

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/346732659>

# Single cell protein: Sources, mechanism of production, nutritional value and its uses in aquaculture nutrition

Article in *Aquaculture* · January 2021

DOI: 10.1016/j.aquaculture.2020.735885

CITATIONS

74

READS

8,562

6 authors, including:



**Muhammad Sharif**

University of Agriculture Faisalabad

25 PUBLICATIONS 176 CITATIONS

[SEE PROFILE](#)



**Amjad Islam Aqib**

Cholistan University of Veterinary and Animal Sciences- Bahawalpur- Pakistan

114 PUBLICATIONS 544 CITATIONS

[SEE PROFILE](#)



**Muhammad Saeed**

Cholistan University of Veterinary and Animal Sciences

154 PUBLICATIONS 2,169 CITATIONS

[SEE PROFILE](#)

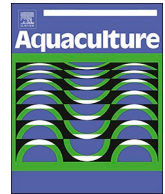
Some of the authors of this publication are also working on these related projects:



SARS-CoV-2 (COVID-19) Pandemic [View project](#)



Natural Products in Poultry [View project](#)



## Review

## Single cell protein: Sources, mechanism of production, nutritional value and its uses in aquaculture nutrition

Muhammad Sharif<sup>a</sup>, Muhammad Hammad Zafar<sup>a</sup>, Amjad Islam Aqib<sup>b,\*</sup>, Muhammad Saeed<sup>b</sup>, Mayada R. Farag<sup>c</sup>, Mahmoud Alagawany<sup>d,\*</sup>

<sup>a</sup> Institute of Animal and Dairy Sciences, University of Agriculture, Faisalabad, Pakistan

<sup>b</sup> Cholistan University of Veterinary and Animal Sciences, Bahawalpur 63100, Pakistan

<sup>c</sup> Forensic Medicine and Toxicology Department, Veterinary Medicine Faculty, Zagazig University, Zagazig 44511, Egypt

<sup>d</sup> Department of Poultry, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt

## ARTICLE INFO

## Keywords:

Single cell protein

Sources

Mechanism of production

Nutritional benefits

## ABSTRACT

Single cell protein (SCP) is a bulk of dried cells which can also termed as bioprotein, microbial protein or biomass. SCP is produced by the microorganisms such as algae, yeast, fungi and bacteria, however, fungi and bacteria are the major producers of this protein. High production of proteins from these sources was mainly due to their fast growth rate and relatively higher protein level in their chemical structure. Some algal species were also used for this purpose which specifically cultivated in the aquatic medium. In addition to high content of protein, SCP also contains carbohydrates, nucleic acids, fats, minerals and vitamins. Additionally, SCP has a high level of essential amino acids such as lysine, methionine, and threonine. This source of protein (SCP) has been proved a good replacement of other expensive protein sources like fish and soybean meals. Therefore, conclusion can be made that SCP can easily replace traditional (plant and animal) protein sources in human, animal as well as fish diets without any detrimental effect. Finally, in this review, we focus on new feeding trials on SCP in some aquaculture species, such as Atlantic salmon, white leg shrimp and rainbow trout.

## 1. Introduction

Fish are an important source of dietary protein for humans, providing more than 3.1 billion people with about 20% of their average per capita animal protein intake (Abd El-Hack et al., 2016; FAO, 2016; Alagawany et al., 2020a, 2020b, 2020c). Production of nutritious and harmless compounds from different biodegradable waste is one of the basic principles in waste management. In ancient times, wastes were treated with various chemicals for their decomposition but this method was not satisfactory for better disposal (Kost et al., 2013). Now, it is possible to convert various wastes into valuable food or feed products for humans and animals which is not only an environment friendly way but also a healthy business activity (Browne et al., 2011). Many types of wastes are still adding pollution to the environment or being processed into low quality products such as biogas, biofuels and bioenergy (Lipinsky, 1981). Different methods and techniques are emerging and providing opportunity to develop high quality products such as single cell oil, single cell protein, chemicals, enzymes and many others. Single cell protein is one of these high quality dietary products obtained from wastes (El-Bakry et al., 2015; de Finco et al., 2017; FitzPatrick et al.,

2010; Werpy et al., 2004).

Single cell protein (SCP) is a bulk of dried cells (biomass) which is produced by algae, yeast, bacteria and fungi. It can also be termed as bioprotein, microbial protein or biomass (Saeed et al., 2016). These microbes can be utilized as protein rich supplements or ingredients in humans and animals' diet (Ritala et al., 2017). Single cell protein can be a good alternative to plant protein sources as they do not require large area of land or large reservoirs of water for its production (Mekonnen and Hoekstra, 2014). Unlike plant sources, their production is also independent of seasonal and climatic variations and it can be produced throughout the year. Moreover, they do not emit greenhouse gasses to the environment as in the case of plant protein sources (Vermeulen et al., 2012).

To reduce the production cost of single cell proteins, the most important thing is the selection of cheap and suitable substrates or biodegradable agro-industrial byproducts as a nutrient source for the microorganisms to grow and produce tons of protein (Anupama and Ravindra, 2000). For this purpose, different substrates were used and compared in the past. Some of the commonly used substrates are apple pomace, yam peels, citrus pulp, potato peels, pineapple waste, papaya

\* Corresponding authors.

E-mail addresses: [amjadwaseer@cuvas.edu.pk](mailto:amjadwaseer@cuvas.edu.pk) (A.I. Aqib), [mmalagawany@zu.edu.eg](mailto:mmalagawany@zu.edu.eg) (M. Alagawany).

<https://doi.org/10.1016/j.aquaculture.2020.735885>

Received 27 July 2020; Received in revised form 20 August 2020; Accepted 24 August 2020

Available online 11 September 2020

0044-8486/ © 2020 Elsevier B.V. All rights reserved.

waste etc. (Nasseri et al., 2011). It is also very important to choose suitable waste product for the proliferation of single cell protein producing microorganism. Nowadays, for both research and industrial purpose, availability of microorganisms is not an issue as many strains of bacteria, algae, fungi and yeast can be cultured in the laboratory by different ways. Mainly, microorganisms do not depend on substrate properties with exception of few. However, substrate availability is very limited regarding the consumer and concerned economy (Ritala et al., 2017). In this review, different points such as production of single cell protein, mechanism of production, methods of production, nutritional values, beneficial uses in fish nutrition, and criteria for microbe's selection and available substrates will be discussed.

## 2. Single cell protein

The word single cell protein (SCP) is considered to be the most appropriate term as it is produced from single celled organisms (Jamal et al., 2008). It was found that yeast is capable of producing about 250 tons of protein in 24 h (Aggelopoulos et al., 2014).

The term of SCP was introduced for the first time in 1968 when scientists were together in a meeting to discover the best alternate of the terminologies which were used in common practice i.e. microbial protein at Massachusetts Institute of Technology, United States (Paraskevopoulou et al., 2003). Single cell protein is produced by various microbial species which include algae, bacteria, fungi and yeast. Among them, fungi and bacteria are the major producers of that protein (Anupama and Ravindra, 2000) as they characterised by fast rate of growth and higher protein content (Gao et al., 2007). Some algal species that are cultivated specifically in the aquatic media were also used for this purpose (Votolina et al., 2005).

In addition to high protein content which is 60–82% on dry matter basis, SCP also consists of carbohydrates, nucleic acids, fats, minerals and vitamins (Jacob-Lopes et al., 2006). Another advantage associated with SCP is that it is rich in various essential amino acids such as lysine, methionine which are not present in sufficient amounts in most animal and plant sources (Gao et al., 2012). SCP has been reported to be a good replacement of expensive protein sources like fish meal and soybean meal (Goldberg, 2013). Protein presents in appreciable amounts in microbial cells which are formed from different sources of inorganic nitrogen such as ammonia (Nasseri et al., 2011) and presents also in algae (Ghasemi et al., 2011). The use of different organic sources such as waste products from industry and agriculture is also very common for SCP production (Gervasi et al., 2018).

