requiring less time and rapid production in comparison to crop and animal farming. The conventional crop farming requires massive land and water resources (12). The SCP production in bioreactors, gives high yield due to lack of competitive pests and weeds. SCP production is an ecofriendly way without harming environment and cause less climate change (13). Additionally, food sector waste is difficult to dispose hence can be used for this noble purpose of SCP production (14). This way, food waste management to valuable conversion is achieved. There are diverse microbial strains and species used for SCP purpose, having specific food and agriculture waste target as a substrate. It implies that if SCP is used for human consumption, the substrate selected for specific SCP strains must comply food safety regulations (15). It is necessary to search for other potential microbial strains in order to find best possible SCP sources for better food safety and security.

The purpose of this review is to discuss the potential microbial strains of fungi, yeast and bacteria which produce large quantity of proteins for animal feed and human food purpose. For conducting this study, the specific key words were searched on web of Science, google scholar, ScienceDirect and Scopus as a methodological approach for literature survey. This study provides detailed account on the utilization of organic waste as microbial nutrient sources for SCP production. In the last section, the nutritional benefits and associated challenges of single cell protein production are discussed.

HISTORY OF SINGLE CELL PROTEINS (SCP)

From centuries, so many organisms have been in regular use as a source of food. It has been found that SCP based organisms proved to be lifesaving food source in poor areas where the population faces malnutrition issues. *Spirulina* is one of the best examples, being grown in African Lake Chad for compensating the protein shortfall for local communities (16). The Germans used fungal species of *Candida* Genus as a source of food during World War I. From that time, variety of fungal, bacterial and yeast species were cultured, identified and used as a source of proteins. The SCP concept arose from these methods and are now widely in use (17).

PRODUCTION PROCESS

The SCP is produced using variety of substrates such as methanol, ethanol, alkanes, acetic acid, simple sugars, starch, cellulose, whey, molasses, milk and cheap fruit wastes (18). Suitable waste is selected on the basis of its availability, low cost and requirement of oxygen for fermentation process (18). The bacterial, fungal and yeast organisms use potential substrate as a source of growth in order to increase the mass of cells and accumulate good percent of proteins (SCP) via fermentation as main process (19) as expressed in Fig. 1.

Furthermore, the biomass produced from the process, is gently harvested upon completion of the fermentation process which is then used as a source of proteins for feed and food purpose (20). As a matter of fact, due to increasing population, the demand of protein is increasing, however, the current sources in the form of livestock and dairy products seem unsustainable hence new venues are in quite need to be exploited for meeting the protein demands for human and animals (21). Additionally, the SCP is considered a potential side product along with sequential extraction of other bioproducts from selected biomass for lowering the process cost, otherwise, several extraction processes seem unprofitable for investors. The residual biomass can be used as animal feed to make the process more profitable (22).

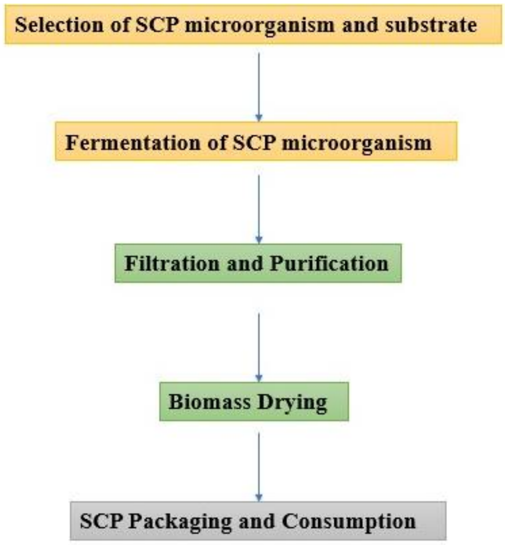

Fig. 1. Steps of SCP production from different microbial sources

POTENTIAL CARBON SUBSTRATES AND SCP ORGANISMS

There are several SCP sources such as bacteria, fungi and yeast.

