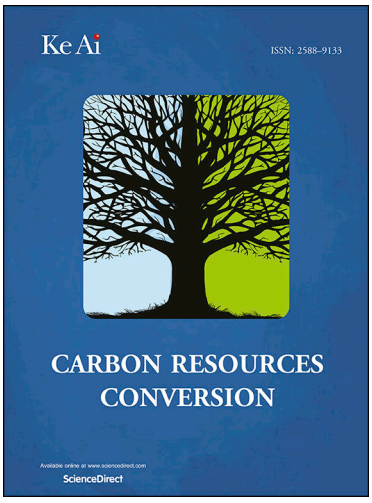
Journal Pre-proofs



Review Article

Recent advances in the production of single cell protein from renewable resources and applications

Danai Ioanna Koukoumaki, Erminta Tsouko, Seraphim Papanikolaou, Zacharias Ioannou, Panagiota Diamantopoulou, Dimitris Sarris

PII: S2588-9133(23)00057-1
DOI: https://doi.org/10.1016/j.crcon.2023.07.004
Reference: CRCON 195

To appear in: *Carbon Resources Conversion*

Received Date: 9 June 2023
Revised Date: 17 July 2023
Accepted Date: 24 July 2023

Please cite this article as: D.I. Koukoumaki, E. Tsouko, S. Papanikolaou, Z. Ioannou, P. Diamantopoulou, D. Sarris, Recent advances in the production of single cell protein from renewable resources and applications, *Carbon Resources Conversion* (2023), doi: https://doi.org/10.1016/j.crcon.2023.07.004

Recent advances in the production of single cell protein from renewable resources and applications

Danai Ioanna Koukoumaki[1], Erminta Tsouko[1], Seraphim Papanikolaou[2], Zacharias Ioannou[1], Panagiota Diamantopoulou[3], Dimitris Sarris[1]*

[1]Laboratory of Physico-Chemical and Biotechnological Valorization of Food By-Products, Department of Food Science & Nutrition, School of Environment, University of the Aegean, Leoforos Dimokratias 66, Myrina 81400, Lemnos, Greece

[2]Department of Food Science and Human Nutrition, Agricultural University of Athens, 75, Iera Odos, 11855 Athens, Greece

[3]Laboratory of Edible Fungi, Institute of Technology of Agricultural Products, Hellenic Agricultural Organization-Dimitra, 1, Sof. Venizelou, 14123 Lykovrysi, Greece

*Corresponding Author email: dsarris@aegean.gr

Danai Ioanna Koukoumaki email: danaikouk@aegean.gr

Erminta Tsouko: etsouko@aegean.gr

Seraphim Papanikolaou: spapanik@aua.gr

Zacharias Ioannou: zioan@aegean.gr

Panagiota Diamantopoulou: pdiamantopoulou@elgo.gr

Recent advances in the production of single cell protein from renewable resources and applications

Danai Ioanna Koukoumaki[1], Erminta Tsouko[1], Seraphim Papanikolaou[2], Zacharias Ioannou[1], Panagiota Diamantopoulou[3], Dimitris Sarris[1]*

[1]Laboratory of Physico-Chemical and Biotechnological Valorization of Food By-Products, Department of Food Science & Nutrition, School of Environment, University of the Aegean, Leoforos Dimokratias 66, Myrina 81400, Lemnos, Greece

[2]Department of Food Science and Human Nutrition, Agricultural University of Athens, 75, Iera Odos, 11855 Athens, Greece

[3]Laboratory of Edible Fungi, Institute of Technology of Agricultural Products, Hellenic Agricultural Organization-Dimitra, 1, Sof. Venizelou, 14123 Lykovrysi, Greece

*Corresponding Author email: dsarris@aegean.gr

Abstract

Single Cell Protein (SCP) refers to dry cells of microorganisms, and it constitutes a highly promising and alternative protein source for multiple applications. SCP presents a rich nutritional profile containing valuable amino acids and fatty acids, nucleic acids, minerals, and several vitamins. Several businesses worldwide have introduced SCP into their production cycles, hence expanding the scope of its application in value added market chains such as the edible food packaging. SCP is produced by a plethora of microorganisms, including fungi, yeasts, bacteria, and algae while many of them are Generally Recognized as Safe (GRAS). Selected microbial strains present satisfying growth capability with high yields when cultivated in renewable feedstock. Thus, production rates and process sustainability could be enhanced via the valorization

of industrial and agricultural wastes as the nutrient sources, combined with optimization of process parameters, i.e fermentation mode and feeding strategy, pH, temperature, C/N ratio, agitation rate and oxygen supply. This review addresses the latest developments made towards the SCP production, highlighting efficient microbial SCP producers, and production systems that valorize solid and liquid streams from several agricultural wastes. Potential applications, challenges in sensorial-, and safety-aspects as well as consumers perception issues of SCP incorporation into food-related matrices are also discussed.

Keywords: alternative protein, sustainability, sensorial aspects, safety, food and feed, packaging applications

1. Introduction

The modernization of world and the necessity for prosperous societies have increased the standards of living and thus food safety and quality levels. Predictions for global population growth up to 9.7 billion by 2050 [1] will inevitably lead to increased competition for land, water, and energy. Sustainable food systems based on renewable food ingredients are vital to be developed. As a result, issues related to greenhouse gas emissions (GHG) [2], increased usage of fresh water (~ 70%) for agriculture [3] and GHG emissions related to livestock production (up to 18%) as well as ammonia utilization could be overcome or at least deteriorated [4].

Alternative proteins sources such as plant by-products, insects, and microorganisms have attracted scientific attention since they do not demand arable land and they can be produced

utilizing renewable feedstock (waste and/or by-product streams) derived from numerous agri-food residues. Among these non-conventional sources, microorganisms present enhanced protein contents. Dry cell biomass has been described as 'single cell protein' (SCP) or 'microbial protein' [5]. SCP is primarily sourced from microorganisms, and it constitutes an eco-friendly substitute for animal-derived proteins. The global demand for proteins is continuously growing while advances in the food processing sector are likely to trigger SCP importance, although the latter still holds a small market share. More specifically, the market of SCP is forecast to exceed $18.5 billion by 2030 [6]. Recent studies have evaluated a variety of microorganisms such as bacteria [7], [8], [9], algae [10], [11], [12], yeasts [13], [14], [15] and other fungi [16],[17],[18] for SCP production. Production of SCP is characterized by convenience, due to fast growth of microbial cells, high yields, and use of a wide range of fermentation substrates [19]. High promising fermentation media for SCP production should be non-toxic, non-exotic, non-seasonal, renewable, and cost-effective [20]. Industrial waste streams, such as paper and pulp effluents [21], methanol, oil [22], latex waste [23], and crude glycerol [24], have been efficiently utilized as unconventional substrates to produce SCP. Other food industry-derived streams i.e waste cooking oil, seems to be also promising [24], [25], while fruit waste (peels or extracts) [26], [27], [28], [29], leaf juice, poultry and slaughterhouse waste [20] and lignocellulosic wastes [30] have been already used for SCP production as demonstrated in Figure 1.

SCP can be involved into a plethora of applications including both agricultural and commercial sector. More specifically, SCP has been used as a protein source for fish-meals [31], [32], foam-stabilizing agent [33], in paper and leather processing [34], potential packaging material [35] and as a good candidate for animal feed supplements [36] due to its nutritional value (Figure 2) as well as due to the potential influence in reduction of enteric CH4 emissions in

ruminant animals [37]. Moreover, it has been proposed that SCP could be used as resilient food for catastrophes [38] or even as alternative food source in space missions [39], [40].

This review deals with the latest advances made towards the SCP production, reporting the microbial strains that have been so far used for its biosynthesis. Different production systems that valorize renewable streams derived from several agricultural wastes are highlighted. Potential applications of SCP are also included. Challenges in sensorial-, and safety-aspects as well as consumers perception issues of SCP incorporation into food-related matrices are also emphasized.

2. SCP producers

Many fungal species including *Kluyveromyces* [41], *Candida* [42], *Saccharomyces*, *Meyerozyma* [43], *Pichia*, *Galactomyces* [15], *Nectaromyces* [14], *Rhodotorula* [44], *Aspergillus* [45], *Fusarium* [46], *Aureobasidium*, *Neurospora* and *Trichoderma* spp. [16] have been employed for the production of SCP due to their chemical composition, nutrients (vitamin B-complex and folic acid) and amino acid profile (rich in essential amino acids i.e lysine and threonine), which complies with standards of FAO [47], [48]. Fungi can reach protein contents from 30% to 50% in case that the fermentation optimization targets cells protein increase [47]. Numerous studies concluded that fungi and yeasts are ideal candidates to produce SCP (Table 1), single cell oil [49] and other added-value compounds [50]. However, factors such as high nucleic acid content of fungi and yeasts (up to 10%) and low cell-wall digestibility [34], limit their wide applicability and must be taken into consideration prior to their use. Difficulties in digestibility of cell walls could lead to low bioavailability of proteins, allergies, intestines problems or even skin problems [51].

Several species of bacteria have been used as a source of protein, as they accumulate significant protein contents (50–80% of dry weight), present fast growth rates, and they can grow

on a wide variety of substrates, such as sugars, starches, and organic wastes [34]. Recent studies indicated purple phototrophic bacteria species such as *Rhodospirillum*, *Rhodobacter* and *Rhodopseudomonas*, as very appealing candidates for SCP production [52], [5], [53]. Bacterial SCP production should comply with certain criteria to be commercialized. To specify, the overall performance in terms of growth rate, pH tolerance, heat and oxygen requirements during fermentation, foam generation, purity and chemical profile of the final product is of particular importance [34]. The main disadvantage is the fact that bacterial SCP is characterized by a high nucleic acid content and low familiarity of consumers to this novel material. It is notable that in aquatic habitats such as fresh and marine water and multiple wastewaters, bacteria are the main decomposers of organic matter while algae are the main absorbers of nutrients. Sial et al. [54] studied extensively the interaction between algae and bacteria on biomass accumulation, wastewater treatment as long as current biotechnological applications, and demonstrated that the algal-bacterial complexes could lead to higher algal biomass accumulation compared to mono-algal cultures.