## 3. Sources for single cell protein

### 3.1. Bacterial sources

Generation time of bacteria is very short as their cell masses multiply quickly within 20 to 120 min (Bamberg, 2000). They also have the ability of growing on wide range of raw materials (Øverland et al., 2010) and edible substrates like starches and sugars (Suman et al., 2015). Bacteria can easily multiply on wastes of organic matter and petrochemicals i.e. ethanol, methanol and nitrogen. They can also multiply in natural water supplemented with minerals and nutrients which are helpful to fulfill deficiency of nutrients needed for their growth. Some bacteria like *Methylophilus* spp. has a very short generation time of 2 h and is also a useful constituent of animal feed. Moreover, their protein is chemically better than any yeast or fungi (Majekodunmi et al., 2011). List of substrates for various bacterial species is illustrated in Table 1.

Numerous bacterial species are present which have been included in animal feed since long time and have the ability of producing SCP in large quantities like *Brevibacterium*, *Methylophilus methylitropous*, *Bacillus megaterium*, *Acinetobacter calcoaceticus*, *Acromobacter delvace*, *Aeromonas hydrophilla*, *Cellulomonas* spp. *Bacillus subtilis*,

**Table 1**

List of substrates for various bacterial species.<sup>1</sup>

Substrates for bacteria to produce SCP	
Bacteria	Substrates
Various bacterial species	Waste of fruit processing
<i>Methylococcaceae</i> family	C-1 compounds
<i>Bacillus cereus</i>	Ram horn
<i>Rhodopseudomonas gelatinosus</i>	Wheat bran
<i>Methylomonas</i> species	Methane broth
<i>Brevibacterium</i> spp.	C-1 to C-4 compounds
<i>Ralstonia</i> species	Natural gas
<i>Bacillus licheniformis</i>	Potato waste
<i>Streptomyces</i> species	Methanol
<i>Corynebacterium ammoniagenes</i>	Fructose and Glucose
<i>Escherichia coli</i>	Ram horn
<i>Cellulomonas</i> species	Agro-industrial wastes
<i>Corynebacterium glutamicum</i>	Glucose
<i>Methanomonas methanica</i>	Methane
<i>Cupriavidus necator</i>	Synthetic growth media
<i>Methylophilus methanotrophus</i>	Methanol
<i>Bacillus pumilus</i>	Potato processing waste
<i>Rhodopseudomonas palustris</i>	Rubber waste
<i>Bacillus subtilis</i>	Ram horn
<i>Rhizospheric diazotrophs</i>	Brewery wastewater
<i>Pseudomonas fluorescens</i>	Animal waste & Manure

(Kadim et al., 2015; Lang et al., 1999; Mahasneh, 1997; Queiroz et al., 2007; Raja et al., 2008; Ravinder et al., 2003; Sousa et al., 2008; Turnbull et al., 1992; Ugalde and Castrillo, 2002; Zepka et al., 2008).

*Methylomonas methylotrophus*, *Thermomonospora fusca*, *Lactobacillus* spp. *Rhodopseudomonas capsulate*, *Flavobacterium* species and *Pseudomonas fluorescens* (Ashok et al., 2000; Dharumadurai et al., 2011; Piper, 2004).

### 3.2. Algal sources

Certain types of microalgae are cultivated for animal and human consumption and usually have healthy protein contents which can go up to 70%. Apart from the proteins, they are excellent sources of fats mainly omega-3 fatty acids, mineral salts, vitamins and chlorophyll (Sousa et al., 2008). However, they contain relatively low amount of nucleic acid content which ranges from 3% to 8% (Nasseri et al., 2011). List of substrates for various algal species is shown in Table 2.

An algal species named *spirulina* was harvested by some Mexican and African people near texcoco. It was dried to be used in the human diet. In different parts of the world, biomass of some other species such as *Chlorella* and *Senedessmus* has also been used as a feed source. High protein content, rapid growth, simple cultivation and a good usage of solar energy are the major advantages due to which they are widely accepted as feed ingredient throughout the world (Raja et al., 2008). Green algae are considered as a good antioxidant. Moreover, progenitor cells can be protected by using the diet having algal species named

**Table 2**

List of substrates for various algal species.

Substrates for algae to produce SCP	
Algae	Substrate
<i>Spirulina</i> species	Carbon dioxide
<i>Chlorella salina</i>	Alkaline waste effluent
<i>Caulerpa racemosa</i>	Carbon dioxide
<i>Spirulina maxima</i>	Sunlight and carbon dioxide
<i>Chlorella</i> species.	Carbon dioxide
<i>Sargassum</i>	Carbon dioxide and Sunlight
<i>Dunaliella</i>	Carbon dioxide and Sunlight
<i>Laminaria</i>	Carbon dioxide and Sunlight
Diatoms and <i>Chlorella</i>	Carbon dioxide and Sunlight
<i>Porphyra</i>	Carbon dioxide and Sunlight

(Anupama and Ravindra, 2000)

**Table 3**

List of substrates for various fungal species.

Fungal species	Substrates
<i>Aspergillus flavus</i>	Rice bran
<i>Aspergillus ochraceus</i>	Rice bran
<i>Saccharomyces cerevisiae</i>	Orange pulp, molasses, brewer's spent grain
<i>Yarrowia lipolytica</i>	Inulin, crude oil, glycerol waste hydrocarbons
<i>Aspergillus niger</i>	Apple pomace, Banana waste, Rice bran, Potato starch
<i>Trichoderma virideae</i>	Citrus pulp
<i>Aspergillus ochraceus</i>	Rice bran
<i>Trichoderma harzianum</i>	Cheese whey filtrate
<i>Penicillium citrinum</i>	Rice bran
<i>Aspergillus oryzae</i>	Rice bran
<i>Kluyveromyces marxianus</i>	Orange pulp, molasses, brewer's spent grain, whey, potato pulp
<i>Candida utilis</i>	Poultry litter; Waste capsicum powder
<i>Cladosporium cladosporioides</i>	Rice bran
<i>Monascus ruber</i>	Rice bran
<i>Candida tropicalis</i>	Molasses

(Aruna et al., 2017; Bhalla and Joshi, 1994; Bogdahn, 2015; Deise Maria Fontana et al., 2001; Ferreira et al., 2010; Guo et al., 2019a; Jay et al., 2005; Kam et al., 2012; Øverland et al., 2013; Øvrund Hansen et al., 2019; Paraskevopoulou et al., 2003; Prado-Rubio et al., 2010; Serrano et al., 2006; Singhania et al., 2008; Ugalde and Castrillo, 2002; Yousufi, 2012; Zhou et al., 2017)

*Spirulina maxima* along with nutraceuticals. They also have the ability to prevent fatty liver syndrome (Mahasneh, 1997).

### 3.3. Fungal sources

List of substrates for various fungal species is illustrated in Table 3. Many fungal species are used for the production of single cell proteins. Protein from the different fungal species is preferred over different sources due to its chemical composition and amino acid profile (Ravinder et al., 2003). Fungi contain 30% to 50% protein when they were cultivated mainly for the SCP production. Their amino acid profile also met the standards of FAO. Their protein is rich in lysine and threonine but deficient in cysteine and methionine as they are sulphur containing amino acids and mainly come from plant sources (Nasseri et al., 2011). However, the fungus *K. fragilis* has the ability to produce sulphur containing amino acids when grows on the whey (Ugalde and Castrillo, 2002).