BACTERIAL SCP AND SUBSTRATES

Bacterial SCP usually contains 50% to 80% proteins by dry weight (23) with smaller cell size, low density, high nucleic acid contents and rapid growth using a wide range of cost-effective substrates such as starch, methanol, ethanol and fruit and vegetable wastes (24) as given in Table I. Plant and food waste are mostly used as a source of carbon for the production of SCP, abundantly used sources is agri-waste from food industries (16). More interestingly, several bacterial species such as *Methylomonas methanica, Methyloccus capsulatus and Methylovibrio soehngenii* bio-utilizes methane gas for accumulation of high amount of proteins in their cells (25). Some bacterial species such as *Escherichia coli*, *Rhodopseudomonas palustris, Methylophilus methylotrophus,* and *Haloarcula sp.* have been found best choices for SCP purpose as given in Table I. The suitability of bacterial strains for SCP purpose must quality a certain criterion e.g. oxygen requirement, growth rate, better yield, heat tolerance, genetic and morphological stability and quantity of protein at the end of the fermentation process (26).

Furthermore, Øverland *et al.,*(2010) grown Ralstonia sp., *Methylococcus capsulatus, Brevibacillus agri and Aneurinibacillus sp* (24). on methane gas as a source of carbon and found that *Methylococcus capsulatus* produced around 65-73% proteins as dry cell weight. Similarly, the Imperial Chemical Industries made SCP (Pruteen) animal feed from methanol consuming bacterial strain (*Methylophilus methylotrophus)* which was found to contain about 70% protein content (27). In other studies, the photosynthetic purple non-sulfur bacterial strains such as *Rhodocyclus sp*, *Rhodopseudomonas sp*, and *Rhodobacter sp.* were cultivated on heavy metals containing wastewater with a total yield of 70-72% protein content. The mentioned strains were found to have tremendous tolerance against Mercury, Chromium and other heavy metals present in wastewater (28). Amino acid composition analysis found the presence of all AAs in the samples (23, 29). Kantachote *et al.,* (2005) obtained 67% proteins by fermenting latex-rubber sheet-based wastewater using *Rhodopseudomonas blastica* (30). Ponsano *et al.,* (2003) successfully obtained 67% single cell proteins cultivating a bacterial species *Rhodocyclus gelatinosus* using slaughterhouse wastewater of poultry which was used as animal feed (31). Similarly, *Rhodopseudomonas palustris* was found to yield 65% SCP by cultivation on pineapple waste extract (32). The *Rhodobacter sphaeroides Z08* was cultivated on soybean wastewater and produced 52% SCP (33). By using glutamate malate medium, the *Rhodovulvum sulphidophilum* was observed to yield 16% SCP (34). About 58% SCP was obtained from *Rhodopseudomonas fulvum* and *Rhodopseudomona* using sugar industry wastewater (23). *Rhodobacter sphaeroides* P47 was observed to produce 50% SCP when cultivated in wastewater obtained from seafood processing industry. In addition, *Rhodobacter blasticus* and *Rhodopseudomonas palustris* peoduced 50% SCP using noodle industry wastewater (35). Recently, the Saejung and Thammaratana, cultured *Rhodopseudomonas sp.* CSK01 on urban wastewater and produced 60% SCP (36). Tuna condensate was found to produce 56% SCP from *Rhodocyclus gelatinosus R7* (37). Simulated wastewater was used for cultivation of *Rhodopseudomonas palustris* to produce 45% of SCP.