Microalgae can convert micro-molecules, such as carbon dioxide or ammonium, into valuable macromolecules like proteins. Microalgae show high photosynthetic efficiency and growth rate, which leads to high productivity values of proteins and/or lipids [55]. Specific types of microalgae have SCP contents reaching up to 70%, which favor their cultivation for animal and human consumption [48]. Raji et al. [56] partially replaced fishmeal protein with *Spirulina platensis* and *Chlorella vulgaris* on African catfishes' feed and they investigated the effect of the algae on growth and body composition of the catfishes. They suggested that the optimum percentage of *S. platensis* and *C. vulgaris* was 68.5% and 69.4%, respectively. Although many microalgal species such as *Arthrospira*, *Chlorella*, *Dunaliella*, *Haematococcus*, and

Schizochytrium are characterized as GRAS by the United States Food and Drug Administration (FDA), the food and feed applications of microalgae in commercial formulations demands extensive attention, due to their possible toxicity [55]. According to Roy-Lachapelle et al. [57], the involvement of toxin-producing microalgae or microalgae that derive from cultivation in toxic wastewater, could lead to the formulation of contaminated and thus dangerous for the human health dietary supplements. Moreover, microalgae display high nucleic acid content (up to 6%) [34], which as mentioned before is a limiting factor for their application in feed and food supplements.

3. SCP production using agricultural waste and by-product streams

Agricultural wastes consisted of mono and disaccharide molecules (dairy waste, and molasses), starch-rich sources such as grains, structural polysaccharides (including lignocellulosic side streams), protein or lipid-rich sources (derived from fish feed production and slaughterhouse wastes), or glycerol-based resources, have been proposed for SCP production. Various of these resources belong to food-processing and / or food-deriving residue. These can be either solid (i.e. waste breads, waste sugars, discarded or expired foods, etc), semi-solid (i.e. olive pomaces) or liquid ones (i.e. waste-waters containing significant quantities of sugars, olive-mill wastewaters, cheese whey, etc) [58], [59]. Moreover, these types of residues can also be either hydrophilic (i.e. olive-mill wastewaters, waste breads, etc) or hydrophobic (i.e. used / cooked oils, tallows, stearins, neutralization pastes, etc) [60]. Other types of residues are characterized as agricultural by-products deriving from agro-industrial activities (i.e. lignocellulosic biomass, lignocellulosic wastewaters deriving from paper and pulp industries, volatile fatty acids originated from dark fermentation or other petrochemical processes, various types of sludges, etc) [58], [59]. Finally, residues containing increased concentrations of glycerol, can derive from biofuel production

facilities or oleochemical units [60]. In the following table (Table 2) we can see the various types of agro-residues (solid, semi-solid and liquid ones) according to the previous classification, that have been implemented in the various types and fermentation configurations of SCP production process, and indicative values of dry biomass (dry cell weight; DCW in g/L) achieved in the mentioned cases.

As far as the sugar-containing residues are concerned, mono and disaccharide sources require less pre-treatment prior to their conversion into fermentation media. On the contrary, starch-rich sources, structural polysaccharides sources, protein and lipid rich sources need to be hydrolyzed or to be mechanically, chemically or biochemically pre-treated, prior to use [61]. For instance, pretreatment of the residual biomass is a critical step for the subsequent conversion of cellulose (the main component in structural polysaccharides sources) into glucose. An efficient pretreatment method should decrease the crystallinity of cellulose to further facilitate the action of the hydrolytic enzymes. The pre-treatment stage ranks among the most expensive processes for the conversion of lignocellulosic feedstock into fermentable sugars. Commonly, in order to enhance SCP production, lignocellulosic sources such as hemicellulose are chemically pre-treated [62]. Efficient and cost-effective pretreatment strategies should provide optimal yields of polysaccharides hydrolysis, and minimum formation of inhibitory compounds i.e phenolic compounds, furfural, 5-(hydroxymethyl)furfural (HMF) and acetic acid. As a consequence, the produced fermentation media could lead to enhanced cell growth and products formation [63]. It should be stressed that as far as the fermentation of sugar-based substrates is conducted for SCP production , sufficient oxygen-excess conditions should be provided, given that the process is positively influenced by the aeration imposed into the culture media, exactly as it happens with the process of *de novo* single cell oil (microbial oil) production (*de novo* oil production refers to

the synthesis performed from glucose or similarly catabolized compounds, where $CH_3COSCoA$ constitutes the base-molecule in order for lipid to be synthesized) [64], [65]. Regarding aeration, previous studies have shown that the best aeration rate (volume of air, volume of medium, minute) and produce a higher yield of SCP is 1vvm in case of C. utilis [66] and *K. marxianus* [67]. When hydrophobic compounds (i.e. solid or liquid free fatty acids, triacylglycerols, etc) are implicated as the sole carbon source for microbial cells production (mostly yeasts and fungi), irrespective of the nitrogen availability , it is possible that a portion of carbon flow would not be directed towards the synthesis of (protein-rich) cells. The accumulation of lipids could simultaneously be enhanced (this is the so-called "*ex novo*" lipid accumulation process, in which incorporated aliphatic chains would be directly esterified with cellular glycerol to form storage triacylglycerols) [64], [68], [69]. As for the case of SCP production when sugars and related hydrophilic compounds are used (i.e. polysaccharides, glycerol, etc), when fatty compounds are implicated as carbon microbial sources, it appears that the process is significantly positively influenced by the high dissolved oxygen saturation content into the medium (see biomass production in shake-flask and highly agitated batch bioreactor experiments in which *Yarrowia lipolytica* was used as microbial cell factory when fully saturated industrial free fatty acids were used as substrate – [129]) (Table 2).

3.1 Dairy waste

Dairy waste (sludge and effluents) contains high level of organic matter, oil, fatty acids, and considerable nitrogenous compounds, while dairy wastewater mostly contains high concentration of dissolved organic components, such as lactose, minerals, fat, and whey protein [70]. Depending on the technology used in the milk processing, dairy waste can contain high levels of either lactose or protein, therefore it can be categorized as a monosaccharide- and disaccharide-rich source or protein or lipid-rich source [61]. In 2020, cheese manufacturing in Europe generated 55.5 million

tons of whey [71], leading to the conclusion that treatment of such waste is crucial. Approximately 50% of total milk solids are found in whey, with lactose representing the major fraction, followed by proteins, minerals, non-protein nitrogen and other minor compounds [72], [73]. The challenge for the efficient valorization of whey is to identify microbial strains that can metabolize lactose and bioconvert it into SCP. Yadav et al. [74] investigated the potential of co- culture of *Kluyveromyces marxianus* and *Candida krusei* to amplify COD removal and to produce SCP, while utilizing whey as substrate in batch and continues aerobic fermentations. Results indicated that the co- culture was able to achieve 8.8% higher COD removal efficacy, combined with 19% higher biomass yield and 33% productivity, compared to monocultures. A maximum SCP production equal to 43.4 % was reported for co-cultures. Another study evaluated the SCP production by *K. marxianus* applying different fermentation strategies (batch and continuous mode) while a simultaneous COD removal using cheese whey was conducted [41]. The authors suggested that the aerobic continuous fermentation process with cell recycling could be applied to SCP production. Utilization of waste milk was also examined using strains of *K. lactis* (TY-98) and *Rhodotorula graminis* (TY-99) to produce SCP while the fermentation parameters i.e initial pH, different incubation temperatures and inoculum size in monoculture, mixed culture, and sequential culture were investigated for maximum production [44]. Mixed culture seemed to be a promising approach, as they resulted in the production of 43.8 g/L dry cell weight, under optimized conditions.

The production of microbial mass (i.e. yeast dry cell weight or dried pellets or mycelia) has been reported by various types of yeasts and fungi, cultured on cheese-whey, in several types of fermentation configurations. Besides cell mass, in several cases cheese-whey has been implicated in processes related to the production of microbial cells containing quantities of lipids (the so-

called single cell oils; SCOs) [64], [60] whereas also, besides cell mass, other valuable metabolites (like exo-polysaccharides) have been reported to be produced by these types of compounds [65]. Results demonstrating the production of cell mass during growth on media composed of lactose (and / or cheese-whey) are shown in Table 3.

3.2Sugar Industry Wastes

Molasses is generated from the sugar industry by repeated crystallization during sugar preparation [75]. Global sugar production amounted to roughly 179 million tons in 2020/2021 [76], while an overproduction of sugar beet pulp is predicted in near future due to growing world population. The principal components of molasses are saccharose (30–35%), fructose and glucose (10–25%), non-sugar compounds (2–3%), and minerals [75]. Due to rich nutritional value, molasses is widely used as a fermentation substrate for production of industrially and/or biotechnologically valuable products such as SCP, organic acids, and biohydrogen [24], [77]. Coimbra et al. [43] enriched vinasses substrate with molasses in order to enhance the production of SCP and bio-aroma by several yeast species including *Saccharomyces cerevisiae* CCMA 0186 and CCMA 0188, *Candida parapsilosis* CCMA 0544, *C. glabrata* CCMA 0193, and *Meyerozyma caribbica* CCMA 0198. Cultivation under fed-batch mode indicated that *C. parapsilosis* was the most efficient strain reaching the highest biomass formation of 8.8 g/L, in medium consisted of 50:50 (vinasse:molasses). In another study, *Rhodopseudomonas faecalis*, was cultivated in media containing different types of wastewaters (wastewaters were collected from anaerobic pond, primary mechanically aerated pond and secondary mechanically aerated pond) from sugar processing [78]. Wastewater collected from anaerobic ponds showed a good potential for SCP

production with protein content exceeding 50% and a valuable amino acid profile (rich in essential amino acids).

3.3 Fruit waste

Waste from fruit processing seems to be a serious problem in Middle Eastern and Asian countries. A variety of studies have investigated the potential of fruit peels or generally fruit wastes as substrates for SCP production, as they are considered as lignocellulosic wastes containing simple and complex sugars that can be metabolized by microorganisms [79]. Al-Farsi et al. [46] produced SCP from date waste collected from a date syrup industry, using *Trichoderma reesei* ATCC 13631, *Fusarium venenatum* ATCC 20334, *Thermomyces lanuginosus* ATCC 34626, *Aspergillus oryzae* ATCC 14895, and *Fusarium graminearum* ATCC 20333. Results indicated that protein derived from *A. oryzae* biomass showed a ratio of essential to non-essential amino acids equal to 1:1.2. In another study, a variety of fruit waste materials (including wastes of mangos, prickly custard apples, pineapples, papayas, bananas, mangosteens, cashew apples, cacaos, jackfruits and pomegranates) were used as substrates for SCP production by *S. cerevisiae* [28]. Maximum biomass and SCP production (0.4 g and 48.3%, respectively) were obtained when pineapple wastes were used as the fermentation feedstock. Pineapple waste (60% v/v) have also been used as effective media with maximum yield of SCP up to 3.0 g/L [26] when *S. cerevisiae* was used. In a recent study, Mostafa Kamal et al. [80] attempted to optimize the process variables using response surface methodology to improve the production of SCP of *A. niger* from banana fruit peel on submerged shake flask fermentation. Maximum biomass and SCP production reached 24.7 g/L and 61.2 % w/w respectively, when optimized condition were applied (T= 31.02 °C, pH of 6.19, substrate concentration of 19.92%, 4 days of fermentation).