Single cell protein obtained from the fungi could provide some other nutrients in addition to protein. These nutrients include different vitamins mainly from vitamin B-complex such as riboflavin, niacin, thiamine, biotin, pantothenic acid, choline, pyridoxine, glutathione, p-amino benzoic acid, streptogenin and folic acid (Turnbull et al., 1992). However, a decrease in insulin and blood glucose level was reported after feeding on mycoprotein obtained from *Fusarium venenatum* (Lang et al., 1999). Fungi have relatively high amount of nucleic acid content as compared to algae and it ranges from 7% to 10% (Nasseri et al., 2011).

A process termed as “Pekilo” was established in Finland to obtain single cell protein from the fungi to feed the animals. A filamentous fungus named *Paecilomyces varioti* was cultivated on different sugars like pentoses along with wood hydrolysates or waste from sulphites. Then the fermentation resulted in the production of SCP as a final product (Ugalde and Castrillo, 2002).

### 4. Mechanism of production

Actually, microorganisms utilize the available wastes and use them as growth medium to increase their cell masses which are made up of the SCP (Queiroz et al., 2007). Fermentation either submerged or solid in nature is the main process responsible for SCP production (Kadim

et al., 2015). After the completion of the fermentation process, the available biomass is harvested which can further be utilized as a protein source (Zepka et al., 2008). Then this source undergoes further processing techniques like purification, cell disruption, washing followed by protein extraction (John et al., 2011) to give generally high production rates along with better yield and makes the production control relatively easier.

Waste materials from the agricultural industry have been reported to be a good substrate for SCP production. When SCP produced by these wastes and microbes the resultant protein enriched product was of a good quality and economical value to be used in animal feed. Further processing makes it edible even for the human beings (Yunus et al., 2015). There are various microbes which are involved in the bio-conversion of different substrates and industrial wastes into the valuable products.

Jacob-Lopes et al. (Jacob-Lopes et al., 2006) used cyanobacterium for the production of SCP. Nasseri et al. (Nasseri et al., 2011) used different substrates for the production of SCP and found that substrates with carbon skeleton were very useful for this purpose. Moreover, cellulose and hemicellulose also have been proven to be useful substrates as well. Similarly, (Ashok et al., 2000) also found that the fermentation of different substrates like hemicellulose and cellulose from the plants nail, hair, feather and different nitrogenous compounds from animals could be useful substrates for the SCP production following different hydrolyzation methods i.e. physical, chemical and enzymatic.

### 5. Criteria for the selection of microorganism

The fast growing microorganisms are required for getting massive output (biomass weight produced per unit time). However, high output produces more quantities of RNA in the cell, RNA production is not desirable as it acts as anti-nutritional factor in the final product. Biomass yield coefficient is the total weight of newly produced cells per unit of consumed substrate (Waldron and Lacroute, 1975). This condition is very useful especially when substrate is expensive (Boehlke and Friesen, 1975). If the growth rate is slow, more amount of substrate will be consumed with less output and this will affect the yield coefficient adversely (Leuenberger, 1972).

Different organisms have different tolerant levels against heat shock. When cells from different microbes are subjected to the heat shock, they develop resistance to different stresses. This is lethal in normal conditions but a phenomenon is adopted by the cells called acquired stress tolerance. Stress tolerance can be developed by application of different treatments like chemicals or heat. Application of these treatments also leads to the production of a special type of protein termed as heat shock proteins (Tamura et al., 1998). A mild type of heat shock at 37 °C for 30 min develops oxidative stress in both types of cells i.e. aerobic and anaerobic. Sensitivity to both oxidative and heat stress is totally dependent on lipid composition of the membrane (Steels et al., 1994). Every organism has different ability to cope with the increase in temperature which occurs during exothermic fermentation. This also reduces the needs for cooling. The ability of a microorganism to show its growing potential at a particular temperature overcomes the need for heating or cooling. Wider tolerable temperature range for the microorganisms lessens the need of temperature control during the fermentation process.

The pH of growth medium also tends to change during process of fermentation. In most of the cases, buffer is added to the media along with the association of the fermenter with pH control. However, ability of microbes to tolerate a wide range of pH can overcome the need of pH control. Fungi usually require lower pH than bacteria for their growth. Therefore, bacterial growth can be inhibited during cultivation by maintaining low pH in the media. So, by adopting these procedures, different costs regarding sterilization and aseptic measures can be minimized (Serrano et al., 2006).

## 6. Substrates for single cell protein production

Substrates are selected on the basis of their abundance in the proximity of the production area. Design and strategy of production process also depend on the nature of substrate. Carbohydrate derived sources with carbon as essential components are widely used for this purpose (Nasseri et al., 2011). The reason for using these substrates is due to microbial dependence on the monosaccharides and disaccharides for their multiplication which serve as building blocks of substrates. Moreover, carbohydrates are easily available and widely distributed in nature and have high energy value. So, the abundance of these substances makes them a good choice for substrate (Ugalde and Castrillo, 2002).

Many other substrates such as molasses, whey, starch, alkanes and hydrocarbons are also used for this purpose. Some microorganisms have some additional growth and maintenance requirements like vitamins and minerals which should be properly fulfilled to obtain maximum output (Jay et al., 2005).

Limitations regarding photosynthetic and non-photosynthetic microorganisms do not exist for SCP production as all types of microbes can take part in this process. Microorganisms require nitrogen for their multiplication and production of SCP. Different sources of nitrogen like ammonia, ammonium salt or nitrate are consumed by microbes as nitrogen source (Ugalde and Castrillo, 2002). Proper carbon to nitrogen ratio should be also maintained in the growth media. Ratio of 10:1 is reported as the most appropriate results as the same ratio presents in the microorganism. However, this ratio is different among different microbes. Higher ratio will cause disappearance of ammonia before all sugars are consumed and the required biomass will not be obtained. While at the ratio of 1:1, most of the ammonia cannot enter the cells and will be wasted (Bamberg, 2000).

Cost of the substrates used in production of SCP usually represents 45–75% of the total cost of production. Major portion of the cost is taken by the carbon source while ammonia contributes only 7–15% of the total substrate. Carbon dioxide present in the atmosphere is free, but high energy is required for agitation purpose. Agitation is helpful to dissolve it into dense cultures of algae (Kam et al., 2012). Different agricultural and industrial wastes are more abundance and relatively cheaper than the other sources. These wastes also represent 20–30% of the total production cost. However, solid wastes from the agriculture sector such as celluloses require special treatment before its usage for human beings and this is very expensive. Industrial wastes have high levels of biological oxygen demand which can cause environmental pollution if disposed without any treatment (Anupama and Ravindra, 2000).

Using industrial and agricultural wastes for SCP production of not only reduces the biological oxygen demand by almost 80% but also minimizes the treatment cost required for their disposal (Ravinder et al., 2003). Hydrocarbons along with their derivatives are also used as substrates and represent 30–70% of the total production cost. They are usually obtained from natural gas or oil which is non-renewable in nature and so expensive. Another drawback of using them as substrates is their use as petrochemicals and fuels and their usage is also politically controversial (Ritala et al., 2017).

