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Review

Influencing factors on single-cell protein production by submerged fermentation: A review

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ABSTRACT

Since more than twenty years ago, some species of bacteria and fungi have been used to produce protein biomass or single-cell protein (SCP), with inexpensive feedstock and wastes being used as their sources of carbon and energy. The role of SCP as a safe food and feed is being highlighted more because of the worldwide protein scarcity. Even though SCP has been successfully commercialized in the UK for decades, study of optimal fermentation conditions, various potential substrates, and a broad range of microorganisms is still being pursued by many researchers. In this article, commonly used methods for the production of SCP and different fermentation systems are briefly reviewed, with submerged fermentation being highlighted as a more commonly used method. Emphasis is given to the effect of influencing factors on the biomass yield and productivity in an effort to provide a comprehensive review for researchers in related fields of interest.
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Contents

1. Introduction

Extreme population growth leads to lower quality of life, poverty, and starvation. Thus, mankind has been trying to overcome crises through technological advances that can help more access to food. In this era of a globalized society, reliable estimates could be made of the

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available resources for human subsistence eventually through technology revolution. Indeed, two major mechanisms result in an important increase in the future demand for food and water: First, population growth with a factor of 1.4, which means that by 2050, the population will increase to approximately 9.3 billion people [1]; second, the fact that the living standard will increase and approximately 3 billion people will belong to this expanding middle class by 2050, mainly because of economic growth in the developing countries, which can lead to change in lifestyle and diet. An increase of 50% in protein demand [2] and 102% in meat product demand will be the consequence of these changes [3]. Therefore, facing such worldwide issues, protein production has been the subject of various research investigations. One of the most advantageous approaches among these research studies is access to single-cell proteins (SCPs) produced from an agricultural waste source by fermentation [4,5]. As defined in the literature, SCPs are the dried cells of microorganisms such as fungi, algae, and bacteria that are used as a protein supplement in human foods or animal feeds. By utilizing waste products and inexpensive feedstock as the source of carbon and energy, microorganisms can be used to grow biomass or protein concentrates [4,5].

Even though man has been using microorganisms in the production of food and animal feed for centuries, the technology of SCP production as food has been developed during the last 100 years. Actually, its large-scale production was developed in the 20th century and particularly after the First World War [6]. Considering the vast range of existing microorganisms, investigation of their optimal condition for producing higher quantity and from nutritional aspect, better quality of SCP remains an attracting field for research throughout the world. Efforts are being made to determine alternative substrates and methods capable of eliminating the issues of the nutritional sources and methods currently in use for the production of SCP to a better acceptance of this valuable nutrient supplement throughout the world [7].

This review is focused on SCP production from different carbon sources by submerged fermentation, and emphasis is given to assessment of the influencing factors on the produced biomass, which is considered as the final product.

2. Experimental design in SCP production

Traditionally, optimization in analytical chemistry has been carried out by monitoring the influence of one factor at a time on an experimental response. Although only one parameter is changed, the others are kept at a constant level. This optimization technique is called one-variable-at-a-time. Its major disadvantage is that it does not include the interactive effects among the variables studied. Consequently, this technique does not completely depict the effects of the parameter on the response [8]. Another disadvantage of the one-factor optimization is the increase in the number of experiments necessary to conduct the research, which leads to an increase in time and expenses, as well as an increase in the consumption of reagents and materials [9]. To have a more systematic procedure for conducting experiments, some studies are based on planned experiments by evaluating the effect of several variables and their interaction on the final responses. In fact, the design of an experiment requires identifying important influencing factors and achieving the most valid results from least experimental trials with minimum efforts, resources, and time. Experimental design is a specific set of experiments defined by a matrix composed of different combinations of the level of variables studied [9]. Table 1 summarizes the reported studies that used experimental designs and shows the impact of independent variables on SCP production as response.