3.4 Crop waste

Crop residues constitute cheap, renewable and abundant resources[81]. Production of crop residues is increasing as Cherubin et al. [82] estimated the global crop residues production from 2003 to 2013 and reported that about 3607.6 x 10^6 Mg of crop residues were produced from different crops (i.e cerals, barley, corn, millet, rice, wheat, oats, rye). They include straws, bran, husks, and hulls of wheat and rice, barley straw, barley husk, corncob, corn husks, oat husks, and oat hulls [83]. These materials mainly consist of cellulose, hemicelluloses, and lignin [81]. However, cellulose and hemicelluloses cannot be utilized directly by most microorganisms and therefore, pretreatment and enzymatic hydrolysis that can aid cellulose hydrolysis and conversion of hemicelluloses into monosaccharides are required [84] as shown in Figure 3. Upcraft et al. [85] studied the fermentation of *Fusarium venenatum* on sugars derived from lignocellulosic residues of rice straws (using ionic liquid combined with food-grade Celluclast). A subsequent techno-economic analysis and life cycle assessment based on the proposed biorefinery model showed that the crude mycoprotein paste product could be produced at ~$40.04 per kg-protein. This price could be reduced further by improving saccharification yields and utilization of feedstocks with high cellulose contents. Moreover, life cycle assessment results indicated that lignocellulosic-derived mycoprotein, demonstrated greenhouse gas emissions less than 14% compared to protein from beef. Another type of crop residue such as wheat bran, was utilized by *Candida utilis* and *Rhizopus oligosporus* for biomass production and after fermentation's parameters optimization the maximum SCP yield reached up to 41.0% [86]. In another study, three different crop residues (rye straw, rye bran and oat bran) were tested as substrates for the growth of *Y. lipolytica* [87].

Maximum biomass production of yeast was observed in oat bran hydrolysates while the protein contents in yeast biomass ranged within 30.5–44.5% of dry weight.

3.5 Combined agricultural waste

Aggelopoulos et al. [88] reported that the bioconversion of mixed substrates has several advantages including reduction of transportation and disposal costs. For this reason, they studied the potential growth of several microorganisms (*S. cerevisiae, K. marxianus* and *kefir*) in mixed substrates consisted of orange pulp, potato pulp, molasses, whey, Brewer's spent grains and malt spent rootlets, under solid state fermentation. *K. marxianus* was able to accumulate the highest fat and protein concentration (59.2% w/w on dry basis). In a recent study, combination of vinasse and whey wastewaters was studied as substrate for the cultivation of filamentous fungus *Neurospora intermedia* to produce protein- rich biomass [18]. The highest production of biomass reached 12.0 g/ L, with a SCP content of 45%, while essential amino acid contents were comparable to commercial sources of protein that is applied in aquatic feed production.

3.6 Glycerol

Concentrated glycerol-containing waters, with a concentration of glycerol ranging between 65-85% w/w, called also "crude" or "industrial" glycerol" (or "glycerin"), can derive as the main side products of biodiesel production [89], [60]. The synthesis of 10 kg of biodiesel generates *c.* 1 kg of glycerol (purity ≈90% w/w) as side-product of the process, therefore, with the constantly increasing quantities of produced biodiesel world-wide, very high concentrations of this feedstock are accumulated into the market volume, with an inevitable event the significant drop of the price of this side product [90], [91]. Besides biodiesel production process, significant quantities of

glycerol-containing water can be generated through bioethanol and alcoholic beverages production units; for instance, during bioethanol production process, ethanol is separated via distillation while the liquid fraction of the remaining material (the so-called thin stillage) contains *c*. 2% w/v of glycerol [90]. Likewise, liquid waste streams containing high levels of glycerol (glycerol quantities of 55-90% w/v) are generated in oleochemical facilities in which transformations of vegetable or animal fats are implicated [60], [92]. The last years, therefore, a significant number of reports deal with the utilization of glycerol as renewable carbon source implicated in the Industrial Microbiology, and in many instances, significant dry cell weight (DCW) production was reported using yeast, fungal or heterotrophic grown algal strains employed as cell factories (Table 4). Kurzc et al. [42] studied the use of glycerol (as carbon source) and deproteinized potato wastewaters (as nitrogen source) for *C. utilis* and reported that SCP production reached 40.7% on dry weight basis. In another study, *Y. lipolytica* yeast strain YLY accumulates crude glycerol reaching 19.7 g/L of SCP [24]. Furthermore, as shown in Table 4. when *Cryptococcus curvatus* ATCC 20509 accumulate glycerol in a fed- batch bioreactor, production of dry cell weight reached 118.0 g/L [93].

4. SCP in animal feed supplementation

Several yeast species have been used as potential protein alternative for fishmeal, soybean meal or plant-based protein. In a recent study, yeasts (autolyzed and inactive) of *C. jadinii, B. adeninivorans and W. anomalus* were used to partially substitute (up to 30% and 70%) fishmeal (reference) on salmons diets. Final weight and specific growth rate of salmons with 30% substitution were significantly different compared to the reference diet [32]. Partial or total replacement of fishmeal with the inactivated dry yeast product DY-Pro in fish diets was studied

by Yossa et al. [94]. The entire substitution of fishmeal with DY- Pro improved feed and nutrient utilization, while no significant differences were detected in fishes' weight, and gut length. Also, no negative impacts on the gastrointestinal tract of the fishes were observed. Yan et al. [24] engineered an *Y. lipolytica* strain for overproduction of lipases, and SCP on cost-effective media (molasses, waste cooking oil and crude glycerol). They carried out both in vitro characterization by mimicking a gastro-intestinal environment to determine the essential amino acids of the SCP, and in vivo experiments via oral feeding of fish. *K. marxianus, C. utilis* and *S. cerevisiae* have been studied as a potential protein source (up to 40%) on salmon diets. *S. cerevisiae* showed poor protein properties while *K. marxianus* and *C. utilis,* demonstrated very similar nutrient and amino acid digestibility compared to fishmeal [95]. Also, brewer's yeast was effectively used to replace up to 24% of soybean meal and fishmeal in shrimps feed with no significant differences in final biomass, survival, protein retention efficiency and feed conversion rate [96]. SCP obtained from *Corynebacterium ammoniagenes* was applied to replace up to 40% of shrimps' diet. The best results were obtained at 10% substitution improving final weight, weight gain, specific growth and feed conversion ratio compared to higher substitution percentages [97]. SCP obtained from *Clostridium autoethanogenum* was used to replace plant proteins (up to 200g/kg) in Jian carp fish diets. In this case, final body weight, weight gain, specific growth rate, protein retention value, and protein efficiency ratio were significantly improved in fished that were fed with SCP [98]. In African catfish diets, substitution of fishmeal with 30% microbial protein, indicated sufficient results regarding weight gain, specific growth rate and metabolic growth rate, while whole body protein increased by 8% [99]. Alloul et al. [100] studied purple non-sulfur bacteria as a protein source for shrimps feed. Results showed that higher individual weights, better feed conversion,

and higher tolerance against ammonia were observed in diets containing SCP proteins compared to commercial feed.

5. SCP in food packaging formulation

Food packaging is an indispensable sector of food industry, responsible for food protection or damage, food degradation, hygiene and overall safety before and during storage. The most common materials that are used as food packaging are plastic, paper, glass and steel. In recent years, consumers awareness about climate change, has led to an increasing demand for more sustainable choices [101]. Nowadays, research has focused on biodegradable plastic materials [102] and edible films [103] involving proteins. Proteins could be characterized as ideal candidates to produce packaging materials due to their film-forming capacity, amino acid profile, transparency, gas barrier behavior and protein-antioxidant complexes [104]. Moreover, other functional properties that could influence the final biobased product, could be tailor-made after chemical or mechanical treatment. Usage of proteins in packaging materials displays some limitations including low water barrier properties and brittleness of final formulations [104]. Lately, usage and integration of microorganisms regarding production of sustainable packaging materials has become an emerging strategy. For instance, Khattab et al. [105] managed to produce polyhydroxyalkonates (PHAs) from bacterial strains valorizing cheese whey. In another study, evaluation of edible whey protein films incorporated with mycelial biomass of *Trametes versicolor* was examined [106]. SCP is of particular interest as a potential packaging material, since microbial treatment of biodegradable wastes enables sustainable valorization and enhances circular economy. Singha et al. [35] developed SCP-based films using potato starch (present in cutting waters) as substrate for SCP production and glycerol as plasticizer. The oxygen barrier properties

of SCP-based films were significantly better compared to the common polyethylene packaging material, while biodegradation test revealed a similar degradation pattern in relation to a household compostable bag.

5.1 Functional properties of SCP

Physicochemical and functional properties of proteins are influenced by their source of origin and structure. Proteins are characterized as amphiphilic molecules and they create interfacial layers between hydrophilic and hydrophobic regions [104]. Among various functional properties of proteins, including emulsification, thickening, foaming, and gelation, solubility is the most important as the protein fraction must be soluble to be used in a food system [107]. The main factor that affects the degree of protein solubility is the pH. pH values higher than proteins' isoelectric points favor solubility and induce disulphide bonds leading to more stable protein structures [104]. In an emulsion formulation, protein acts as a stabilizing agent, and its ability is described by protein emulsifying activity. Paraskevopoulou et al. [108] studied the SCP production by *kefir* microorganisms and indicated that its emulsifying properties were similar to those of the defatted soy flour. Regarding foaming properties, proteins should stabilize foams rapidly at various pH range and low concentrations [107]. Kupfer et al. [33] performed characterization of protein PAU5 from *S. cerevisiae* regarding its potential foam-stabilizing properties and consequently, to clarify if there is a direct influence on the gushing potential of sparkling wines, with results showing that PAU5 has foam-stabilizing properties .According to Paraskevopoulou et al. [108] gels refer as aqueous solutions or dispersions of high molecular weight such as proteins, cross-linked to form an interconnected molecular network that restrains the volume of the liquid medium. In the same

study, texture profile of SCP's gels, showed that the produced structures were stronger compared to gels made with soy flour.