## 7. Industrial production of SCP

Fermentation is the main process involved in the production of SCP (Fig. 1). Specific strain of microorganisms is selected according to the situation. Selected strains are grown and multiplied on suitable substrates in technical cultivation process. This leads to cultural growth and increase in the biomass which is then followed by the separation process. Next step is the development which begins with the screening of microbes in which suitable microbial strains are obtained from soil, air and water samples or from swabs of biological or inorganic materials. These are then optimized by mutation, selection or other genetic

protocols (Nasseri et al., 2011). Then the optimization of the conditions needed for proper cultivation is done followed by the determination of cell structures and metabolic pathways. Moreover, apparatus technology and process engineering are adapted to improve the technical performance of the whole phenomena. This will make the final product ready for use on large technical scale. Protection and safety of the innovation helps to overcome important aspects regarding product authorizations and operating. These are also required for the protection of the environment due to the use of living microorganisms in the whole process (Anupama and Ravindra, 2000).

## 8. Nutritional benefits of SCP

Nutritional value of SCP completely depends on its chemical composition from amino acids, nucleic acids minerals, enzymes and vitamins in addition to the high protein content which is relatively cheaper than other plant and animal sources (Bogdahn, 2015). It is reported that dried cells of *Pseudomonas* spp. grew on petroleum based liquid paraffin contain protein as high as 69%. Single cell protein obtained by algae processing is about 40%. Ferreira et al. (Ferreira et al., 2010) stated that protein obtained from microbes contains all essential amino acids depending on the type of substrate used (carbon or nitrogen source) and the type of microorganism grown on a specific media.

Microorganisms like bacteria and yeast have a very short multiplication time as they double their population in just 5–15 min. While, algae and mold species double themselves in 2–4 h. Amino acid profile of SCP from bacteria shows a close resemblance with fish protein. However, protein from yeast resembles to soya protein (Yousufi, 2012). In addition, SCP is reported to be deficient in sulphur containing amino acids such as methionine and cysteine whereas high levels of lysine and other amino acids have been observed. So, supplementation of these two amino acids is required for the use of SCP as a feed ingredient. Microorganisms normally contain Vitamin B12 in significant amounts. Bacteria and algae are reported with high vitamin B12 and vitamin A content respectively. Most common vitamins present in SCP are riboflavin, thiamine, pyridoxine, niacin choline, folic acid, pantothenic acid, biotin, para amino benzoic acid, inositol and B12 (Anupama and Ravindra, 2000).

Most microorganisms have very fast growth rate that yields high amount of biomass as result of their multiplication (algae: 3–6 h, bacteria: 30 min to 2 h, yeast: 40 min to 3 h) (Nasseri et al., 2011). These microorganisms can be used as a whole in contrast to most of the crops and animal protein sources which cannot be used entirely (Zhou et al., 2017). SCP produced from different microbes has high protein content (30–70%) as compared to different green plants and animal sources. Moreover, these proteins have excellent amino acids profile that makes them nutritionally more useful than conventional protein sources (Aruna et al., 2017).

Some microorganisms during their course of SCP production produce significant amounts of vitamins which cannot be produced by the host individual in appropriate amounts. Production of SCP also requires low water content as compared to the plant sources (Majekodunmi et al., 2011). Unlike plant protein sources, it is independent of environmental and climatic variation and can be produced throughout the year as microbes are available round the clock (Bhalla and Joshi, 1994).

## 9. Submerged fermentation

Submerged fermentation is the process in which substrate is always used in the liquid form containing all the necessary nutrients needed for their growth. Whole process is carried out in a fermenter which is operated in a continuous manner and the resultant biomass is harvested continuously using various techniques. Centrifugation or filtration of the final product is done followed by drying. Aeration is also important during whole cultivation operation as heat is generated in this process which needs to be removed with the time by a proper cooling



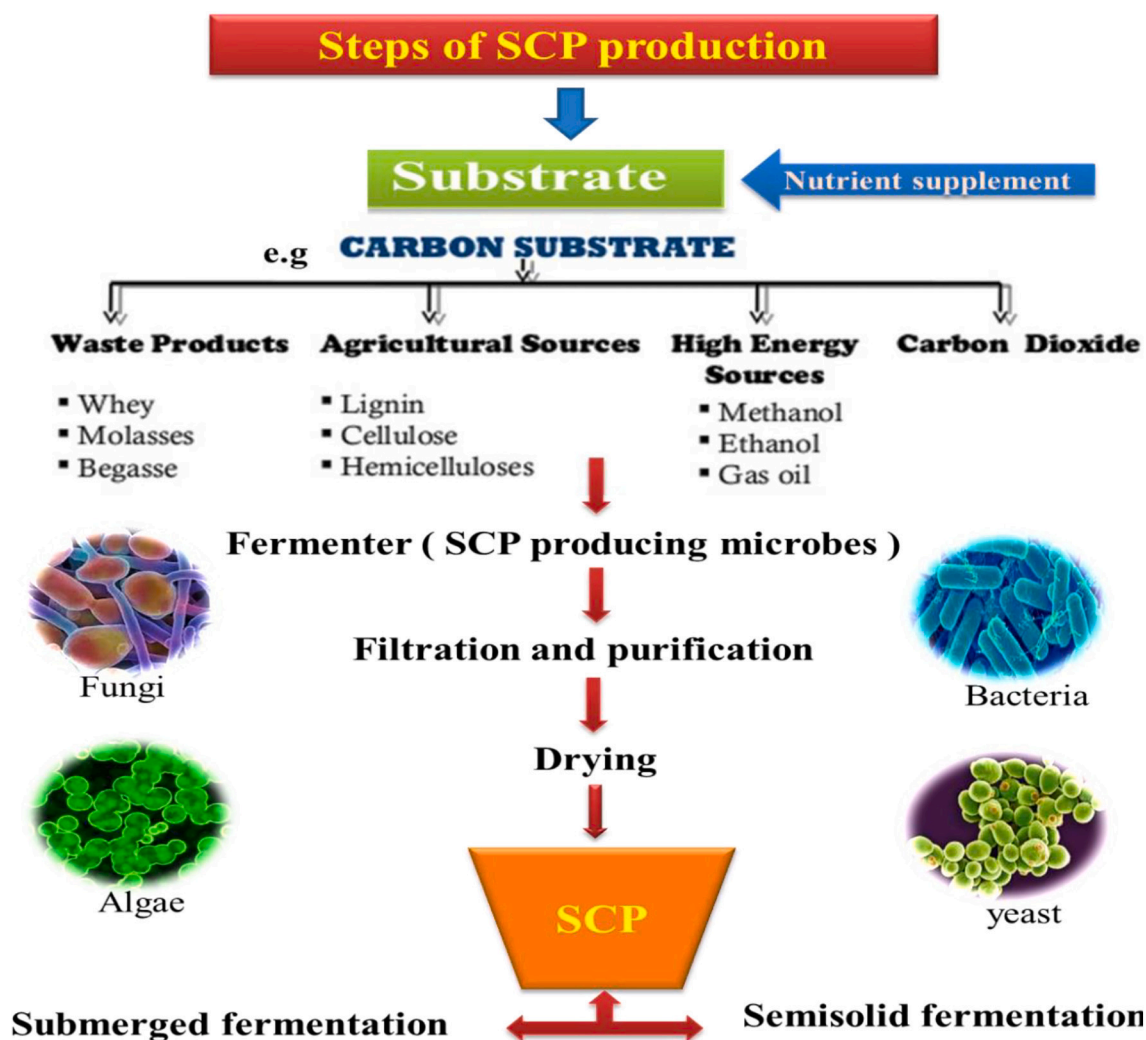


Fig. 1. General steps during industrial production of SCP.

mechanism. Different microorganisms require different methods for their recovery as bacteria can be recovered by centrifugation while filamentous fungi require filtration for their recovery. Recovery of maximum water content is desirable as it contains important soluble nutrients which become available after drying (Nasseri et al., 2011).