Upon using methane as a carbon source, the *Methylocapsa acidiphila* yielded 59% of SCP (38). Two of the methane consuming bacterial strains (*Methylophilus spp and Methylomonas spp*.) produced 41% extra proteins when cultivated on sewage sludge, with having essential amino acids in considerable quantity (21). The *Methylomonas* which is a widely commercialized SCP strain, reached the level of 69% SCP, cultivated with methane gas (39). *A. marina STW181* and *Rhodobacter sphaeroides SS15* were grown on shrimp feed substrate with a total yield of 46% and 53% SCP respectively (40). Furthermore, *Bacillus licheniformis* was grown on potato waste as a carbon source for the production of SCP on 32.8 °C optimal temperature with a 30% yield of protein content (41). In a recent research study, Kornochalert *et al.,* reported 65%, 3%, 8%, 14% and 10% proteins, fats, carbohydrates, ash and moisture contents respectively from *Rhodopseudomonas palustris* strain using pineapple fermented extract (32). Kunasundari *et al.,* (2013) used PHAs as a substrate for the enhanced biomass production of *Cupriavidus necator* for SCP purpose as a feed for rats (42) while Taran and Asadi, used *Haloarcula sp.* to bioutilize petrochemical wastewater for SCP production with an optimum yield of 76.4% (43). After defatting soybean, the obtained soybean hull has been used as cheap carbon source for the production of SCP from *Bacillus subtilis* TK8 and MR10 with a protein yield of 25% and 26% respectively (44). From literature, it is evident that bacterial strains are able to produce high amount of proteins for food and feed purpose however low biomass yield and high nucleic acid content make it a bit infeasible commercially.

Table I. Potential bacterial SCP strains and their nutrient substrate from organic waste

Bacterial Strains	SCP (%)	Substrate	Reference
Rhodopseudomonas sp.	50	Noodle industry Wastewater	(35)
Rhodopseudomonas palustris microbial community of Rhizospheric diazotrophs	55	wastewater from Brewery	(45)
Methylophilus methylotrophus	70	Methyl alcohol	(22)
Rhodobacter sphaeroides	52	Wastewater from Soyabean	(33)
Methilomonas.sp	56	sewage sludge supernatant and biomethane	(21)
Methilomonas.sp	69	Methane	(39)
Rhodopseudomonas blastica	67	Latex rubber Wastewater	(32)
Afifella marina STW181	46	Commercial shrimp feed	(40)
Methylophilus methylotrophus	81	Methanol	(24)
Rhodopseudomonas sp.	60	Municipal wastewater	(36)
Rhodocyclusg elatinosus	56	Tuna condensate	(37)
Methylophilus sp	24	Methane	(21)
Rhodovulvum sulphidophilum	15	Medium of Glutamate malate	(34)
Haloarcula sp. IRU1	76	Petrochemical wastewater	(43)
Metilococ capsulatus, Methylomonas and Methylophilus spp.	41	Liquid and Gaseous sewage products	(21)
Methylocapsa acidiphila	59	Methane gas	(38)
Cupriavidus necator	40–46	Synthetic growth medium	(42)
Rhodopseudomon palustris	65	Pineapple extract	(32)
Rhodocyclus gelatinosus	68	Wastewater from Poultry slaughterhouse	(31)
Bacillus subtilis sp	26	Soybean hull	(44)

Bacillus licheniformis	38	Potato starch processing waste	(41)
Methylococcus capsulatus	66–73	Methane gas	(24)