5.2 Nutritional profile and safety aspects of SCP

As mentioned above, SCP is characterized by high nutritional value. Amino acid profile, specifically the essential amino acid composition, is the most important criteria for the evaluation of the nutritional importance of SCP. A variety of studies have indicated that SCP, derived from several microorganisms consists of essential and non-essential amino acids. According to Agboola et al. [32], yeasts are particularly rich (>20 g/kg dry matter) in leucine, lysine, aspartic acid, and glutamic acid. Yeast species of *Cyberlindnera jadinii, Blastobotrys adeninivorans and Wickerhamomyces anomalus* showed similar amino acid profile to conventional fishmeal and soybean meal when they were cultivated in hydrolysates of pre-treated wood and chicken products. Moreover, Razzaq et al. [13] investigated the nutritional properties of SCP from *S. cerevisiae* cultivation on sugar-beet bagasse and they demonstrated that SCP contained a broad spectrum of essential amino acids like leucine (~43.5 g/kg), valine (~38.3 g/kg), and lysine (~31.4 g/kg). Similarly, in another study, SCP from *S. cerevisiae* using sugarcane bagasse, contained 17 amino acids including almost all essential amino acids (except threonine and tryptophan) while it was suggested that the amino acid profile of SCP and soya protein were quite comparable [62]. Another study evaluating the nutritional properties of photo hydrogenotrophic bacteria *(Rh. capsulatus, Rh. sphaeroides and Rps. palustris)* led to the conclusion that these kinds of bacteria are able to produce 37.5-42.5% of total essential amino acids [5]. Rasouli et al. [10] investigated the use of microbial biomass (bacteria, algae, and co-cultures) as potential candidate to recover nutrients from industrial wastewater and upcycle them to SCP, which is suitable for animal feed. Results

indicated that *C. sorokiniana* biomass contained essential amino acids for animal feed and *Methylococcus capsulatus* showed similar amino acid profiles compare to *C. sorokiniana* but in higher concentrations. However, amino acid profile of co- culture determined as most suitable for animal diets, due to its similarities to other commercialized protein sources, such as soy bean meal. Likewise, Amorim et al. [55] suggested that microalgae, like *C. vulgaris* and *A. platensis*, showed amino acid profiles similar to soybean which is currently the main source of protein used in feed. Although SCP's amino acid content is similar to the FAO guidelines, SCP derived from certain species presents high nucleic acid content which could cause problems such as gout and kidney stones [109]. The recommended amount of SCP supplemented to human diet should have nucleic acid contents below 2% [20]. During human metabolism, nucleic acid is converted into purines. The latter raise the uric acid levels in plasma leading to several side-effects [34]. Moreover, studies have reported that SCP's usage could lead to allergies, toxicity or even contamination with other microbes [110].

6. Sensorial aspects and consumer perception

In recent years, there has been a shift towards investigating the usage of alternative protein sources and how they could possibly affect consumers' perception. Examples of alternative protein sources are plant- based protein, insect protein, in vitro or cultured meat, and SCP [111]. However, regarding consumer perception and acceptance, several factors must be taken into consideration. For instance, when older adults' attitudes to accept the consumption of a variety of alternative protein sources, such as plant-based protein, insects, SCP, and in vitro meat were investigated, gender and country of residence were found to influence acceptance [111]. Specifically, plant-based protein was the most acceptable alternative reaching 58%, followed by SCP (20%), insect-

based protein (9%), and in vitro meat-based protein (6%). Siegrist & Hartmann [112] evaluated the influence of cultured meat among ten countries, in parameters of perceived naturalness, disgust and food disgust sensitivity, trust and food neophobia. Results of this study indicated that perceived naturalness and disgust evoked by cultured meat were significant parameters in the acceptance of culture meat among all countries, while there are large cultural differences regarding the acceptance of this alternative protein. Regarding factors that influence consumer perception about insect- based foods, it seems that neophobia, disgust, visibility of insects in food products and familiarity have key roles [113]. According to Saint-Eve et al. [114], the addition of pea flour on plant-based snacks affected the liking in terms of texture such as "crispy" and "puffy", while flavor perception seems to be a hindrance of acceptance. The development of food products incorporating SCP still requires investigation regarding consumers attitudes and acceptability, as few studies have investigated this factor. In a recent study, SCP enriched bread was prepared by adding SCP, produced by food wastes, at different concentrations (4-12%) and sensory analysis in parameters of color (crust and crumb), aroma, flavor, taste, texture, and overall acceptability was conducted. Results showed that SCP concentration up to 4% can be added into the wheat flour without drastically affecting the organoleptic properties of the resulting bread [115]. Muniz et al. [116], studied protein enrichment of by-products (guava peels and cashew bagasse) using *S. cerevisiae* on cereal bars for human nutrition. Results showed an increase in the protein content and all cereal bars presented average scores of 7/10 for sensorial attributes and average 4/5 for purchase intention. Fradinho et. [117] evaluated *A. platensis* biomass at 2% incorporation in terms of color, odor, flavor, extensibility, texture, overall acceptability and buying intension in pasta samples, with the results showing that around 58% of consumers reported positive buying intension. Likewise, the addition of *Arthrospira platensis* (4%) to a white chocolate formulation

increased its protein, amino acid, lipid, and mineral contents without modifying its sensory acceptance [118]. Garcia- Segovia et al. [119] investigated the influence of microalga- based breadsticks on consumer perception and acceptability. Results showed that a sensory and emotional vocabulary generated by trained panel including terms which describing sensory characteristics including “bitter”, “salty”, “crunchy”, “hardness”, “intense aroma”, “green particles”, “roast”, “smooth texture”, “golden surface”, “algae flavor”, as well as terms associated to composition, nutritional characteristics and usage including “addictive”, “expensive”, “nutritive”, “with fiber” and so on. Moreover, it was indicated that microalgae breadsticks were as acceptable as the control breadsticks and consumers consider that the product is healthier, and they would understand if it had greater expense.

7. Concluding remarks

The interest of researchers and businesses in SCP production is continuously increasing due to the inability to meet the protein requirements of the world's growing population. The valorization of renewable resources generated from various waste streams of the food and agricultural sector, could favor the SCP production in terms of socio-, economic- and environmental-sustainability. Especially in case that the SCP production is involved within biorefinery schemes, circular bio-economy concepts could be boosted, encouraging the further expansion of the SCP market in animal feed, innovative food formulations and bioactive food packaging. Important agro-industrial residues and side streams like olive-processing wastes, sugar- and/or lipid-rich resides, glycerol deriving from agro-industrial facilities, lignocellulosic solid resides or waste-waters, etc, [120], [121], [68], [122], [123] are important candidates, the valorization of which would certainly have much to offer on the environmental sustainability and

the economic viability of the fermentative SCP production process. The selection of highly efficient microbial cells including algae, fungi, yeasts, and bacteria is a great challenge for enhanced SCP production enclosing value-added properties which are greatly dependent on the cultivation and processing conditions. The production of microbial biomass (from selected fungi and algae i.e. *Zygomycetes*) accumulating simultaneously significant Polyunsaturated fatty acids PUFAs and/or polysaccharides (i.e. β-glycans) as well as important nutraceutical compounds (i.e. ergothioneine, ergosterol, etc) could further increase the nutritional and economic value of the microbial cells produced [124], [125], [69]. The presence of anti-nutritional factors that is, nucleic acids, in SCP, that are found in higher amounts compared to meat- and plant-based protein sources, is an emerging field of scientific investigation with much space for optimization and innovation. The implementation of advanced downstream processes that are green and circular, combined with novel physical processing for nucleic acids' elimination, might add extra value to SCP and expand its application pool. SCP presents a very attractive nutritional profile while it could stand as an alternative to partially replace so far established protein sources such as soymeal and fishmeal mainly in animal diets. SCP production valorizing renewable feedstock could address both food waste management and protein shortage issues of the modern society while consumers acceptance should be increased for the effective commercialization of this alternative.

Acknowledgements

This research was funded by the project "Infrastructure of Microbiome Applications in Food Systems-FOODBIOMES" (MIS 5047291), which is implemented under the Action "Regional Excellence in R&D Infrastructures", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund).

References

[1] United Nations (2019) https://www.un.org/development/desa/en/news/population/world-population-prospects-2019.html. Assessed: 05/10/2023.

[2] Shukla P.R., Skea J., Calvo Buendia E., Masson-Delmotte V., Pörtner H.-O., Roberts D. C., Zhai P., Slade R., Connors S., van Diemen R., Ferrat M., Haughey E., Luz S., Neogi S., Pathak M., Petzold J., Portugal Pereira J., Vyas P., Huntley E., Kissick K., Belkacemi M., Malley J.. IPCC: Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems. In press (2019) 1-894.

[3] FAO. Water for Sustainable Food and Agriculture :A report produced for the G20 Presidency of Germany. (2017) 1-27.

[4] Cai, Y., Tang, R., Tian, L. & Chang, S. X. Environmental impacts of livestock excreta under increasing livestock production and management considerations: Implications for developing countries. Current Opinion in Environmental Science and Health. 24 (2021) 100300.

[5] Spanoghe, J., Vermeir, P. & Vlaeminck, S. E. Microbial food from light, carbon dioxide and hydrogen gas: Kinetic, stoichiometric and nutritional potential of three purple bacteria. Bioresource Technology. 337 (2021) 125364.

[6] Global Market Insights (2022) https://www.globenewswire.com/en/news-release/2022/09/28/2523971/0/en/Single-Cell-Protein-Market-to-surpass-18-5Bn-by-2030-says-Global-Market-Insights-Inc.html. Assessed: 05/10/2023.

[7] Kerckhof, F. M. et al. From Biogas and Hydrogen to Microbial Protein Through Co-Cultivation of Methane and Hydrogen Oxidizing Bacteria. Frontiers in Bioengineering and Biotechnology. 9 (2021) 1-17.

[8] Zha, X., Tsapekos, P., Zhu, X., Khoshnevisan, B., Lu, X., & Angelidaki, I. Bioconversion of wastewater to single cell protein by methanotrophic bacteria. Bioresource Technology. 320 (2021) 124351.

[9] Yang, X., Jiang, Y., Zou, R., Xu, M., Su, Y., Angelidaki, I., and Zhang, Y. Green electricity-driven simultaneous ammonia recovery and in-situ upcycling for microbial protein production. Chemical Engineering Journal. 430 (2022) 132890.

[10] Rasouli, Z., Valverde-Pérez, B., D'Este, M., De Francisci, D., and Angelidaki, I. Nutrient recovery from industrial wastewater as single cell protein by a co-culture of green microalgae and methanotrophs. Biochemical Engineering Journal. 134 (2018) 129–135.

[11] Hülsen T., Hsieh K., Lu Y., Tait S., Batstone D. J.. Simultaneous treatment and single cell protein production from agri-industrial wastewaters using purple phototrophic bacteria or microalgae – A comparison. Bioresource Technology. 254 (2018) 214-233.