#### 10. Semisolid fermentation

In semisolid fermentation process, substrate preparation is an important factor as it is used in solid state. This type of fermentation requires high capital investment and operating cost. Cultivation is done through many operations like multiphase system mixing, stirring, transport of oxygen molecules from gas bubbles to microorganisms through the liquid phase and ultimately transfers heat from liquid phase to the surroundings. A specific type of bioreactor is designed for identification of energy and mass transportation phenomena termed as U-loop fermenter (Prado-Rubio et al., 2010).

Single cell protein production by semisolid fermentation involves some basic steps such as preparation of suitable media having a specific carbon source, proper sterilization of the media and the fermenter to avoid any microbial contamination, biomass production using desirable strain of a particular microorganism, separation of the final product and its processing. Different types of carbon sources are used for this purpose including gaseous hydrocarbons, n-alkenes, ethanol, methanol, carbon oxide, polysaccharides, molasses, effluents of various breweries and other solid compounds (Nasseri et al., 2011).

#### 11. Solid state fermentation

Solid state fermentation is carried out by using different types of fermenter designs, a variety of microorganisms and different environmental conditions during fermentation. Resultant products can be SCP, enzymes, feeds, organic acids, ethanol, pigments, B complex vitamins or flavors. The process requires substrate in pure solid form like wheat bran or rice bran. Selected substrate is spread on the flatbeds after inoculation with microorganisms. Proper moisture level (60–65%) is maintained to get optimum yield (Singhania et al., 2008). Microorganisms show their maximum growth potential in liquid culture medium. Continuous oxygen supply to microbes is maintained in liquid phase which is achieved by stirring the fermentation medium. Proper regulation of temperature, ionic strength, soluble oxygen, nutrients control and pH are the factors which help the synthesis of desired metabolites (Deise Maria Fontana et al., 2001).

#### 12. SCP feeding trial in fish

Several yeast meals have been studied such as *Candida utilis*, *S. cerevisiae*, and *K. marxianus* (Øverland et al., 2013; Øvrund Hansen et al., 2019). *Saccharomyces cerevisiae* was found to be a poor protein meal, while (Øverland et al., 2013) used 40% of *K. marxianus* and *C. utilis* instead of fishmeal in rations. For shrimp, several products of *S. cerevisiae* were used in diets (from 15 to 24%, depending on the product) to replace fishmeal or soybean meal (up to 24%) with no impact

on growth rate (Guo et al., 2019a, 2019b; Jin et al., 2018). Several different methanotroph-based SCP meals have been studied successfully on Atlantic salmon. Salmon fed rations contained 36% SCP (bacterial protein meal) displayed a higher feed efficiency ratio and growth rate than the control ration, but digestibility of nutrient was reduced. In another study, (Aas et al., 2006) stated that SCP (*Methylococcus capsulatus*) can make up to 38% of the dietary protein in trout rations and 52% in salmon rations without any adverse effect on growth performance. Interestingly, the use of SCP (*M. capsulatus*) in soybean meal (SBM) diets prevented the development of SBM-induced enteritis in salmon fish, suggesting further benefits of microbial protein products (Romarheim et al., 2011). KnipBio Meal (*Methylobacterium extorquens*) could be used up to 55% instead of fishmeal in salmon rations without adverse impacts on growth (Tlustý et al., 2017) and up to 10% instead of SBM in trout rations (Hardy et al., 2018). In shrimp, use of a mixture of two purple non-sulphur bacteria (1%) in the ration improved the growth than control (Chumpol et al., 2018). The SCP (*Corynebacterium ammoniagenes*) could be used up to 10–20% (Hamidoghli et al., 2019), and *Methylobacterium extorquens* SCP was found to be able to completely replace fishmeal in shrimp rations (Hardy et al., 2018). (Dantas Jr et al., 2016) used a biofloc meal up to 30% in shrimp diets instead of fishmeal. Effect of using some sources of SCP in fish feeding is illustrated in Fig. 2.

### 13. Challenges of using SCP

Apart from having very attractive characteristics, SCP also has some limitations to be included in humans or animal diet. Main anti-nutritional factor is the high concentration of nucleic acid which is more abundant in SCP than other conventional protein sources. This high nucleic acid content enhances the uric acid in the serum that ultimately leads to the formation kidney stones. Most of the nitrogen (70–80%) presents in the form of amino acids while rest is in the form of nucleic acids which is a key property of the fast growing microorganisms. Moreover, it also consists of cell wall which is non-digestible in case of simple stomach animals and birds. It is further reported that live cells of

microbes should become inactive before consumption due to development of unpleasant color and taste. If unprocessed product is used before killing the active microbes then incidence of skin and gastrointestinal infections is increased which sometimes leading to nausea and vomiting. Filamentous fungi have high growth rates than yeasts along with more risk of contamination than any other microorganism. In case of bacteria, more RNA content, risk of contamination and endotoxins are the most limiting factors.

Some toxic substances i.e. mycotoxins, cyanotoxins can be produced by certain microorganisms used during SCP production. This can be counteracted by selecting proper microorganism for processing. Due to the substances present in the substrate and some produced by the microorganisms, the final product sometimes leads to indigestion. Apart from toxic substances, some carcinogenic substances may be produced when microbes undergo mutation during processing and formation of final product which could be toxic for both humans and livestock. Moreover, algae is free of toxins, however, it has a very slow growth rate or low density like 1–2 g per liter of the substrate. All of the above problems can be avoided by carefully optimizing the fermentation protocol during SCP production. Moreover, the selection of active microorganism along with suitable substrate is also helpful in counteracting the above limitations and makes the usage of SCP beneficial. Furthermore, anti-nutritional factors i.e. nucleic acids can also be removed by applying different physical and chemical treatments during processing (Dantas Jr et al., 2016).

### 14. Conclusion

Single cell proteins exhibit very attractive characteristics as a nutrient supplement for both animals and plants. It can be produced at any time during a year because of their independence from seasonal and climatic variations. It could be produced from various cost free substrates. Therefore, conclusion can be made that it can easily replace conventional animal and protein sources in both humans and animal diets without any negative impact.