FUNGAL SCP AND SUBSTRATES

There are so many identified fungal species used for the purpose of SCP as given in Table II. Among these strains, the *Aspergillus niger*, *Fusarium venenatum* and *Pleurotus floria* are on the top of list due to presence of high percentage of proteins (46). The SCP fungal strains are observed to contain about 63% protein content. The fungal SCP qualifies the FAO standards for amino acids profile in human food. The fungal SCP is rich in having high amount of threonine and lysine, however poor in having the methionine and cysteine (sulfur containing amino acids) (6). It has been found that the fungal SCP is a rich source of vitamin B-complex. Fungi has high percentage of nucleic acid (7-10%) compared to algae (6, 47, 48). One of the drawbacks of fungi is the presence of higher nucleic acid content (49). Whey has been found a tremendous source of SCP production with enhanced quantity of sulfur containing amino acids by K. fragilis (50). The SCP from fungi is not only good source of protein but also provide essential nutrients such as vitamins and folic acid (51). In a recent study, Liu *et al.,* (2013) successfully transformed potato waste into SCP via two-step process obtained from starch processing industry (52). The waste fiber and starch were fermented by *Aspergillus niger* followed by next level fermentation by *Bacillus licheniformis* in wastewater. The main purpose was to enhance the quality of SCP with a protein yield of 28% for animal feed. More interestingly, Valentino *et al.,* (2016) used rice bran as a substrate for SCP synthesis by using nine fungal strains such as *Aspergillus ochraceus*, *Aspergillus flavus*, *Cladosporium cladosporioides*, *Aspergillus niger*, *Monascus ruber*, *Fusarium sp1,* Penicillium *citrinum*, Fusarium sp2 and *Fusarium semitectum* (53). The CPC (crude protein content) of the bioprocess was evaluated after every 20 days of the SSF process for SCP potential which indicated an increased level of protein of rice bran. The highest CPC was 10.6% from *A.niger* followed by *Aspergillus flavus* with 10.46%. Other species produced CPC in the range of 10.25% to 5.25%. The results indicated that investigated strains were good source SCP production (53). Ahmadi *et al.,* (2010) produced SCP by using wheat straw as carbon source with fungal strain *Pleurotus florida* and obtained 62% protein as a whole (54). Chiou *et al.,* (2001)obtained about 50% proteins by cultivating *Aspergillus niger* in waste liqueur (55). The deoiled rice bran was used to grow *Aspergillus oryzae* to enhance protein content to 23.3% followed by 23.5% on veg and fruit waste (46). Wiebe, successfully produced SCP by *Fusarium venenatum* using glucose as carbon source to reach a protein level of 44% (56). The Şişman *et al.,* (2013) obtained 34% SCP from *Trichoderma harzianum* using whey as substrate (57). De Gregorio *et al.,* (2002) were able to successfully produce 25% SCP from *Aspergillus niger* respectively, using lemon juice from its pulp) (58).

Table II. Fungal sources of SCP along-with their potential carbon substrates

Fungal source	SCP (%)	Substrate	Reference
Trichoderma harzianum	34%	Cheese whey filtrate	(57)
Monascus ruber	9%	Rice bran	(53)
Fusarium venenatum	44%	Glucose	(56)
Fusarium semitectum and sp1 and sp2	10%	Rice bran	(53)
Aspergillus oryzae	24%	Rice bran	(46)
Aspergillus ochraceus	10%	Rice bran	(53)
Aspergillus flavus	10%	Rice bran	(53)
Aspergillus niger	50%	Waste liquor	(55)
Aspergillus niger	25.6%	Citrus pulp	(58)
Aspergillus niger	38%	Potato starch processing waste	(41)
Penicillium citrinum	10%	Rice bran	(53)
Pleurotus florida	63%	Wheat straw	(54)
Aspergillus niger	11%	Rice bran	(53)
Cladosporium cladosporioides	10%	Rice bran	(53)

YEAST SCP AND SUBSTRATES

Yeast is a wonderful SCP source from long time due to tremendous nutritional quality. The Germans during World War 1 used *Candida utilis* in soups and sausages. Presently, it is used animal feed and aa a vegetarian seasoning agent (59). The Yeasts are quite larger in size than that of bacteria, as well as high malic acid and lysine, can grow in acidic environment and having low nucleic acid content (60). However, yeasts have lower SCP content (65%) in comparison to bacteria with about 80% SCP (6, 61). Furthermore, the presences of carcinogenic and toxic compounds such as citrinin, aflatoxins and zearalenone should also be kept in mind when tend to use yeasts and fungi as SCP source. Research shows that the main problem with yeast SCP is the presence of nucleic acid in large quantity and worse digestibility of cell walls (18, 61).

Various research studies have been conducted globally to evaluate the potential of yeasts as SCP source, as given in Table III. Aggelopoulos *et al.*, (2014) in their specific study, evaluated the potential of *Saccharomyces cerevisiae, Kefir sp*, and *Kluyveromyces marxianus* using brewer potato, orange waste and whey as a carbon source with a total protein yield of 23%, 39%, and 34% from *Kefir sp*, *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* respectively (62). The use of mixed culture of *Candida krusei* and *Kluyveromyces marxianus* were grown on whey in batch and continuous fermentation systems for quality enhancement of SCP with a total yield of protein content of 43% and 47.5% in *Kluyveromyces marxianus* and *Candida krusei* respectively (63). Pineapple juice was used to cultivate *S. cerevisiae* with a yield of 48% bioproteins (64).