[12] Haris, N., Manan, H., Jusoh, M., Khatoon, H., Katayama, T., and Kasan, N.A. Effect of different salinity on the growth performance and proximate composition of isolated indigenous microalgae species. Aquaculture Reports. 22 (2022) 100925.

[13] Razzaq, Z. U., Khan, M. K. I., Maan, A. A., & Rahman, S. ur. Characterization of single cell protein from Saccharomyces cerevisiae for nutritional, functional and antioxidant properties. Journal of Food Measurement and Characterization. 14(5), (2020) 2520–2528.

[14] Zhang, L., Zhou, P., Chen, Y.C., Cao, Q., Liu, X.F., and Li, D. The production of single cell protein from biogas slurry with high ammonia-nitrogen content by screened Nectaromyces rattus. Poultry Science. 100 (2021) 1-10.

[15] Zhou, P., Zhang, L., Ding, H., Gao, X., Chen, Y., and Li, D. Optimization of culture conditions of screened Galactomyces candidum for the production of single cell protein from biogas slurry. Electronic Journal of Biotechnology. 55 (2022) 47–54.

[16] Alhomodi, A.F., Zavadil, A., Berhow, M., Gibbons, W.R., and Karki, B. Application of Cocultures of Fungal Mycelium during Solid-State Fermentation of Canola Meal for Potential Feed Application. JAOCS, Journal of the American Oil Chemists' Society. 98 (2021) 509–517.

[17] Salgado, C.L., Muñoz, R., Blanco, A., and Lienqueo, M.E. Valorization and upgrading of the nutritional value of seaweed and seaweed waste using the marine fungi Paradendryphiella salina to produce mycoprotein. Algal Research 53 (2021).

[18] Hashemi S.S, Karimi K., Taherzadeh M. J. Valorization of vinasse and whey to protein and biogas through an environmental fungi-based biorefinery. 303 (2022) 114-138.

[19] KV, N.S Application of Microbes in Environmental and Microbial Biotechnology, Environmental and Microbial Biotechnology. 10 (2022) 323-338.

[20] Raziq, A. Single cell protein (SCP) production and potential substrates: A comprehensive review. Pure and Applied Biology. 9 (2020) 1743-1754.

[21] Khumchai J., Wongchai A., Onuma R., Sabour A., Alshiekheid M., Narayanan M., Karuppusamy I., Pugazhendhi A., Brindhadevi K., Chi N., T., L. A viable bioremediation

strategy for treating paper and pulp industry effluents and assessing the prospect of resulted bacterial biomass as single cell protein (SCP) using indigenous bacterial species. Chemosphere. 304 (2022) 135246.

[22] Spalvins, K., Zihare, L. & Blumberga, D. Single cell protein production from waste biomass: Comparison of various industrial by-products. Energy Procedia. 147 (2018) 409–418.

[23] Kornochalert, N., Kantachote, D., Chaiprapat, S. & Techkarnjanaruk, S. Use of Rhodopseudomonas palustris P1 stimulated growth by fermented pineapple extract to treat latex rubber sheet wastewater to obtain single cell protein. Annals of Microbiology. 64 (2014) 1021–1032.

[24] Yan, Jinyong, Bingnan Han, Xiaohua Gui, Guilong Wang, Li Xu, Yunjun Yan, Catherine Madzak, et al. 2018. "Engineering Yarrowia Lipolytica to Simultaneously Produce Lipase and Single Cell Protein from Agro-Industrial Wastes for Feed." Scientific Reports. 8 (2018) 1-10.

[25] Spalvins, K., Geiba, Z., Kusnere, Z., & Blumberga, D. Waste Cooking Oil as Substrate for Single Cell Protein Production by Yeast Yarrowia lipolytica. Environmental and Climate Technologies. 24(3) (2020) 457–469.

[26] Mensah, J.K.M., and Twumasi, P. Use of pineapple waste for single cell protein (SCP) production and the effect of substrate concentration on the yield. Journal of Food Process Engineering. 40 (2017) 1-9.

[27] Zhou, Y. M., Chen, Y. P., Guo, J. S., Shen, Y., Yan, P., & Yang, J. X. Recycling of orange waste for single cell protein production and the synergistic and antagonistic effects on production quality. Journal of Cleaner Production. 213 (2019) 384–392.

[28] Dunuweera, A.N., Nikagolla, D.N., and Ranganathan, K. Fruit Waste Substrates to Produce Single-Cell Proteins as Alternative Human Food Supplements and Animal Feeds Using Baker's Yeast (Saccharomyces cerevisiae). Journal of Food Quality. 2021 (2021) 1-6.

[29] Rages, A.A., Haider, M.M., and Aydin, M. Alkaline hydrolysis of olive fruits wastes for the production of single cell protein by Candida lipolytica. Biocatalysis and Agricultural Biotechnology. 33 (2021) 101999.

[30] Saejung, C., and Sanusan, W. Valorization of Lignocellulosic Wastes and Nutrient Recovery by Anoxygenic Photosynthetic Bacteria. Waste and Biomass Valorization. 12 (2021) 4835–4844.

[31] Hansen, J.Ø., Lagos, L., Lei, P., Reveco-Urzua, F.E., Morales-Lange, B., Hansen, L.D., Schiavone, M., Mydland, L.T., Arntzen, M.Ø., Mercado, L., et al. Down-stream processing of baker's yeast (Saccharomyces cerevisiae) – Effect on nutrient digestibility and immune response in Atlantic salmon (Salmo salar). Aquaculture. 530 (2021) 735707.

[32] Agboola, J.O., Lapeña, D., Øverland, M., Arntzen, M.Ø., Mydland, L.T., and Hansen, J.Ø. Yeast as a novel protein source - Effect of species and autolysis on protein and amino acid digestibility in Atlantic salmon (Salmo salar). Aquaculture. 546 (2022) 737312.

[33] Kupfer, V. M., Vogt, E. I., Siebert, A. K., Meyer, M. L., Vogel, R. F., & Niessen, L. Foam-stabilizing properties of the yeast protein PAU5 and evaluation of factors that can influence its concentration in must and wine. Food Research International. 102 (2017) 111–118.

[34] Bratosin, B.C.; Darjan, S.; Vodnar, D.C. Single Cell Protein: A Potential Substitute in Human and Animal Nutrition. Sustainability. 13 (2021) 1-24.

[35] Singha, S., Mahmutovic, M., Zamalloa, C., Stragier, L., Verstraete, W., Svagan, A.J., Das, O., and Hedenqvist, M.S. Novel bioplastic from single cell protein as a potential packaging material. ACS Sustainable Chemistry and Engineering. 9 (2021) 6337–6346.

[36] Uwineza, C.; Sar, T.; Mahboubi, A.; Taherzadeh, M.J. Evaluation of the Cultivation of Aspergillus oryzae on Organic Waste-Derived VFA Effluents and Its Potential Application as Alternative Sustainable Nutrient Source for Animal Feed. Sustainability. 13 (2021) 1-13.

[37] Abbott, D.W., Aasen, I.M., Beauchemin, K.A., Grondahl, F., Gruninger, R., Hayes, M., Huws, S., Kenny, D.A., Krizsan, S.J., Kirwan, S.F., et al. Seaweed and seaweed bioactives for mitigation of enteric methane: Challenges and opportunities. Animals. 10 (2020) 1–28.

[38] García Martínez, J. B., Pearce, J. M., Throup, J., Cates, J., Lackner, M., & Denkenberger, D. C. Methane Single Cell Protein: Potential to Secure a Global Protein Supply Against Catastrophic Food Shocks. Frontiers in bioengineering and biotechnology. 10 (2022) 906704.

[39] Alvarado, K. A., Martinez, J. B. G., Matassa, S., Egbejimba, J., & Denkenberger, D. Food in space from hydrogen-oxidizing bacteria. Acta Astronautica 180 (2021) 260-265.

[40] Alvarado, K. A., García Martínez, J. B., Brown, M. M., Christodoulou, X., Bryson, S., & Denkenberger, D. C. Food production in space from CO2 using microbial electrosynthesis. Bioelectrochemistry. 149 (2023) 1-9.

[41] Yadav, J.S.S., Bezawada, J., Elharche, S., Yan, S., Tyagi, R.D., and Surampalli, R.Y. Simultaneous single-cell protein production and COD removal with characterization of residual protein and intermediate metabolites during whey fermentation by K. marxianus. Bioprocess and Biosystems Engineering. 37 (2014) 1017–1029.

[42] Kurcz, A., Błażejak, S., Kot, A.M., Bzducha-Wróbel, A., and Kieliszek, M. Application of Industrial Wastes for the Production of Microbial Single-Cell Protein by Fodder Yeast Candida utilis. Waste and Biomass Valorization. 9, (2018) 57–64.

[43] Coimbra, J.M., Cristina dos Reis, K., Schwan, R.F., and Silva, C.F. Effect of the Strategy of Molasses Supplementation in Vinasse to High SCP Production and Rose Flavor Compound. Waste and Biomass Valorization. 12 (2021) 359–369.

[44] Myint, K.T., Otsuka, M., Okubo, A., Mitsuhashi, R., Oguro, A., Maeda, H., Shigeno, T., Sato, K., and Nakajima-Kambe, T. Isolation and identification of flower yeasts for the development of mixed culture to produce single-cell protein from waste milk. Bioresource Technology Reports. 10 (2020) 100401.

[45] Chakraborty, A., Paul, B.N., Bhattacharyya, D.K., Bhowal, J. Evaluation of fungal single cell protein as aqua feed on carcass analysis and growth performance of Cirrhinus reba fingerlings. Aquaculture Research. 53 (2022) 3140–3153.

[46] Al-Farsi1 M., Bakir Al. A., Marzouqi H. Al and Thomas R. Production of Single Cell Protein from Date Waste. Materials Research Proceedings. 11 (2019) 302-312.

[47] Nasseri A.T, Rasoul- Amini S., Morowvat M.H., Ghasemi. Y. Single Cell Protein: Production and Process. American Journal of Food Technology. 6 (2011) 103- 116.

[48] Sharif, M., Zafar, M.H., Aqib, A.I., Saeed, M., Farag, M.R., and Alagawany, M. Single cell protein: Sources, mechanism of production, nutritional value and its uses in aquaculture nutrition. Aquaculture. 531(2021) 735885.

[49] Sarris, D., Sampani, Z., Rapti, A., and Papanikolaou, S. Valorization of Crude Glycerol, Residue Deriving from Biodiesel- Production Process, with the Use of Wild-type New Isolated Yarrowia lipolytica Strains: Production of Metabolites with Pharmaceutical and Biotechnological Interest. Current Pharmaceutical Biotechnology. 20 (2019) 881–894.