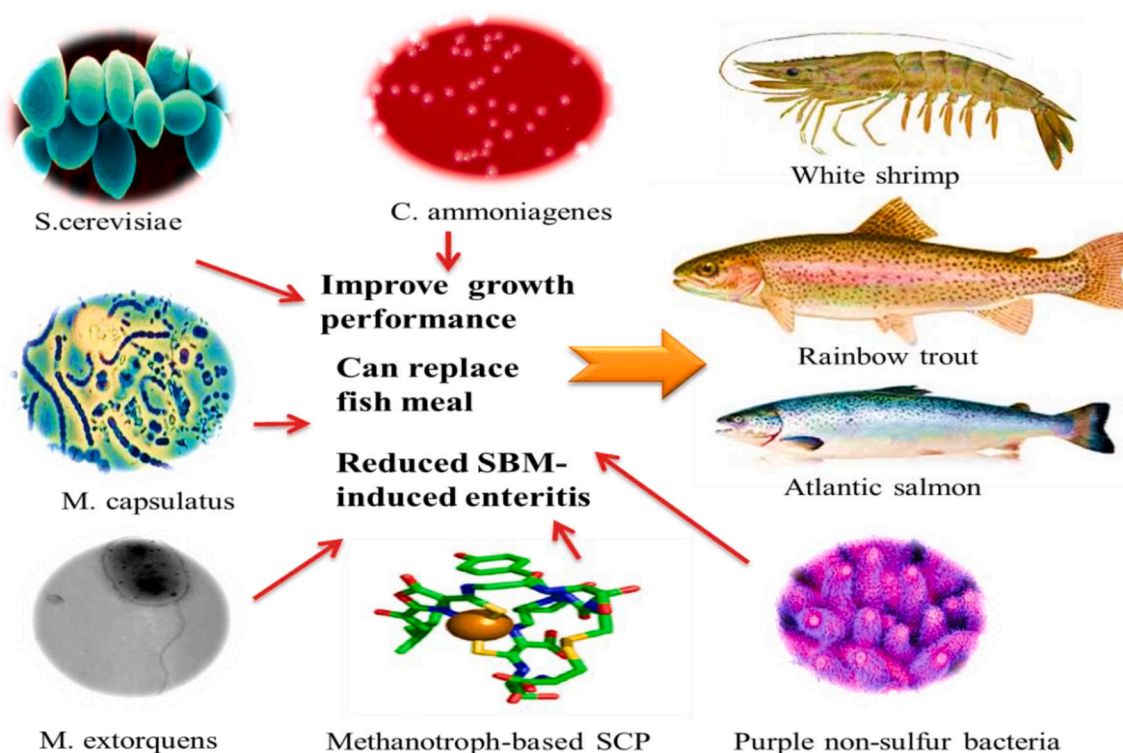


Fig. 2. Effect of using some sources of SCP in fish feeding.

## Funding source

Authors did not receive any funding from any source.

## Declaration of Competing Interest

Authors' declare no conflict of interest.