3. Influencing factors on SCP production

The yield (g/L) and productivity ($g/L \cdot h^{-1}$) of SCP production are strongly dependent on culture medium composition and environmental conditions [10]. According to literature, substantial effort has been made to evaluate factors that affect the growth of SCP, such as pH, temperature, incubation period, dissolved oxygen, aeration rate, and nutritional requirements such as carbon and nitrogen sources to cultivate and to use this information to optimize the culture conditions [11]. The effect of these factors on SCP growth is individually discussed below.

3.1. Carbon source

The production of SCP can be performed using readily available food waste, which had previously been considered of low or no value. However, waste materials and inexpensive feedstock, especially agricultural wastes, have recently been recognized as raw materials of potential value to produce platform bioactive compounds by fermentation [12]. The common materials used as substrates for the production of SCP from different microorganisms include the residue of orange peel, wheat straw, sweet orange, sugarcane, paper mill waste, rice husk, cassava waste, wood shavings, sawdust, corn cobs, sugar beet pulp, coconut waste, grape waste, mango waste, etc. [13,14]. Lignocellulosic biomass such as cellulose and hemicellulose waste has been used as a suitable substrate for increasing SCP production [15]. However, several compositional and structural characteristics provide resistance to biological degradation, thus limiting the bioconversion of lignocellulosic substrates.

Thus, the raw materials are hydrolyzed by physical, chemical, and enzymatic methods before being used as substrates [16,17]. In fact, pretreatment methods became fundamental to break the resistant layer of lignin by reducing the crystallinity of cellulose, thus increasing the availability of carbohydrates (amorphous cellulose and hemicelluloses) to be used by microorganisms. Physical pretreatments include mechanical (i.e., grinding, chipping, milling, knife mill, scissors, etc.), microwave, ultrasound, steam explosion, and liquid hot water. In contrast, chemical pretreatments are purely initiated by chemical reactions to disrupt of the biomass structure. Biological pretreatments can be performed by applying either commercial enzymes or fungi to the lignocellulose material [18]. In addition to conventional materials such as molasses, fruit and vegetable wastes and unconventional substrates such as petroleum by-products, natural gas, ethanol, and methanol have been used [11,13]. The technology proteins-from-oil process was developed by British Petroleum for producing SCP with yeast using waxy n-paraffin, a product produced by oil refineries, as the substrate [19,20].

The degree of SCP production depends on the type of substrate used and also on media composition [14]. It is extremely important to use the correct substrate because it directly affects the outcome of fermentation. Each type of substrate fermentation technique has to be optimized because organisms react differently to each substrate, and thus, the rate of utilization of nutrients varies in each substrate.

In fact, utilization of waste in the production of SCP not only can play an efficient role in controlling environmental pollution but is also an effective strategy to reduce the cost of SCP production [21].

Waste materials, however, need to have some criteria to be a useful substrate for the production of microbial protein. These criteria include being nontoxic, abundant, regenerable, nonexotic, and inexpensive. They should also be capable of supporting the rapid growth and multiplication of the microorganisms and a high-quality biomass production. In other words, the use of organic wastes as a substrate in the fermentation processes can be accepted as one of the solutions to reduce the total price of the culture, as well as an environmentally friendlier method of removing these residues. Fig. 1 shows the different substrates used as the carbon source and also various microorganisms used for SCP production [22]. Table S1 summarizes studies that reported on using different substrates as the carbon source and the impact of different influencing variables of fermentation on the biomass yield and productivity. For most of the

Table 1
Experimental designs of studies on the effect of the fermentation condition on SCP production.