Candida tropicalis effectively utilized molasses to produce SCP with a yield of 56.4% protein content hence it was found that this strain is a potential candidate for SCP purpose if cultivated on molasses (65). Jalasutram *et al.*, (2013) used poultry litter for production of 48% SCP from *Candida utilis* (66). Another study shows the use of sugarcane bagasse as a substrate for SCP production from *Candida tropic* with a total yield of 31% proteins containing all essential amino acids (67). Capsicum powder medium has proven to be an effective carbon sources containing sufficient nutrients, was used to cultivate *Candida utilis* for SCP synthesis. The strain yielded about 29% SCP for animal feed purpose (68). Liu *et al.*, (2014) used potato starch industry waste and its wastewater into SCP in a motivation to reduce environmental pollution (52). The *Candida utilis* effectively converted the potato waste and wastewater to 46% and 49% SCP respectively (69). *P. kudriavzevii* has been investigated with using biogas slurry as nutrient source with a yield of 39.4% protein content (70).

Hashem *et al.*, used partially spoiled dates as a source of carbon for production of SCP from two selected yeast strains Zygosaccharomyces *rouxii* and *Hanseniaspora uvarum* (71). It was seen that both the strains produced 49 g/L SCP when grown for 60 h. *Y. lipolytica* successfully produced 12.6% proteins, cultivated in waste cooking oil (72). Furthermore, the cheese whey was fermented to SCP by *Kefir* microorganisms with a yield of 54% (73). Cui *et al.*, (2011) investigated the potential of insulin containing stuff to produce 54% SCP from *Yarrowia lipolytica* yeast (74). The *Debaryomyces hansenii*, was cultivated on brewer spent grain to produce SCP with an optimum yield of 32% as a whole (75). The *S. cerevisiae* yielded 68.8% proteins upon cultivation in date juice (76). Most recent study show that olive fruit waste was found to trigger the accumulation of 70% proteins in *C. lypolytica* species (77). On the whole, yeast can be a major source of protein if cultivated on relatively cheap carbon source.

Table III. Yeast sources of SCP along-with their potential carbon sources

Yeast	SCP (%)	Substrate	Reference
Candida krusei	48	Whey	(63)
C. lypolytica	70	Olive fruit waste	(77)
Candida tropicalis	56	Molasses	(65)
Saccharomyces cerevisiae	39	Brewer's spent grain and Orange pulp	(62)
P. kudriavzevii	39.4	Biogas slurry	(70)
Candida utilis	29	Capsicum powder waste	(68)
Candida utilis	48	Poultry litter	(66)
Candida tropicalis	31	Cane Bagasse	(67)
S. cerevisiae	68.8	Date juice	(76)

Hanseniaspora uvarum	49	Spoiled date palm fruit	(71)
Candida utilis	49	Wastewater from potato industry	(69)
Kefir sp.	54	Whey	(73)
Kluyveromyces marxianus	43	Whey	(63)
Debaryomyces hansenii	32	Spent grains from brewing industry	(75)
N rattus	35.9	Biogas slurry	(78)
Kefir sp.	23	Potato pulp, molasses, Orange pulp, whey and brewer's spent grain	(62)
Y. lipolytica	12.6	Waste cooking oil	(72)
Candida utilis	46	Potato starch industry waste	(52)
Zygosaccharomyces rouxi	49	Spoiled date palm fruit	(71)
Yarrowia lipolytica	48–54	Crude oil, Inulin and glycerol waste	(74)
Kluyveromyces marxianus	34	Molasses, Orange pulp, whey, brewer's spent grain and potato pulp	(62)
S. cerevisiae	48.3	Fruit waste (pineapple)	(64)

NUTRITIONAL BENEFITS OF SCP

Wu *et al.,* found that human protein demand is 35% from animal source while the rest 65% is plant based on global level (79). Predictive statistics says that per capita meat intake will rise to 51.5 kg in 2050 compared to 40 kg in 2013. Due to this reason the global meat demand and production may increase to 494 million tons in comparison to 288.0 million tons of 2013. In this scenario, the alternate SCP maybe an excellent solution for global protein demand. The reason is its easy production process, lower production cost and better nutritional value (9).