## References

- Aas, T.S., Grisdale-Helland, B., Terjesen, B.F., Helland, S.J., 2006. Improved growth and nutrient utilisation in Atlantic salmon (*Salmo salar*) fed diets containing a bacterial protein meal. *Aquaculture* 259, 365–376.
- Abd El-Hack, M., Alagawany, Farag M.M., Tiwari, R., Karthik, K., Dhama, K., Zorriehzahra, J., Adel, M., 2016. Beneficial impacts of thymol essential oil on health and production of animals, fish and poultry: a review. *J. Essent. Oil Res.* 28 (5), 365–382.
- Aggelopoulos, T., Katsieris, K., Bekatorou, A., Pandey, A., Banat, I.M., Koutinas, A.A., 2014. Solid state fermentation of food waste mixtures for single cell protein, aroma volatiles and fat production. *Food Chem.* 145, 710–716. <https://doi.org/10.1016/j.foodchem.2013.07.105>.
- Alagawany, M., Farag, M.R., Abdelnour, S.A., Elnesr, S.S., 2020a. A review on the beneficial effect of thymol on health and production of fish. *Rev. Aquac.* <https://doi.org/10.1111/RAQ.12490>.
- Alagawany, M., Farag, M.R., Salah, A.S., Mahmoud, M.A., 2020b. The role of oregano herb and its derivatives as immunomodulators in fish. *Rev. Aquac.* <https://doi.org/10.1111/raq.12453>.
- Alagawany, M., Abd El-Hack, M.E., Farag, M.R., Shaheen, H.M., Abdel-Latif, M.A., Noreldin, A.E., Khafaga, A.F., 2020c. The applications of *Origanum vulgare* and its derivatives in human, ruminant and fish nutrition – a review. *Ann. Anim. Sci.* <https://doi.org/10.2478/aoas-2020-0004>.
- Anupama, Ravindra, P., 2000. Value-added food: single cell protein. *Biotechnol. Adv.* 18, 459–479. [https://doi.org/10.1016/s0734-9750\(00\)00045-8](https://doi.org/10.1016/s0734-9750(00)00045-8).
- Aruna, T.E., Aworh, O.C., Raji, A.O., Olagunju, A.I., 2017. Protein enrichment of yam peels by fermentation with *Saccharomyces cerevisiae* (BY4743). *Ann. Agric. Sci.* 62, 33–37.
- Ashok, R.S., Nigam, P., Vanete, T., Luciana, P.S., 2000. Bio resource technology. *J. Am. Sci.* 16, 8–35.
- Bamberg, J., 2000. *British Petroleum and Global Oil 1950–1975: The Challenge of Nationalism*. Cambridge University Press.
- Bhalla, T.C., Joshi, M., 1994. Protein enrichment of apple pomace by co-culture of cellulolytic moulds and yeasts. *World J. Microbiol. Biotechnol.* 10, 116–117. <https://doi.org/10.1007/BF00357577>.
- Boehlke, K.W., Friesen, J.D., 1975. Cellular content of ribonucleic acid and protein in *Saccharomyces cerevisiae* as a function of exponential growth rate: calculation of the apparent peptide chain elongation rate. *J. Bacteriol.* 121, 429–433.
- Bogdahn, I., 2015. Agriculture-Independent, Sustainable, Fail-Safe and Efficient Food Production by Autotrophic Single-Cell Protein. <https://doi.org/10.7287/peerj.preprints.1279v2>.
- Browne, J., Nizami, D.A.-S., Thamsiroj, T., Murphy, J., 2011. Assessing the cost of biofuel production with increasing penetration of the transport fuel market: a case study of gaseous biomethane in Ireland. *Renew. Sust. Energ. Rev.* 15, 4537–4547. <https://doi.org/10.1016/j.rser.2011.07.098>.
- Chumpol, S., Kantachote, D., Nitoda, T., Kanzaki, H., 2018. Administration of purple nonsulfur bacteria as single cell protein by mixing with shrimp feed to enhance growth, immune response and survival in white shrimp (*Litopenaeus vannamei*) cultivation. *Aquaculture* 489, 85–95.
- Dantas Jr., E.M., Valle, B.C.S., Brito, C.M.S., Calazans, N.K.F., Peixoto, S.R.M., Soares, R.B., 2016. Partial replacement of fishmeal with biofloc meal in the diet of postlarvae of the Pacific white shrimp *Litopenaeus vannamei*. *Aquac. Nutr.* 22, 335–342.
- de Finco, A.M.O., Mamani, L.D.G., de Carvalho, J.C., de Melo Pereira, G.V., Thomaz-Soccol, V., Soccol, C.R., 2017. Technological trends and market perspectives for production of microbial oils rich in omega-3. *Crit. Rev. Biotechnol.* 37, 656–671. <https://doi.org/10.1080/07388551.2016.1213221>.
- Deise Maria Fontana, C., Fernando Hercule, V., Iracema de Oliveira, M., Lúcia Helena, P., 2001. Solid-state fermentation of *Bacillus thuringiensis* *tolworthii* to control fall armyworm in maize. *Electron. J. Biotechnol.* 4, 2.
- Dharumadurai, D., LAWANYA, S., Saha, S., Thajuddin, N., Annamalai, P., 2011. Production of single cell protein from pineapple waste using yeast. *Innov. Rom. Food Biotechnol.* 8, 26.
- El-Bakry, M., Abraham, J., Cerda, A., Barrena, R., Ponsá, S., Gea, T., Sánchez, A., 2015. From wastes to high value added products: novel aspects of SSF in the production of enzymes. *Crit. Rev. Environ. Sci. Technol.* 45, 1999–2042. <https://doi.org/10.1080/10643389.2015.1010423>.
- FAO, 2016. The State of World Fisheries and Aquaculture 2016. Contributing to Food Security and Nutrition for all. [Cited 14 Oct 2017.] Available from URL. <http://www.fao.org/3/a-i5555e.pdf>.
- Ferreira, I.M.P.L.V.O., Pinho, O., Vieira, E., Taveira, J.G., 2010. Brewer's *Saccharomyces* yeast biomass: characteristics and potential applications. *Trends Food Sci. Technol.* 21, 77–84. <https://doi.org/10.1016/j.tifs.2009.10.008>.
- FitzPatrick, M., Champagne, P., Cunningham, M.F., Whitney, R.A., 2010. A biorefinery processing perspective: treatment of lignocellulosic materials for the production of value-added products. *Bioresour. Technol.* 101, 8915–8922. <https://doi.org/10.1016/j.biortech.2010.06.125>.
- Gao, L., Chi, Z., Sheng, J., Ni, X., Wang, L., 2007. Single-cell protein production from Jerusalem artichoke extract by a recently isolated marine yeast *Cryptococcus aureus* G7a and its nutritive analysis. *Appl. Microbiol. Biotechnol.* 77, 825–832. <https://doi.org/10.1007/s00253-007-1210-7>.
- Gao, Y., Li, D., Liu, Y., 2012. Production of single cell protein from soy molasses using *Candida tropicalis*. *Ann. Microbiol.* 62, 1165–1172. <https://doi.org/10.1007/s13213-011-0356-9>.
- Gervasi, T., Pellizzeri, V., Calabrese, G., Di Bella, G., Cicero, N., Dugo, G., 2018. Production of single cell protein (SCP) from food and agricultural waste by using *Saccharomyces cerevisiae*. *Nat. Prod. Res.* 32, 648–653. <https://doi.org/10.1080/14786419.2017.1332617>.
- Ghasemi, Y., Rasoul-Amini, S., Morowvat, M.H., 2011. Algae for the production of SCP. *Bioprocess Sci. Technol. Nov. Sci. Publ. Inc* 163–184.
- Goldberg, I., 2013. *Single Cell Protein*. Springer Science & Business Media.
- Guo, J., Qiu, X., Salze, G., Davis, D.A., 2019a. Use of high-protein brewer's yeast products in practical diets for the Pacific white shrimp *Litopenaeus vannamei*. *Aquac. Nutr.* 25, 680–690. <https://doi.org/10.1111/anu.12889>.
- Guo, J., Reis, J., Salze, G., Rhodes, M., Tilton, S., Davis, D.A., 2019b. Using high protein distiller's dried grain product to replace corn protein concentrate and fishmeal in practical diets for the Pacific white shrimp *Litopenaeus vannamei*. *J. World Aquacult. Soc.* 50, 983–992. <https://doi.org/10.1111/jwas.12606>.
- Hamidoghli, A., Yun, H., Won, S., Kim, S., Farris, N.W., Bai, S.C., 2019. Evaluation of a single-cell protein as a dietary fish meal substitute for whiteleg shrimp *Litopenaeus vannamei*. *Fish. Sci.* 85, 147–155.
- Hardy, R.W., Patro, B., Pujol-Baxley, C., Marx, C.J., Feinberg, L., 2018. Partial replacement of soybean meal with *Methylobacterium extorquens* single-cell protein in feeds for rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aquac. Res.* 49, 2218–2224. <https://doi.org/10.1111/are.13678>.
- Jacob-Lopes, E., Zepka, L.Q., Queiroz, M.I., Netto, F.M., 2006. Protein characterisation of the *Aphanthece* Microscopic *Nägeli* cyanobacterium cultivated in parboiled rice effluent. *Food Sci. Technol.* 26, 482–488.
- Jamal, P., Alam, M., SALLEH, N., 2008. Medai optimization for bioproteins productions from cheaper carbon source. *J. Eng. Sci. Technol.* 3, 124–130.
- Jay, J.M., Loessner, M.J., Golden, D.A., 2005. Indicators of food microbial quality and safety. *Mod. Food Microbiol.* 1, 473–495.
- Jin, M., Xiong, J., Zhou, Q.-C., Yuan, Y., Wang, X.-X., Sun, P., 2018. Dietary yeast hydrolysate and brewer's yeast supplementation could enhance growth performance, innate immunity capacity and ammonia nitrogen stress resistance ability of Pacific white shrimp (*Litopenaeus vannamei*). *Fish Shellfish Immunol.* 82, 121–129. <https://doi.org/10.1016/j.fsi.2018.08.020>.
- John, R.P., Anisha, G.S., Nampoothiri, K.M., Pandey, A., 2011. Micro and macroalgal biomass: a renewable source for bioethanol. *Bioresour. Technol.* 102, 186–193. <https://doi.org/10.1016/j.biortech.2010.06.139>.
- Kadim, I.T., Mahgoub, O., Baqir, S., Faye, B., Purchas, R., 2015. Cultured meat from muscle stem cells: a review of challenges and prospects. *J. Integr. Agric.* 14, 222–233.
- Kam, S., Kenari, A.A., Younesi, H., 2012. Production of single cell protein in stickwater by *Lactobacillus acidophilus* and *Aspergillus Niger*. *J. Aquat. Food Prod. Technol.* 21, 403–417. <https://doi.org/10.1080/10498850.2011.605539>.
- Kost, C., Mayer, J.N., Thomsen, J., Hartmann, N., Senkpiel, C., Philipps, S., Nold, S., Lude, S., Saad, N., Schlegel, T., 2013. Levelized cost of electricity renewable energy technologies. *Fraunhofer Inst. Sol. Energy Syst. ISE* 144.
- Lang, V., Bellisle, F., Alamowitch, C., Craplet, C., Bornet, F.R., Slama, G., Guy-Grand, B., 1999. Varying the protein source in mixed meal modifies glucose, insulin and glucagon kinetics in healthy men, has weak effects on subjective satiety and fails to affect food intake. *Eur. J. Clin. Nutr.* 53, 959–965. <https://doi.org/10.1038/sj.ejcn.1600881>.
- Leuenberger, H.G., 1972. Cultivation of *Saccharomyces cerevisiae* in continuous culture. II. Influence of the crabtree effect on the growth characteristics of *Saccharomyces cerevisiae* grown in a glucose limited chemostat. *Arch. Mikrobiol.* 83, 347–358.
- Lipinski, E.S., 1981. Chemicals from biomass: petrochemical substitution options. *Science* 212, 1465–1471. <https://doi.org/10.1126/science.212.4502.1465>.
- Mahasneh, I.A., 1997. Production of single cell protein from five strains of the microalga *Chlorella* spp. (Chlorophyta). *Cytobios* 90, 153–161.
- Majekodunni, A., Ajiboye, E., Odaibo, A., 2011. Single cell proteins: as nutritional enhancer. *Adv. Appl. Sci. Res.* 2, 396–409.
- Mekonnen, M.M., Hoekstra, A.Y., 2014. Water footprint benchmarks for crop production: a first global assessment. *Ecol. Indic.* 46, 214–223. <https://doi.org/10.1016/j.ecolind.2014.06.013>.
- Nasseri, A.T., Rasoul-Amini, S., Morowvat, M.H., Ghasemi, Y., 2011. Single cell protein: production and process. *Am. J. Food Technol.* 6, 103–116.
- Øverland, M., Tauson, A.-H., Shearer, K., Skrede, A., 2010. Evaluation of methane-utilising bacteria products as feed ingredients for monogastric animals. *Arch. Anim. Nutr.* 64, 171–189. <https://doi.org/10.1080/17450391003691534>.
- Øverland, M., Karlsson-Drangsholt, A., Mydland, L., Romarheim, O., Skrede, A., 2013. Evaluation of *Candida utilis*, *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* yeasts as protein sources in diets for Atlantic salmon (*Salmo salar*). *Aquaculture* 402–403, 1–7. <https://doi.org/10.1016/j.aquaculture.2013.03.016>.
- Øvrum Hansen, J., Hofossæter, M., Sahlmann, C., Ånestad, R., Revoco-Urzuza, F.E., Press, C.M., Mydland, L.T., Øverland, M., 2019. Effect of *Candida utilis* on growth and intestinal health of Atlantic salmon (*Salmo salar*) parr. *Aquaculture* 511, 734239. <https://doi.org/10.1016/j.aquaculture.2019.734239>.
- Paraskevopoulou, A., Athanasiadis, I., Kanellaki, M., Bekatorou, A., Bilekas, G., Kiosseoglou, V., 2003. Functional properties of single cell protein produced by kefir microflora. *Food Res. Int.* 36, 431–438. [https://doi.org/10.1016/S0963-9969\(02\)00000-0](https://doi.org/10.1016/S0963-9969(02)00000-0).