[50] Tzirita, M., Kremmyda, M., Sarris, D., Koutinas, A.A., and Papanikolaou, S. Effect of Salt Addition upon the Production of Metabolic Compounds by Yarrowia lipolytica Cultivated on Biodiesel-Derived Glycerol Diluted with Olive-Mill Wastewaters. Energies. 12 (2019) 1-19.

[51] García-Garibay, M., Gómez-Ruiz, L., Cruz-Guerrero, A.E., and Bárzana, E. Single Cell Protein: Yeasts and Bacteria. In Encyclopedia of Food Microbiology: Second Edition (2014) 431–438.

[52] Capson-Tojo, G., Batstone, D.J., Grassino, M., Vlaeminck, S.E., Puyol, D., Verstraete, W., Kleerebezem, R., Oehmen, A., Ghimire, A., Pikaar, I., et al. Purple phototrophic bacteria for resource recovery: Challenges and opportunities. Biotechnology Advances. 43 (2020) 107567.

[53] Alloul, A., Cerruti, M., Adamczyk, D., Weissbrodt, D.G., and Vlaeminck, S.E. Operational strategies to selectively produce purple bacteria for microbial protein in raceway reactors. Environmental Science and Technology. 55 (2021) 8278–8286.

[54] Sial, A., Zhang, B., Zhang, A., Liu, K.Y., Imtiaz, S.A., and Yashir, N. Microalgal–Bacterial Synergistic Interactions and Their Potential Influence in Wastewater Treatment: a Review. Bioenergy Research. 14 (2021) 723–738.

[55] Amorim, M.L., Soares, J., Coimbra, J.S. dos R., Leite, M. de O., Albino, L.F.T., and Martins, M.A. Microalgae proteins: production, separation, isolation, quantification, and application in food and feed. Critical Reviews in Food Science and Nutrition. 61 (2021)1976–2002.

[56] Raji, A.A., Junaid, O.Q., Milow, P., Taufek, N.M., Fada, A.M., Kolawole, A.A., Alias, Z., and Razak, S.A. Partial replacement of fishmeal with Spirulina platensis and Chlorella vulgaris and its effect on growth and body composition of African catfish Clarias gariepinus (Burchell 1822). Indian Journal of Fisheries. 66 (2019) 100–111.

[57] Roy-Lachapelle, A., Solliec, M., Bouchard, M.F., and Sauvé, S. Detection of cyanotoxins in algae dietary supplements. Toxins. 9 (2017) 1-17.

[58] Koutinas, A. A., Vlysidis, A., Pleissner, D., Kopsahelis, N., Lopez Garcia, I., Kookos, I. K., Lin, C. S. K. Valorization of industrial waste and by-product streams via fermentation for the production of chemicals and biopolymers. Chemical Society Reviews. Royal Society of Chemistry. 43 (2014) 2587-2627.

[59] Qin, L., Liu, L., Zeng, A. P., & Wei, D. From low-cost substrates to Single Cell Oils synthesized by oleaginous yeasts. Bioresource Technology. 245 (2017) 1507-1519.

[60] Papanikolaou, S. & Aggelis, G. Microbial products from wastes and residues. FEMS Microbiology Letters. 367 (2020) 1-4.

[61] Spalvins, K., Ivanovs, K., and Blumberga, D. Single cell protein production from waste biomass: Review of various agricultural by-products. Agronomy Research. 16 (2018) 1493–1508.

[62] Samadi, S., Mohammadi, M., and Najafpour, G.D. Production of single cell protein from sugarcane bagasse by Saccharomyces cerevisiae in tray bioreactor. International Journal of Engineering, Transactions B: Applications. 29 (2016) 1029–1036.

[63] Tsouko, E., Papanikolaou, S., & Koutinas, A. A. (2016). Production of fuels from microbial oil using oleaginous microorganisms. Handbook of biofuels production. Woodhead Publishing (2016) 201-236.

[64] Athenaki M., Gardeli C., Diamantopoulou P., Tchakouteu S.S., Sarris D., Philippoussis A., Papanikolaou S.Lipids from yeasts and fungi: physiology, production and analytical considerations, Journal of Applied Microbiology. 124 (2018) 336–367.

[65] Vasilakis, G., Karayannis, D., Massouras, T., Politis, I., & Papanikolaou, S. Biotechnological conversions of Mizithra second cheese whey by wild-type non-conventional yeast strains: Production of yeast cell biomass, single-cell oil and polysaccharides. Applied Sciences. 12 (2022) 11471.

[66] Rajoka, M. I., Ahmed, S., Hashmi, A. S., & Athar, M. Production of microbial biomass protein from mixed substrates by sequential culture fermentation of Candida utilis and Brevibacterium lactofermentum. Annals of Microbiology 62(3) (2012) 1173–1179.

[67] Anvari, M., & Khayati, G. Submerged yeast fermentation of cheese whey for protein production and nutritional profile analysis. Advance Journal of Food Science and Technology. 3(2) (2011) 122-126.

[68] Kothri M, Mavrommati M, Elazzazy AM, Baeshen MN., Moussa T. AA, Aggelis G.. Microbial sources of polyunsaturated fatty acids (PUFAs) and the prospect of organic residues and

wastes as growth media for PUFA-producing microorganisms. FEMS Microbiology Letters. 367 (2020) 1-11.

[69] Bellou, S., Triantaphyllidou, I. E., Aggeli, D., Elazzazy, A. M., Baeshen, M. N., & Aggelis, G. Microbial oils as food additives: recent approaches for improving microbial oil production and its polyunsaturated fatty acid content. Current opinion in Biotechnology 37, (2016) 24-35.

[70] Ahmad, T., Aadil, R.M., Ahmed, H., Rahman, U. ur, Soares, B.C.V., Souza, S.L.Q., Pimentel, T.C., Scudino, H., Guimarães, J.T., Esmerino, E.A., et al. Treatment and utilization of dairy industrial waste: A review. Trends in Food Science and Technology. 88 (2019) 361–372.

[71] Eurostat (2021) https://ec.europa.eu/eurostat/en/web/products-eurostat-news/-/ddn-20211118-1. Assessed: 05/10/2023.

[72] Lappa, I.K., Papadaki, A., Kachrimanidou, V., Terpou, A., Koulougliotis, D., Eriotou, E., and Kopsahelis, N. Cheese whey processing: Integrated biorefinery concepts and emerging food applications. Foods. 8 (2019) 1-37.

[73] Tsermoula, P., Khakimov, B., Nielsen, J. H. & Engelsen, S. B. WHEY - The waste-stream that became more valuable than the food product. Trends in Food Science and Technology. 118 (2021) 230–241.

[74] Yadav, J.S.S., Bezawada, J., Ajila, C.M., Yan, S., Tyagi, R.D., and Surampalli, R.Y. Mixed culture of Kluyveromyces marxianus and Candida krusei for single-cell protein production and organic load removal from whey. Bioresource Technology. 164 (2014) 119–127.

[75] Jamir L., Kumar V., Kaur J., Kumar S. & Singh H. Composition, valorization and therapeutical potential of molasses: a critical review, Environmental Technology Reviews. 10:1 (2021) 131-142.

[76] Statista (2022) https://www.statista.com/statistics/495973/sugar-production-worldwide/#statisticContainer. Assessed: 05/10/2023.

[77] Arslan1 N. P. and Aydogan M.N. Evaluation of Sheep Wool Protein Hydrolysate and Molasses as Low-Cost Fermentation Substrates for Hyaluronic Acid Production by Streptococcus zooepidemicus ATCC 35246. Waste and Biomass Valorization. 12 (2021) 925-935.

[78] Saejung, C., and Salasook, P. Recycling of sugar industry wastewater for single-cell protein production with supplemental carotenoids. Environmental Technology (United Kingdom). 41 (2018) 59–70.

[79] Thiviya, P., Gamage, A., Kapilan, R., Merah, O., and Madhujith, T. Single Cell Protein Production Using Different Fruit Waste: A Review. Separations. 9 (2022) 1-17.

[80] Kamal, M.M., Ali, M.R., Shishir, M.R.I., Saifullah, M., Haque, M.R., and Mondal, S.C. Optimization of process parameters for improved production of biomass protein from Aspergillus niger using banana peel as a substrate. Food Science and Biotechnology. 28 (2019) 1693–1702.

[81] Kamusoko, R., Jingura, R.M., Parawira, W., and Chikwambi, Z. Strategies for valorization of crop residues into biofuels and other value-added products. Biofuels, Bioproducts and Biorefining. 15 (2021) 1950–1964.

[82] Cherubin, M. R., Oliveira, D. M. D. S., Feigl, B. J., Pimentel, L. G., Lisboa, I. P., Gmach, M. R., Cerri, C. C. Crop residue harvest for bioenergy production and its implications on soil functioning and plant growth: A review. *Scientia Agricola*, *75*(3) (2018) 255–272.

[83] Deshwal G.K., Alam T., Panjagari N.R., Bhardwaj A. Utilization of Cereal Crop Residues, Cereal Milling, Sugarcane and Dairy Processing By-Products for Sustainable Packaging Solutions. Journal of Polymers and the Environment (2021).

[84] Yang F., Jin Z., Nawaz M., Xiao Y., Jiang Y., Hu J., Lib J. and Gao M.T. Oligosaccharides in straw hydrolysate could improve the production of single-cell protein with Saccharomyces cerevisiae. Journal of the Science of Food and Agriculture. (2021) 1-9.

[85] Upcraft T., Tu W.C, Johnson R., Finnigan T., Van Hung N., Hallett J. and Guo M. Protein from renewable resources: mycoprotein production from agricultural residues. Green Chemistry. 23 (2021) 5150-5165.

[86] Yunus F.N., Nadeem M. and Rashid F. Single-cell protein production through microbial conversion of lignocellulosic residue (wheat bran). Institute of Brewing& Distilling. 121 (2015) 553-557.

[87] Drzymała, K., Mirończuk, A.M., Pietrzak, W., and Dobrowolski, A. Rye and oat agricultural wastes as substrate candidates for biomass production of the non-conventional yeast Yarrowia lipolytica. Sustainability (Switzerland). 12 (2020) 1-12.

[88] Aggelopoulos, T., Katsieris, K., Bekatorou, A., Pandey, A., Banat, I.M., and Koutinas, A.A. Solid state fermentation of food waste mixtures for single cell protein, aroma volatiles and fat production. Food Chemistry. 145 (2014) 710–716.

[89] Papanikolaou, S., & Aggelis, G. (2009). Biotechnological valorization of biodiesel derived glycerol waste through production of single cell oil and citric acid by Yarrowia lipolytica. Lipid technology. 21 (2009) 83-87.