The main aim of SCP production is to find out a protein substitute of meat in order to cope the hunger and food scarcity problem worldwide. It is worth mentioning that the potential SCP sources must be of high nutritional value in order to qualify the criteria of human food and animal feed in terms of having good amino acid profile, safe to consume and better digestibility (80).

Finnigan *et al.,* (2024) analyzed the micro and macro nutrients of SCP in terms of beta carotene, vitamin A, carbohydrate content, lipids, proteins, folic acid, biotin, B12, thiamine and riboflavin etc. (81). Additionally, the nutritional value of SCP also depends on the chemical composition (14). The proteins of microbes usually contain all the essential amino acids for specific use. The microbial SCP is advantageous because the bacteria and yeasts can multiply fast in just 5 to 20 min time while molds can double its population in 2 to 4 h. The composition of SCP amino acids closely resembles that of fish proteins while the protein of yeasts resembles soy proteins. However, it has been found that the microbial SCP is deficient in having sulfur containing amino acids (cysteine and methionine) whereas, containing high level of lysine. Additionally, microbial SCP is a rich source of vitamin B12, mostly thiamine, pyridoxine, folic acid, riboflavin, niacin, biotin and amino benzoic acid (5). As a matter of fact, the growth rate of majority of microorganisms is very fast with a good biomass yield (6). The whole biomass of a microbe can be used as a protein source compared to animal and plant proteins, which is usually not entirely used. Similarly, the microbial SCP contains large quantity of proteins (up-to 70%) in comparison to animal and plant proteins (12). Furthermore, the microbial proteins have tremendous amino acid profile, making it nutritionally superior to animal and plant-based proteins (82). Some SCP organisms also produce certain number of vitamins which cannot be produced by host individual. SCP production needs less water content in comparison to plant sources (83). Microbial SCP can tolerate the climatic and environmental changes better than plants hence can be grown round the year.

The yeast-based SCP is in use for aquaculture feed due to its tremendous nutritional profile and being produced cost effectively on commercial scale (84). Some yeast species also play a role as probiotics such as *Debaryomyces hansenii* and *Saccharomyces cerevisiae* for survival of host individual (85, 86). Compared to vegetable sources, the microbial SCP tend to contain large amount of riboflavin, folic acid, carotene and thiamine. *Spirulina* and *Chlorella* species produce vitamin B12 in considerable quantity (87). The SCP process has some challenges, for example the need of low-cost raw materials, protein quality and the ease of difficulty in production process. The SCP usually contains 16% nucleic acid (47) which is quite high for human consumption as human nutrition must contain it in the range of 2%. The excess quantity of purines upon breakdown cause increased uric acid level which as a result leads to kidney stones and gout situation in consumer (6). Another challenge is the taste and aroma of SCP. The consumable SCP needs to be conditioned by aroma and taste which make the production cost a bit higher while making the process least efficient (10). Some SCP causes specific allergic reactions in consumers due to their sensitive digestive system (22). Furthermore, the raw materials used as a carbon source may have unknown chemical substances which create health problems (10).