Experimental design	Carbon source	Microorganism	Independent variables	Dependent variables	References
Plackett-Burman design	Date extract	*Fusarium venenatum*	Jaggery water, Date extract, KH_2PO_4, K_2HPO_4, $MgSO_4$, Inoculum size, Incubation time	Yield productivity	[25]
Response surface methodology, central composite design	Jaggery water, date extract	*Fusarium venenatum*	Jaggery water Date ext. conc. KH_2PO_4 K_2HPO_4 Inoculum size Time	Yield	[24]
Fraction of the full factorial methodology	Glucose	*Saccharomyces cerevisiae*	Glucose conc., ammonium sulfate, iron sulfate glycine, glucose	Biomass production	[10]
Mathematical model	Cheese whey	*Kluyveromyces fragilis*	Lactose concentration retention times 12, 18, and 24 h; air flow rates, mixing speeds	Biomass output	[35]
Polynomial regression model, central composite design	Wheat flour	*Mucor hiemalis*	Wheat flour (g/L), NH_4NO_3 (g/L), KH_2PO_4 Time	Biomass production	[44]
Taguchi as a fractional factorial statistical method	Glucose	*Aspergillus niger*	Glucose con., $MgSO_4$ con., KH_2PO_4 g/L, pH	SCP production Yield	[47]
Face-centered central composite design (FCCD)	Date juice	*Fusarium venenatum*	Conc. of date sugar, conc. of nitrogen $NH_4H_2PO_4$, seed size	Yield of mycoprotein production *w*/*w* protein/biomass	[43]
Plackett-Burman design	Date sugar	*Fusarium venenatum*	Date sugar conc., (NH_4) H_2PO_4, KH_2PO_4, temperature, time, seed age, seed size	Biomass and protein production	[42]
Orthogonal Test	Yam starch	Active dry yeast	Liquid volume initial pH, culture time inoculum size	SCP	[61]
Taguchi	Methanol	Selected *Methylobacterium* strains	Methanol, aeration, agitation pH	SCP and total protein content	[45]

filamentous fungal species, fruit wastes were used as the carbon source, such as lemon pulp for *Aspergillus niger* [23] and date waste for *Fusarium venenatum, Fusarium graminearum,* and *Aspergillus oryzae* [24,25,26,27,28]. In addition to fruit wastes, mono and disaccharides such as glucose, fructose, sucrose, maltose, and lactose were used for *Fusarium moniliforme* and *Fusarium oxysporum*. Similarly, yeast species were mostly exposed to fruit waste and cheese whey as carbon sources. For instance, date waste was used for SCP production from *Trichoderma reesei* and *Thermomyces lanuginosus* [24]. Other fruit wastes such as Beles fruit peels, banana skin, mango waste, sweet orange peel, pomegranate rind, apple waste, pineapple waste, orange plantain, beet pulp, cactus pear, and virgin grape marc were used as substrates for *Saccharomyces cerevisiae*, *Candida utilis*, and *Candida tropicalis* [29,30,31,32,33,34,35]. Industrial wastes such as oil-rich manufacturing wastewater, cheese whey, defatted rice polishing, raw glycerol from biodiesel production, and wheat flour were used for *Kluyveromyces marxianus*, *Clavaria versatilis*, *Kluyveromyces lactis*, *S. cerevisiae*, *Mucor hiemalis*, *Kluyveromyces fragilis*, *Torulopsis cremoris*, and *Yarrowia lipolytica* [36,37,38,39,40,41,42,43]. For bacterial species such as *Bacillus cereus*, *Bacillus subtilis*, *Bacillus coagulans, Bacillus licheniformis, Bacillus stearothermophilus, Escherichia coli, and Brevibacterium lactofermentum*, ram horn hydrolysate, beet pulp hydrolysate and molasses, liquid whey, and glucose were used as the carbon source [33,43,44,45,46]. The concentration of the carbon source used as