[90] Sarantou S, Stoforos NG, Kalantzi O, Papanikolaou S.. Biotechnological valorization of biodiesel-derived glycerol: Trials with the non-conventional yeasts Yarrowia lipolytica and Rhodosporidium sp. Carbon Resources Conversion. 4 (2021) 61-75.

[91] Filippousi R., Tsouko E., Mordini K., et al., Sustainable arabitol production by a newly isolated Debaryomyces prosopidis strain cultivated on biodiesel-derived glycerol. Sustainable arabitol production by a newly isolated *Debaryomyces prosopidis* strain cultivated on biodiesel-derived glycerol. Carbon Resources Conversion. 5 (2022) 92-99.

[92] Diamantopoulou P, Papanikolaou S.. Biotechnological production of sugar-alcohols: Focus on Yarrowia lipolytica and edible/medicinal mushrooms. Process Biochemistry. 124 (2023) 113-131.

[93] Meesters, P.A.E.P., Huijberts, G.N.M. and Eggink, G.. High cell density cultivation of the lipid accumulating yeast *Cryptococcus curvatus* using glycerol as a carbon source. Appl Microbiol Biotechnol. 45 (1996) 575-579.

[94] Yossa, R., Greiling, A.M., Basiita, R.K., Sakala, M.E., Baumgartner, W.A., Taylor, A., and Gatlin, D.M. Replacing fishmeal with a single cell protein feedstuff in Nile tilapia Oreochromis niloticus diets. Animal Feed Science and Technology. 281 (2021) 115089.

[95] Øverland, M., Karlsson, A., Mydland, L.T., Romarheim, O.H., and Skrede, A. Evaluation of Candida utilis, Kluyveromyces marxianus and Saccharomyces cerevisiae yeasts as protein sources in diets for Atlantic salmon (Salmo salar). Aquaculture. 402–403 (2013) 1-7.

[96] Guo, J., Qiu, X., Salze, G., and Davis, D.A. Use of high-protein brewer's yeast products in practical diets for the Pacific white shrimp Litopenaeus vannamei. Aquaculture Nutrition. 25 (2019) 680–690.

[97] Hamidoghli, A., Yun, H., Won, S., Kim, S.K., Farris, N.W., and Bai, S.C. Evaluation of a single-cell protein as a dietary fish meal substitute for whiteleg shrimp Litopenaeus vannamei. Fisheries Science. 85 (2019) 147–155.

[98] Li, M., Liang, H., Xie, J., Chao, W., Zou, F., Ge, X., and Ren, M.. Diet supplemented with a novel Clostridium autoethanogenum protein have a positive effect on the growth performance, antioxidant status and immunity in juvenile Jian carp (Cyprinus carpio var. Jian). Aquaculture Reports. 19 (2021) 100572.

[99] Adeoye, A.A., Akegbejo-Samsons, Y., Fawole, F.J., Olatunji, P.O., Muller, N., Wan, A.H.L., and Davies, S.J. From waste to feed: Dietary utilisation of bacterial protein from fermentation of agricultural wastes in African catfish (Clarias gariepinus) production and health. Aquaculture. 531 (2021) 735850.

[100] Alloul, A., Wille, M., Lucenti, P., Bossier, P., Van Stappen, G., and Vlaeminck, S.E. Purple bacteria as added-value protein ingredient in shrimp feed: Penaeus vannamei growth performance, and tolerance against Vibrio and ammonia stress. Aquaculture. 530 (2021) 735788.

[101] Trajkovska Petkoska, A., Daniloski, D., D'Cunha, N. M., Naumovski, N. & Broach, A. T. Edible packaging: Sustainable solutions and novel trends in food packaging. Food Research International. 140 (2021) 109981.

[102] Efthymiou, M.N., Tsouko, E., Papagiannopoulos, A., Athanasoulia, I.G., Georgiadou, M., Pispas, S., Briassoulis, D., Tsironi, T., and Koutinas, A. Development of biodegradable films using sunflower protein isolates and bacterial nanocellulose as innovative food packaging materials for fresh fruit preservation. Scientific Reports. 12 (2022) 1-13.

[103] Papadaki, A., Kachrimanidou, V., K. Lappa, I., Andriotis, H., Eriotou, E., Mandala, I., and Kopsahelis, N. Tuning the physical and functional properties of whey protein edible films: Effect of pH and inclusion of antioxidants from spent coffee grounds. Sustainable Chemistry and Pharmacy. 27 (2022) 100700.

[104] Efthymiou, M. N., Tsouko, E., Vlassi, E., Papagiannopoulos, A., Koutinas, A., & Pispas, S.. Bio-based and Nanostructured Hybrids for Green and Active Food Packaging. In Bio-and Nano-sensing Technologies for Food Processing and Packaging Royal Society of Chemistry (2022) 81-128.

[105] Khattab, A.M., Esmael, M.E., Farrag, A.A., and Ibrahim, M.I.A. Structural assessment of the bioplastic (poly-3-hydroxybutyrate) produced by Bacillus flexus Azu-A2 through cheese whey valorization. International Journal of Biological Macromolecules. 190 (2021) 319–332.

[106] Papadaki, A., Lappa, I.K., Kachrimanidou, V., Gonou-Zagou, Z., and Kopsahelis, N. Trametes versicolor as a Natural Source of Bioactive Compounds for the Production of Whey Protein Films with Functional Properties: A Holistic Approach to Valorize Cheese Whey. Waste and Biomass Valorization. 13 (2022) 3989–3998.

[107] Patias, L.D., Maroneze, M.M., Siqueira, S.F., de Menezes, C.R., Zepka, L.Q., and Jacob-Lopes, E. Single-Cell Protein as a Source of Biologically Active Ingredients for the Formulation of Antiobesity Foods. Alternative and Replacement Foods.11 (2018) 317–353.

[108] Paraskevopoulou, A., Athanasiadis, I., Kanellaki, M., Bekatorou, A., Blekas, G., and Kiosseoglou, V. Functional properties of single cell protein produced by kefir microflora. Food Research International. 36 (2003) 431–438.

[109] Ritala, A., Häkkinen, S.T., Toivari, M., and Wiebe, M.G. Single cell protein-state-of-the-art, industrial landscape and patents 2001-2016. Frontiers in Microbiology. 8 (2017) 1-18.

[110] Hadi, J. and Brightwell G. "Insect Protein and Single-Cell Protein." Foods. 10 (2021) 1–29.

[111] Grasso, A.C., Hung, Y., Olthof, M.R., Verbeke, W., and Brouwer, I.A. Older consumers' readiness to accept alternative, more sustainable protein sources in the European Union. Nutrients. 11(2019) 1-18.

[112] Siegrist, M., and Hartmann, C. Perceived naturalness, disgust, trust and food neophobia as predictors of cultured meat acceptance in ten countries. Appetite 155 (2020) 104814.

[113] Wendin, K.M., and Nyberg, M.E. Factors influencing consumer perception and acceptability of insect-based foods. Current Opinion in Food Science. 40 (2021) 67–71.

[114] Saint-Eve, A., Granda, P., Legay, G., Cuvelier, G., and Delarue, J. Consumer acceptance and sensory drivers of liking for high plant protein snacks. Journal of the Science of Food and Agriculture. 99 (2019) 3983–3991.

[115] Khan, M.K.I., Asif, M., Razzaq, Z.U., Nazir, A., and Maan, A.A. Sustainable food industrial waste management through single cell protein production and characterization of protein enriched bread. Food Bioscience. 46 (2022) 101406.

[116] Muniz, C.E.S., Santiago, Â.M., Gusmão, T.A.S., Oliveira, H.M.L., Conrado, L. de S., and Gusmão, R.P. de Solid-state fermentation for single-cell protein enrichment of guava and cashew by-products and inclusion on cereal bars. Biocatalysis and Agricultural Biotechnology. 25 (2020) 101576.

[117] Fradinho, P., Niccolai, A., Soares, R., Rodolfi, L., Biondi, N., Tredici, M.R., Sousa, I., and Raymundo, A. Effect of Arthrospira platensis (spirulina) incorporation on the rheological and bioactive properties of gluten-free fresh pasta. Algal Research 45 (2020) 101743.

[118] Özbal, B.; Çelekli, A.; Gün, D.; Bozkurt, H. Effect of Arthrospira platensis incorporation on nutritional and sensory attributes of white chocolate. Int. J. Gastron. Food Sci. 28 (2022) 109908.

[119] García-Segovia, P., García Alcaraz, V., Tárrega, A., and Martínez-Monzó, J. Consumer perception and acceptability of microalgae based breadstick. Food Science and Technology International. 26 (2020) 493–502.

[120] Tsioulpas A, Dimou D, Iconomou D, Aggelis G.. Phenolic removal in olive oil mill wastewater by strains of *Pleurotus* spp. In respect to their phenol oxidase (laccase) activity. *Bioresource Technology*. 84 (2002) 251–257.

[121] Economou CN, Aggelis G, Pavlou S, Vayenas DV. Modeling of single-cell oil production under nitrogen-limited and substrate inhibition conditions. Biotechnology Bioeng. 108 (2011) 1049–1055.

[122] Valdés G, Mendonça RT & Aggelis G. Lignocellulosic biomass as a substrate for oleaginous microorganisms: A review. Applied Sciences. 10 (2020) 1-40.

[123] Dritsas P, Aggelis G. Studies on the co-metabolism of glucose and glycerol in the fungus Umbelopsis isabellina. Carbon Resour Convers. In press (2023).

[124] Bellou, S., Baeshen, M. N., Elazzazy, A. M., Aggeli, D., Sayegh, F., & Aggelis, G.. Microalgal lipids biochemistry and biotechnological perspectives. Biotechnology advances. 32(8), (2014)1476-1493.

[125] Tsouko, E., Papadaki, A., Papanikolaou, S., Danezis, G. P., Georgiou, C. A., Freire, D. M., & Koutinas, A. Enzymatic production of isopropyl and 2-ethylhexyl esters using γ-linolenic acid rich fungal oil produced from spent sulphite liquor. Biochemical Engineering Journal. 169, (2021) 107956.

[126] Sorour, M., Ramadan, B., Mehanni, A., and Abdelsabour, N.. Single cell protein production using low quality fruits of some dates. Archives of Agriculture Sciences Journal. 4 (2021) 115–126.

[127] Svensson, S. E., Bucuricova, L., Ferreira, J. A., Filho, P. F. S., Taherzadeh, M. J., & Zamani, A. (2021). Valorization of bread waste to a fiber-and protein-rich fungal biomass. Fermentation. 7 (2021) 1-14.