CHALLENGES AND FUTURE DIRECTIONS

There are several documented challenges in the microbial SCP production, though promising source of proteins but scaling-up from lab scale to full production scale have shown challenges (88). The purity of product, optimal fermentation parameters, and economic feasibility are among the key changes faced. Additionally, the commercial scale production needs massive investments in development of technology, infrastructure and optimal process (89). It is important to realize the proper management to enhance the potential of SCP production for benefits on global scale (90). There is a need of regulatory framework to monitor the SCP production and consumption in a safe way, ensuring the product quality standards. In case of animal feed, strict regulatory guidelines for nutritional standards are essential to ensure animal health (91). It is needful to establish certain limits for possible contaminants such as mycotoxins, undesired microbes and heavy metals in the animal feed SCP (92). Similar approach must be adopted in case of SCP for human food in order to exclude the possibility of any potential allergens, posing threat to human health (93). The presence of high concentration of nucleic acid in SCP source is another big challenge (94). Elevated nucleic acid concentration may increase the uric acid level in body accompanied by kidney stone formation (95). This increased level of uric acid may also cause hyperuricemia (96). It is necessary to conduct proper analytical assays for quantification of nucleic acid content in the SCP source organisms coupled with animal-based trials and other clinical studies to analyze the effect of SCP on uric acid level, overall kidney function and stone formation etc. Furthermore, in future, the required epidemiological studies must be conducted on novel SCP source for further insights into the impacts on masses health and cellular mechanisms in order to understand the potential risks and overall safety profile (97). There is a risk of gastro and skin infections from active microbes present in SCP source which may cause clinical complications in the consumers hence need to be analyzed properly in future research (98).

The production of toxic substances such as mycotoxins and related cellular toxins are of great concern in the context of SCP based organisms hence further research must be conducted for identification and careful selection of microorganism which is inevitable to overcome potential risks. In some cases, the SCP may cause indigestion problems coupled with production of carcinogenic compounds during processing stage. This needs proper research evaluation to minimize the risks. The SCP for food and feed should be analyzed for absence of potentially toxic substances, present in the substrates or produced by SCP organism during the production process (99). The SCP consumption may also cause allergic reactions and gastrointestinal disorders which need to be assessed and assayed before utilization of such products to ensure proper regulatory measures (100). Furthermore, high yielding, nonpathogenic and genetically improved microbes must be identified for SCP production in order to cope the global malnutrition problem (101, 102).

Another subject of interest is the use of genetic manipulation of microorganisms for the production of SCP. Several studies have worked with modified microorganisms for the production of SCP. For example, Cernak *et al.*, (2018) used the CRISPR-Cas9 technique in the yeast *Kluyveromyces marxianus* to impart the ability to produce lipids and acquire thermotolerance, opening future research for the use of this yeast on an industrial scale in food production (103). Genetic and biosynthetic manipulation of SCP microbes may lead to enhanced production of other bioactive and nutritional compounds which are not present naturally (104). A SCP-based fishmeal has been produced from genetically modified *Yarrowia lipolytica* yeast for accumulation of increased level of astaxanthin which is positive improvement in the nutritional value (105). However, it needs regulatory mechanism to avoid any negative impacts of genetic manipulation. In case if the genetically modified organism is part of the final consumable product, strict and stringent regulations are necessary on global level for its safety (106). In future, the abundantly found, lignocellulosic material seems better choice as an alternative substrate for SCP fermentation process which is currently not in use on commercial scale (107). However, safety of the lignocellulosic raw material must be assessed first hence the regulations are needed to ensure the absolute safety of such raw material for SCP production (22). Similarly, the health and safety aspects of mycoproteins must also be evaluated at all levels (4). On the whole, research studies must be conducted about the composition of SCP organisms and safety measures need to be taken for well-being of the human society.

CONCLUSION

An interest in production of SCP by using cost-effective carbon sources is continuously increasing. The utilization of different food and agricultural waste streams can produce microbial SCP in sustainable and ecofriendly way. The use of efficient microbes for SCP production can be accompanied with accumulation of polyunsaturated fatty acids and other important nutraceuticals. It is necessary to identify potential SCP microbes with decreased amount of nucleic acids. The composition analysis and safety regulation need further research for each SCP source for sustainable food system. The future research regarding genetic manipulation of specific bacterial and yeast SCP organisms would enhance their nutritional and safety value. It is also necessary to identify other SCP strains with better biomass yield in order to fulfill the protein needs of human population. On the whole, utilization of renewable feedstocks for production of SCP from microbial systems could meet the proteins needs of human and animals alongside managing the hazardous and CO_2 emitting agricultural and food wastes.

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