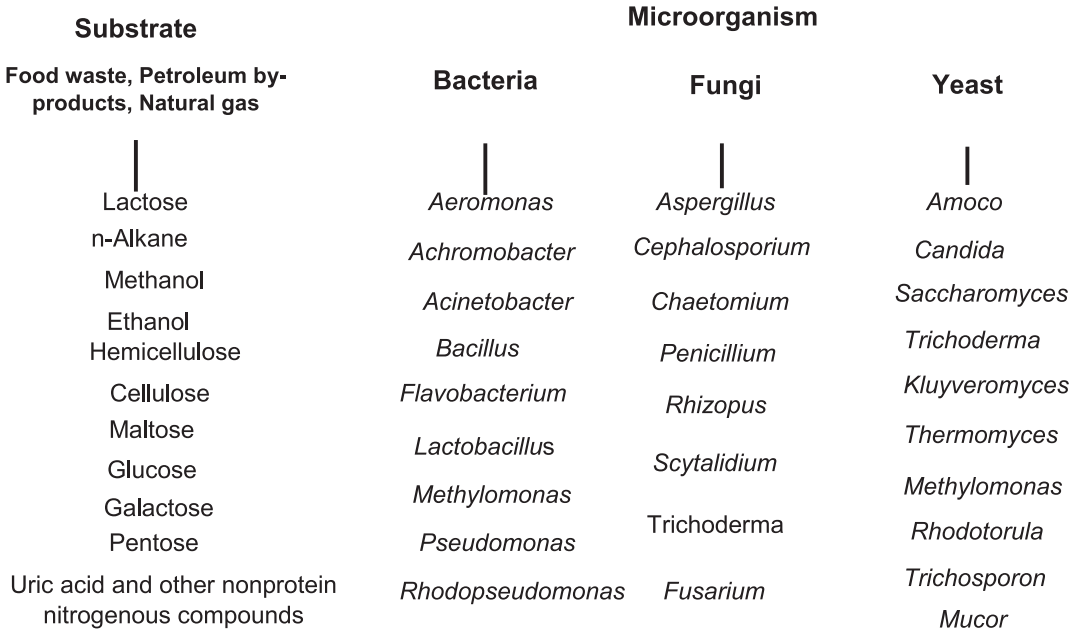

Fig. 1. Microorganisms and substrates used for SCP production.

substrates ranged from 4 to 192 g/L, which produced 21.89 g/L and 19.8–23 g/L biomass yield by *M. hiemalis* and *S. cerevisiae*, respectively [29,44]. As shown in Table S1, fungi such as *F. venenatum*, with a carbon source concentration of 20 g/L (taken from date waste sugar), yielded approximately 0.35 g/g biomass [22]. Additionally, *S. cerevisiae*, using a significantly higher level of sugar (taken from virgin grape marc), i.e., 192 g/L, yielded lower biomass, that is, approximately 0.12 g/g [29]. In another study conducted by Ardestani and Alishahi [45], *A. niger* used 70 g/L glucose to yield 0.62 g/g biomass, which is considered as a high yield. A report by Ghaly et al. [35] showed that *K. fragilis* can produce a biomass yield as high as 0.74 g/g (37 g/L) using 34.3 g/L lactose. As seen and stated earlier, chemical structure and type of substrate can directly affect cell growth and fermentation outcome. A clear example is given by a comparison of the two yeasts *K. fragilis* and *C. utilis*. Although they were exposed to two different carbon sources, i.e., cheese whey and defatted rice polishing, both of them produced quite high biomass yield of 0.74 g/g and 0.65 g/g, respectively [37,40]. Thus, it is extremely important to optimize the fermentation techniques for each type of substrate used and culture nutrients to attain the highest advantage of the process.

3.2. Nitrogen source

Because of the structural properties, nitrogen source is one of the most important factors during the synthesis of protein by microorganisms. Nitrogen sources that are useful for growth include ammonia, ammonium salts, urea, nitrate, and organic nitrogen in different substrates such as wastes. In addition, sometimes it is suggested to add mineral nutrient supplement to the culture medium to restore the deficiency of nutrients with concentration insufficient to support growth.