- 00176-X.
- Piper, S., 2004. Continuous Cultures of *Methylococcus Capsulatus*. Center of Microbial Biotechnology (Biocentrum)-Technical University of Denmark.
- Prado-Rubio, O.A., Jørgensen, J.B., Jørgensen, S.B., 2010. Systematic model analysis for single cell protein (SCP) production in a U-loop reactor. In: *Computer Aided Chemical Engineering*. 28. Elsevier, pp. 319–324.
- Queiroz, M.I., Lopes, E.J., Zepka, L.Q., Bastos, R.G., Goldbeck, R., 2007. The kinetics of the removal of nitrogen and organic matter from parboiled rice effluent by cyanobacteria in a stirred batch reactor. *Bioresour. Technol.* 98, 2163–2169. <https://doi.org/10.1016/j.biortech.2006.08.034>.
- Raja, R., Hemaiswarya, S., Kumar, N.A., Sridhar, S., Rengasamy, R., 2008. A perspective on the biotechnological potential of microalgae. *Crit. Rev. Microbiol.* 34, 77–88. <https://doi.org/10.1080/10408410802086783>.
- Ravinder, R., Linga, V., Ravindra, P., 2003. Studies on *Aspergillus oryzae* mutants for the production of single cell proteins from deoiled rice bran. *RNA* 41, 243–246.
- Ritala, A., Häkkinen, S.T., Toivari, M., Wiebe, M.G., 2017. Single cell protein-state-of-the-art, industrial landscape and patents 2001–2016. *Front. Microbiol.* 8, 2009. <https://doi.org/10.3389/fmicb.2017.02009>.
- Romarheim, O.H., Øverland, M., Mydland, L.T., Skrede, A., Landsverk, T., 2011. Bacteria grown on natural gas prevent soybean meal-induced enteritis in Atlantic salmon. *J. Nutr.* 141, 124–130. <https://doi.org/10.3945/jn.110.128900>.
- Saeed, M., Yasmin, I., Murtaza, M.A., Fatima, I., Saeed, S., 2016. Single cell proteins: a novel value added food product. *Pakistan J. Food Sci.* 26, 211–217.
- Serrano, R., Martín, H., Casamayor, A., Ariño, J., 2006. Signaling alkaline pH stress in the yeast *Saccharomyces cerevisiae* through the Wsc1 cell surface sensor and the Slt2 MAPK pathway. *J. Biol. Chem.* 281, 39785–39795. <https://doi.org/10.1074/jbc.M604497200>.
- Singhania, R., Patel, A., Soccol, C., Pandey, A., 2008. Recent advances in solid-state fermentation. *Biochem. Eng. J.* 44, 13–18. <https://doi.org/10.1016/j.bej.2008.10.019>.
- Sousa, I., Gouveia, L., Batista, A., Raymundo, A., Bandarra, N., 2008. Microalgae in novel food products. *Algae Nutr. Pollut. Control Energy Sour.* 75–112.
- Steels, E.L., Learmonth, R.P., Watson, K., 1994. Stress tolerance and membrane lipid unsaturation in *Saccharomyces cerevisiae* grown aerobically or anaerobically. *Microbiology* 140, 569–576. <https://doi.org/10.1099/00221287-140-3-569>.
- Suman, G., Nupur, M., Anuradha, S., Pradeep, B., 2015. Single cell protein production: a review. *Int. J. Curr. Microbiol. App. Sci.* 4, 251–262.
- Tamura, K., Miyashita, M., Iwahashi, H., 1998. Stress tolerance of pressure-shocked *Saccharomyces cerevisiae*. *Biotechnol. Lett.* 20, 1167–1169.
- Thlusty, M., Rhyne, A., Szczebak, J.T., Bourque, B., Bowen, J.L., Burr, G., Marx, C.J., Feinberg, L., 2017. A transdisciplinary approach to the initial validation of a single cell protein as an alternative protein source for use in aquafeeds. *PeerJ* 5, e3170. <https://doi.org/10.7717/peerj.3170>.
- Turnbull, W.H., Leeds, A.R., Edwards, D.G., 1992. Mycoprotein reduces blood lipids in free-living subjects. *Am. J. Clin. Nutr.* 55, 415–419. <https://doi.org/10.1093/ajcn/55.2.415>.
- Ugalde, U.O., Castrillo, J.I., 2002. Single cell proteins from fungi and yeasts. In: Khachatourians, G.G., Arora, D.K.B.T.-A.M. (Eds.), *Agriculture and Food Production*. Elsevier, pp. 123–149. [https://doi.org/10.1016/S1874-5334\(02\)80008-9](https://doi.org/10.1016/S1874-5334(02)80008-9).
- Vermeulen, S.J., Campbell, B.M., Ingram, J.S.I., 2012. Climate change and food systems. *Annu. Rev. Environ. Resour.* 37, 195–222. <https://doi.org/10.1146/annurev-environ-020411-130608>.
- Voltoлина, D., Gómez-Villa, H., Correa, G., 2005. Nitrogen removal and recycling by *Scenedesmus obliquus* in semicontinuous cultures using artificial wastewater and a simulated light and temperature cycle. *Bioresour. Technol.* 96, 359–362. <https://doi.org/10.1016/j.biortech.2004.04.004>.
- Waldron, C., Lacroute, F., 1975. Effect of growth rate on the amounts of ribosomal and transfer ribonucleic acids in yeast. *J. Bacteriol.* 122, 855–865.
- Werpy, T., Petersen, G., Aden, A., Bozell, J., Holladay, J., White, J., Manheim, A., Eliot, D., Lasure, L., Jones, S., 2004. *Top Value Added Chemicals From Biomass. Volume 1 - Results of Screening for Potential Candidates From Sugars and Synthesis Gas*.
- Yousufi, M.K., 2012. To determine protein content of single cell protein produced by using various combinations of fruit wastes and two standard food fungi. In: *Int. J. Adv. Biotechnol. Res.* 3. pp. 533–536.
- Yunus, F.-N., Nadeem, M., Rashid, F., 2015. Single-cell protein production through microbial conversion of lignocellulosic residue (wheat bran) for animal feed. *J. Inst. Brew.* 121, 553–557. <https://doi.org/10.1002/jib.251>.
- Zepka, L., Jacob-Lopes, E., Goldbeck, R., Queiroz, M., 2008. Production and biochemical profile of the microalgae *Aphanotece microscopica* Ngeli submitted to different drying conditions. *Chem. Eng. Process. Process Intensif.* 47, 1305–1310. <https://doi.org/10.1016/j.cep.2007.04.013>.
- Zhou, Y.-M., Chen, Y.-P., Shen, Y., 2017. Single cell protein-feed: Taking orange waste as raw material for fermentation. In: *Advanced Materials and Energy Sustainability*. World Sci, pp. 323–335. [https://doi.org/10.1142/9789813220393\\_0041](https://doi.org/10.1142/9789813220393_0041).