Similar to carbon source, utilization of different nitrogen sources could lead to different yield of SCP production. For instance, urea as a nitrogen source for *C. utilis* had an adverse effect on its growth (12.7 g/L cell mas) compared to organic sources such as corn steep liquor (CSL), a low cost by-product of the starch industry; yeast extract; peptone; and soybean meal (20.7, 18.4, 16.3, and 14.3 g/L cell mass, respectively) or inorganic sources such as ammonium sulfate and ammonium chloride (16.2 and 15.3 g/L cell mass, respectively) [46]. In another report by Taran and Bakhtiyari [47], the halophile *Haloarcula* sp. could grow on NH_4Cl and peptone, but not much SCP was produced using yeast extract, peptone, and tryptone as nitrogen sources. A 0.8% (*w*/*v*) concentration of nitrogen source was more effective in biomass production than other concentrations. The maximal yield of SCP was obtained when tryptone was supplemented at a concentration of 0.8% (*w*/*v*) [47]. In another report by Adoki [32], ammonium sulfate was a better nitrogen source for the growth of *C. utilis* than ammonium nitrate. In their study, ammonium nitrate and ammonium sulfate were used as nitrogen sources for other yeast species such as *M. hiemalis, T. cremoris, K. marxianus,* and *S. cerevisiae*. Ouedraogo et al. [48] observed that with a concentration of 0.75 g of peptone, the maximum growth of *C. utilis* cells as yeast biomass (3.25 g/L) was obtained. However, they observed that using yeast extract as a nitrogen source with 0.5% concentration resulted in maximal biomass (4.56 g/L) production. A gradual increase in growth was observed with an increase in nitrogen source. Nevertheless, an adverse effect was observed after it reached to 4.56 g/L, and the use of higher amounts of yeast extract resulted in lesser growth. Yeasts such as *C. utilis* have been fed with a variety of organic (CSL, yeast extract, soybean meal, and peptone) and inorganic (ammonium sulfate, ammonium chloride, and urea) nitrogen sources, and CSL appeared to generate the highest yield for this microorganism [49].

Nitrogen sources for bacterial SCP as seen in Table S1 were whey for *B. subtilis* [41]; ram horn hydrolysate for *B. subtilis, B. cereus, and E. coli* [39]; and CSL for *B. lactofermentum* [30].

As shown in Table S1, thus far, date extract was used for *F. venenatum* as a nitrogen source [22,23,24,25]. In addition, dihydrogen ammonium phosphate ($NH_4H_2PO_4$) was used for *F. venenatum* by Hosseini et al. [22] and Hosseini and Khosravi-Darani [23]. Sodium nitrate and nitrite, urea, yeast extract, peptone, and a range of different amino acids were used as inorganic and organic nitrogen sources for the growth of *F. moniliforme*, with peptone being the best source in terms of the production of highest yield and productivity [50]. Ardestani and Alishahi [45] used lemon pulp as the nitrogen and carbon source for the growth of *A. niger*. According to a report by Haddish [26], the use of inorganic nitrogen sources compared to organic sources could reduce the protein percentage in the produced SCP by *S. cerevisiae* using Beles fruit as the substrate. Adoki [32] used orange, plantain, and banana wastes as substrates and observed that the use of ammonium sulfate had a better effect on the yield of SCP production by *Candida* spp. than that of ammonium nitrate.

Zheng et al. [33] recommended a ratio of N:C as high as 1:6 and 1:8, starting from a N:C ratio equal to 1:25 for SCP production from *Candida* and *Rhodotorula* species by using oil-rich salad oil manufacturing wastewater as the substrate. In another study, Rajoka et al. [40] compared the effect of some variables including CSL, ammonium sulfate, ammonium nitrate, ammonium dihydrogen phosphate, urea, and sodium nitrate for SCP production by using defatted rice polishing and found out that CSL (5%) had the best effect on SCP from *C. utilis*.

Organic nitrogen sources such as CSL, yeast extract, soybean meal, and peptone (5 g/L, *w*/*v*) and inorganic sources (ammonium sulfate, ammonium chloride, and urea) at a concentration of 2 g/L were used to investigate the effect of different variables on SCP by *C. utilis* by using capsicum powder medium (CPM) [46]. According to their results, urea had an adverse effect on the cell growth of *C. utilis* and CSL (20.7 g/L) had the best effect. The crude protein content of *C. utilis 1769* was 48.2%, which was more than that of *C. utilis OZ993* grown on salad oil manufacturing wastewater reported by Zheng et al. [33] and that of *C. utilis* Y900 grown on pineapple cannery effluent reported by Nigam [49]. To improve cell mass production of *C. utilis* 1769, several organic and inorganic nitrogen sources were added to CPM. The results showed that these nitrogen compounds influenced the yield of cell mass. In addition, CSL supported the maximum cell mass production of *C. utilis* 1769 (20.7 g/L) compared to those of other nitrogen compounds [36,46].

3.3. Inoculum size and age

The attainment of optimum growth at the production stage could depend on the inoculum age and size of the seed culture because they can affect the overall production yield and cost of the fermentation process [51,52,53,54,55]. The optimum inoculum size varied for different microorganisms as shown in Table S1. For instance, Hosseini et al. [22] and Hosseini and Khosravi-Darani [23] used a culture size of 13% and 10% *v*/*v* for the inoculation of *F. venenatum*, thus leading to a biomass yield of 4.84 g/L and approximately 47% protein yield, respectively. In a similar work, Prakash et al. [25] used a culture size of 5% for *F. venenatum* and obtained a 5 g/L biomass yield, which is significantly lower than that reported by Hosseini and Khosravi-Darani [23]. Kurbanoglu and Algur [43] applied a 5% inoculum size for *B. cereus*, and the result was a maximum of 7.3 g/L biomass production. Moreover, inoculum age can play a crucial role in the final biomass yield. Hosseini et al. [22] showed that a 48 h old inoculum provided a better condition for the growth of *F. venenatum*. In a study conducted by Ugwuanyi [42] for SCP production from *B. stearothermophilus*, an inoculum size of 0.5% *v*/*v* and age of 12 h were used, and this provided the highest yield of 2.41 g/L biomass.

Yunus et al. [4] used *C. utilis* and *Rhizopus oligosporus* as the microbial culture and wheat bran as the substrate and found that an inoculum size of 10% *v*/v can lead to the production of the highest level of protein. Pogaku et al. [53] obtained maximum biomass of *A. oryzae* while using

a 3% (*v*/*v*) inoculum size on de-oiled rice bran, which is in accordance with the result of Yunus et al. [4]. Rajoka et al. [40] studied the kinetics of batch SCP production from rice polishing with *C. utilis* in continuously aerated tank reactors and observed that by using a 10% (*v*/*v*) inoculum size, maximum yield of biomass and protein can be obtained. The differences in the maximum yield while using different inoculum sizes may lie in the fact that different microorganisms, substrates, and fermentation techniques were employed [4,43]. In another report by Zhao et al. [46], *C. utilis, C. tropicalis, and S. cerevisiae* were used with a 5% *v*/*v* inoculum size, and *C. utilis* showed the highest yield (14 g/L) compared to the other tested yeasts.

3.4. Aeration

As stated earlier, aeration is an important operation in submerged fermentation for microorganisms to absorb oxygen. In general, the more reduced the substrate, the greater the cell yield and the more oxygen required for the oxidation of the substrate. As reported by Nanou et al. [54], the morphology of microorganisms plays a crucial role in oxygen absorption [56,57]. A study, for instance, has shown that the best aeration rate (volume of air, volume of medium, minute) and dissolved oxygen to produce a higher yield of *C. utilis* is 1 vvm and 50%, respectively [40]. Dissolved oxygen concentration was analyzed for a group of microorganisms (*Rhodotorula rubra, C. tropicalis, C. utilis, Candida boidinii, and Trichosporon cutaneum*), and *C. utilis* showed a better choice than others in terms of higher yield, which was 2 mg/L according to Zheng et al. [33]. Curto et al. [29] used 3 vvm airflow for *S. cerevisiae* in a 15 L Biostat E-level reactor. In another study, Kurbanoglu et al. [43] used an aeration rate of 1.5 vvm for *Bacillus* spp. and *E. coli*. Another recent work was conducted by Anvari and Khayati [37] on *K. lactis, K. marxianus, S. fragilis, K. lactis*, and *S. cerevisiae*, and among them, *K. marxianus* showed the highest yield when 1 vvm aeration was used for a 2 L stirred tank. A study by Ugwuanyi [42] showed that 1 vvm of aeration yielded higher yield than 0.5 vvm of aeration using three different bacteria, i.e., *B. coagulans, B. licheniformis, and B. stearothermophilus*.

3.5. Temperature and pH

Temperature is one of the most influencing factors on the growth of microorganisms and thus on the yield and productivity in the process of SCP production [58,59]. The most common temperature used for the incubation of different microorganisms was room temperature, i.e., 25–27°C (Table S1). However, for some fungi such as *K. fragilis* and *C. utilis*, a temperature between 33 and 35°C was reported as the optimum [35,46]. For bacteria such as *Bacillus* spp. and *E. coli*, Kurbanoglu et al. [43] used a fixed temperature, i.e., 30°C. Gomashe et al. [41] used a temperature of 37°C for *B. subtilis*, and Curto et al. [29] used a temperature of 30°C for the growth of *S. cerevisiae*. However, the optimum temperature for SCP production from *C. utilis* was 25°C and 30°C when using oil-rich salad oil manufacturing wastewater and cactus pear fruit as carbon sources, respectively [31,33]. *F. moniliforme* also showed optimum growth at 28°C with different culture media containing different types of simple carbohydrates [49]. Similarly, Hosseini et al. [22] observed that 28°C is the optimum temperature for the growth of *F. venenatum* when using date sugar as the substrate. *Rhizopus* sp. exhibited different growth morphologies during cultivation at different temperatures. It grew as small mycelial clumps at 30, 35, and 40°C, while it grew as small mycelial pellets at 25 and 45°C. Because of their growth morphology, the cultivation of filamentous fungi has been widely investigated using reactors with a simpler design, such as airlifts and bubble columns, compared to the traditional stirred-tank reactors [59].

A recent study by Ferreira et al. [60] showed that a simpler reactor such as a bubble column can be used for the production of ethanol and biomass with the same performance as that using an airlift, which at a starting point is cost-effective. In addition to temperature, studies have been performed on the effect of pH ranging from 3.5 to 7. In studies that used *S. cerevisiae* for SCP production, a fixed pH of 5.8 (virgin grapes as the substrate) [29] and 6 (glucose as the substrate) was used [11]. In another study, *Neurospora intermedia, Rhizopus* sp., *A. oryzae, F. venenatum,* and *Monascus purpureus* were used to produce biomass at a fixed pH of 5.5 [59]. Nevertheless, a pH range of 3–6.2 was used for SCP production using *Candida* sp. by Adoki [32] and a value of pH 4.2 was determined to be the optimum pH for higher biomass generation. In case of mixed culture of *Candida* sp. and *Brevibacterium*, the pH was fixed at 6 [30]. Similarly, Rajoka et al. [40] used a fixed value of pH, i.e., pH 6 by using defatted rice polishing for SCP production from *C. utilis*. A mixed culture of *K. marxianus* and *Torula* sp., was used at a fixed pH of 4.8 [36]. Among all species, a relatively higher pH of 7.2 was used by Kurbanoglu and Algur [43] for *Bacillus* sp. and *E. coli* to produce SCP by using ram horn hydrolysate [43]. Chen et al. [61] examined the effect of a range of pH (3.5–5.5) as initial fermentation conditions on SCP production from active dry yeast, and the optimum initial pH was observed as pH 5.0.

As shown in Table S1, for most of the performed studies, the pH value was not among independent variables to evaluate the optimum condition for higher biomass and protein production.

Other factors, e.g., enrichment of the culture medium by macronutrients and micronutrients such as amino acids and different metal salts, were also effective on the protein and biomass yields. As shown in Table S1, the highest achievable biomass was reported as 21.9 g/L, which is produced by *M. hiemalis* with wheat flour as the substrate [34]. The amount of wheat flour used as the substrate was low (4 g/L), but it resulted in a very high biomass yield. It should be noted that in this work, vacuum filtration, with an 11 μm pore size filter, was used to filter the biomass, although this does not seem to be a suitable method to separate the flour particles, which have an average particle size of 45 μm, from the biomass. Moreover, the productivity value was not outstanding (0.22 $g/L \cdot h^{-1}$). Further verification is required by performing similar studies to confirm their report. However, *K. fragilis* showed to have a high potential to produce a yield as high as 0.74 g/g along with a high productivity value, i.e., 3 $g/L \cdot h \cdot h^{-1}$ [35]. The filamentous fungus *F. moniliforme* was ranked the third in terms of biomass yield, i.e., 0.74 g/g [49]. Nevertheless, there was no such a high value as its productivity, which was approximately 0.077 $g/L \cdot h^{-1}$ according to Pradeep and Pradeep [50]. Finally, Rajoka et al. [30] reported that *C. utilis* produced 0.65 g/g biomass with 1.24 $g/L \cdot h^{-1}$ productivity, which can be considered a relatively high value compared to that reported in similar studies. Therefore, fungi such as *Mucor*, *Kluyveromyces*, *Fusarium,* and *Candida* were the species with the highest reported biomass yield (Fig. 2).

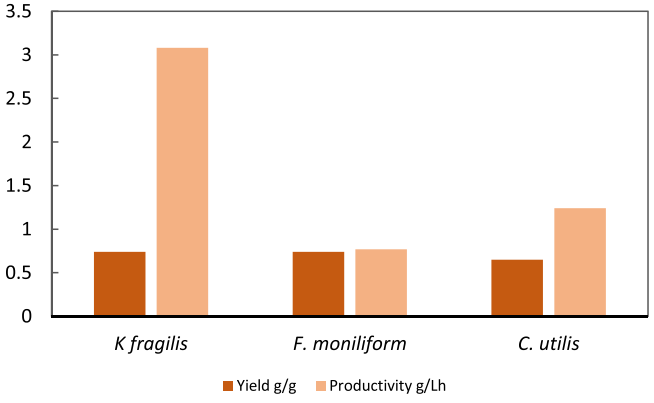

Fig. 2. Highest reported yield (g biomass/g substrate) and productivity ($g/L \cdot h^{-1}$) of SCP.

4. Conclusion

From the literature, it could be concluded that the performed studies were mainly conducted at 28–32°C and a fixed pH and often with cheap fermentable substrates, i.e., waste products of an industry, whose disposal otherwise is costly. Despite the fact the design of an experiment requires identifying important influencing variables and achieving the most valid results from least experimental trials with minimum efforts, resources, and time, many studies in this area have been conducted without a proper systematic experimental design, and this is considered as a serious gap in those studies.

In general, type of microorganism used, incubation temperature, incubation time, shake rate, chemical structure, and availability of carbon source from different substrates were considered and were monitored in various works. However, inoculum size, inoculum age, pH, and aeration rate are also important factors for which their effects on the SCP production need to be investigated. Furthermore, biomass productivity has a crucial role in the economic aspect of the research owing to its inclusion of time factor and could be a more informative dependent variable than biomass yield; however, it is not properly calculated and reported in most studies. In SCP production, there are only few reports that used more than 2 L bioreactors. The future of SCP production will be heavily dependent on reducing production cost and improving quality, which could be achieved by lower feedstock cost, improvement of its nutritional and health quality, and its acceptability by consumers in the society. The use of SCP as a non-animal protein is promising, and further developments are being made for the future. Further research and development will ensure the usage of microbial biomass as SCP or as a diet supplement particularly in developing countries.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Supplementary material

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