[128] Amr Salem Awad, Salah Mosatafa Saad, E. M. and N. Y. A. (2021). Production of Single-Cell Protein from some Fruit peels. Annals of Agricultural Science, Moshtohor. 59(3) (2021) 755–762.

[129] Papanikolaou, S., Chevalot, I., Galiotou-Panayotou, M., Komaitis, M., Marcc, I., & Aggelis, G. Industrial derivative of tallow: a promising renewable substrate for microbial lipid, single-cell protein and lipase production by *Yarrowia lipolytica*. Electron J Biotechnol 10 (2007) 425-435.

[130] Szotkowski, M., Byrtusova, D., Haronikova, A., Vysoka, M., Rapta, M., Shapaval, V., & Marova, I. Study of metabolic adaptation of red yeasts to waste animal fat substrate. Microorganisms. 7 (2019) 1-28.

[131] Moreira, M. D., Melo, M. M., Coimbra, J. M., Reis, K. C. dos, Schwan, R. F., & Silva, C. F.. Solid coffee waste as alternative to produce carotenoids with antioxidant and antimicrobial activities. Waste Management. 82 (2018) 93–99.

[132] Pillaca-Pullo, O. S., Lopes, A. M., Rodriguez-Portilla, L. M. I., & Estela-Escalante, W.. Optimizing medium composition with wastewater from Coffea arabica processing to produce single-cell protein using Candida sorboxylosa. Journal of Chemical Technology and Biotechnology. 98 (2023) 106–116.

[133] Sarris, D., Stoforos, N. G., Mallouchos, A., Kookos, I. K., Koutinas, A. A., Aggelis, G., & Papanikolaou, S.. Production of added-value metabolites by Yarrowia lipolytica growing in olive mill wastewater-based media under aseptic and non-aseptic conditions. Engineering in Life Sciences. 17 (2017) 695–709.

[134] Floetenmeyer, M.D., Glatz, B.A., Hammond, E.G. Continuous culture fermentation of whey permeate to produce microbial oil. Journal of Dairy Science. 68 (1985) 633-637.

[135] Ykema, A., Verbree, E.C., Kater, M.M., Smit, H. Optimization of lipid production in the oleaginous yeast *Apiotrichum curvatum* in whey permeate. Applied Microbiology and Biotechnology. 29 (1988) 211-218.

[136] Vamvakaki, A.-N., Kandarakis, I., Kaminarides, S., Komaitis, M., Papanikolaou, S. Cheese whey as a renewable substrate for microbial lipid and biomass production by Zygomycetes. Engineering in Life Sciences.10 (2010) 348-360.

[137] Seo, Y.H., Lee, I., Jeon, S.H., Han, J.I. Efficient conversion from cheese whey to lipid using *Cryptococcus curvatus*. Biochemical Engineering Journal. 90 (2014) 149-153.

[138] Tchakouteu, S.S., Chatzifragkou, A., Kalantzi, O., Koutinas, A.A., Aggelis, G., Papanikolaou, S. Oleaginous yeast *Cryptococcus curvatus* exhibits interplay between biosynthesis of intracellular sugars and lipids. European Journal of Lipid Science and Technology. 117 (2014) 657-672.

[139] Carota, E., Crognale, S., D'Annibale, A., Gallo, A.M., Stazi, S.R., Petruccioli, M. A sustainable use of Ricotta cheese whey for microbial biodiesel production. Science of the Total Environment. 584 (2017) 554-560.

[140] Vyas, S., & Chhabra, M. Assessing oil accumulation in the oleaginous yeast *Cystobasidium oligophagum* JRC1 using dairy waste cheese whey as a substrate. 3 Biotech. 9 (2019) 1-13.

[141] Papanikolaou, S., & Aggelis, G.. Lipid production by Yarrowia lipolytica growing on industrial glycerol in a single-stage continuous culture. Bioresource technology. 82 (2002) 43-49.

[142] Makri, A., Fakas, S. and Aggelis, G.. Metabolic activities of biotechnological interest in *Yarrowia lipolytica* grown on glycerol in repeated batch cultures. Bioresour Technol 101 (2010) 2351-2358.

[143] Liang, Y., Cui, Y., Trushenski, J., & Blackburn, J. W.. Converting crude glycerol derived from yellow grease to lipids through yeast fermentation. Bioresource technology, 101 (2010) 7581-7586.

[144] Saenge, C., Cheirsilp, B., Suksaroge, T.T. and Bourtoom, T. Potential use of oleaginous red yeast *Rhodotorula glutinis* for the bioconversion of crude glycerol from biodiesel plant to lipids and carotenoids. Proc Biochem. 46 (2011) 210-218.

[145] Cui, Y., Blackburn, J.W. and Liang, Y. Fermentation optimization for the production of lipid by *Cryptococcus curvatus*: Use of response surface methodology. Biomass Bioenerg. 47 (2012) 410-417.

[146] Fontanille, P., Kumar, V., Christophe, G., Nouaille, R. and Larroche, C. Bioconversion of volatile fatty acids into lipids by the oleaginous yeast *Yarrowia lipolytica*. Bioresour Technol. 114 (2012) 443-449.

[147] Xu, J., Zhao, X., Wang, W., Du, W. and Liu, D. (2012) Microbial conversion of biodiesel byproduct glycerol to triacylglycerols by oleaginous yeast *Rhodosporidium toruloides* and the individual effect of some impurities on lipid production. Biochem Eng J. 65 (2012) 30-36.

[148] Celińska, E., & Grajek, W.. A novel multigene expression construct for modification of glycerol metabolism in Yarrowia lipolytica. Microbial Cell Factories. 12 (2013) 1-16.

[149] Duarte, S.H., Ghiselli, S. and Maugeri, F. Influence of culture conditions on lipid production by *Candida* sp. LEB-M3 using glycerol from biodiesel synthesis. Biocatal Agric Biotechnol. 2 (2013) 339-343.

[150] Kitcha, S., & Cheirsilp, B. Enhancing lipid production from crude glycerol by newly isolated oleaginous yeasts: strain selection, process optimization, and fed-batch strategy. Bioenergy Research. 6 (2013) 300-310.

[151] Kitcha, S., & Cheirsilp, B.. Enhanced lipid production by co-cultivation and co-encapsulation of oleaginous yeast Trichosporonoides spathulata with microalgae in alginate gel beads. Applied biochemistry and biotechnology. 173 (2014) 522-534.

[152] Cheirsilp, B., & Louhasakul, Y.. Industrial wastes as a promising renewable source for production of microbial lipid and direct transesterification of the lipid into biodiesel. Bioresource technology, 142 (2013) 329-337.

[153] Ryu, B. G., Kim, J., Kim, K., Choi, Y. E., Han, J. I., & Yang, J. W. (2013). High-cell-density cultivation of oleaginous yeast Cryptococcus curvatus for biodiesel production using organic waste from the brewery industry. Bioresource technology. 135 (2013) 357-364.

[154] Kiran, E. U., Trzcinski, A., & Webb, C.. Microbial oil produced from biodiesel by-products could enhance overall production. Bioresource technology. 129 (2013) 650-654.

[155] Poli, J.S., da Silva, M.A.N., Siqueira, E.P., Pasa, V.M.D., Rosa, C.A. and Valente, P. Microbial lipid produced by Yarrowia lipolytica QU21 using industrial waste: A potential feedstock for biodiesel production. Bioresour Technol. 161(2014) 320-326.

[156] Sestric, R., Munch, G., Cicek, N., Sparling, R. and Levin, D.B. Growth and neutral lipid synthesis by *Yarrowia lipolytica* on various carbon substrates under nutrient-sufficient and nutrient-limited conditions. Bioresour Technol. 164 (2014) 41-46.

[157] Yang, X., Jin, G., Gong, Z., Shen, H., Bai, F. and Zhao, Z.K. Recycling biodiesel-derived glycerol by the oleaginous yeast *Rhodosporidium toruloides* Y4 through the two-stage lipid production process. Biochem Eng J. 91 (2014) 86-91.

[158] Kamal, R., Liu, Y., Li, Q., Huang, Q., Wang, Q., Yu, X., & Zhao, Z. K. Exogenous L-proline improved Rhodosporidium toruloides lipid production on crude glycerol. Biotechnology for biofuels. sarantou13 (2020) 1-11.

[159] Papanikolaou, S., Fakas, S., Fick, M., Chevalot, I., Galiotou-Panayotou, M., Komaitis, M., Marc, I. and Aggelis, G. Biotechnological valorisation of raw glycerol discharged after bio-diesel (fatty acid methyl-esters) manufacturing process: production of 1,3-propanediol, citric acid and single cell oil. Biomass Bioenerg. 32 (2008) 60-71.

[160] Fakas, S., Papanikolaou, S., Batsos, A., Galiotou-Panayotou, M., Mallouchos, A. and Aggelis, G. Evaluating renewable carbon sources as substrates for single cell oil production by *Cunninghamella echinulata* and *Mortierella isabellina.* Biomass Bioenerg. 33 (2009) 573-580.

[161] André, A., Diamantopoulou, P., Philippoussis, A., Sarris, D., Komaitis, M., & Papanikolaou, S.. Biotechnological conversions of bio-diesel derived waste glycerol into added-value compounds by higher fungi: production of biomass, single cell oil and oxalic acid. Industrial Crops and Products. 31 (2010) 407-416.

[162] Liang, Y., Sarkany, N., Cui, Y., Yesuf, J., Trushenski, J., & Blackburn, J. W. (2010). Use of sweet sorghum juice for lipid production by Schizochytrium limacinum SR21. Bioresource Technology. 101 (2010) 3623-3627.

[163] Ethier, S., Woisard, K., Vaughan, D. and Wen, Z. Continuous culture of the microalgae *Schizochytrium limacinum* on biodiesel-derived crude glycerol for producing docosahexaenoic acid. Bioresour Technol . 102 (2011) 88-93.

[164] Bellou, S., Moustogianni, A., Makri, A. and Aggelis, G. Lipids containing polyunsaturated fatty acids synthesized by zygomycetes grown on glycerol. Appl Biochem Biotechnol. 166 (2012) 146-158.

[165] Dedyukhina, E.G., Chistyakova, T.I., Kamzolova, S.V., Vinter, M.V. and Vainshtein, M.B. Arachidonic acid synthesis by glycerol-grown *Mortierella alpine*. Eur J Lipid Sci Technol. 114 (2012) 833-841.

[166] Chang, G., Luo, Z., Gu. S., Wu, Q., Chang, M. and Wang, X. Fatty acid shifts and metabolic activity changes of *Schizochyrium* sp. S31 cultured on glycerol. Bioresour Technol. 142 (2013) 255-260.

[167] Dedyukhina, E.G., Chistyakova, T.I., Mironov, A.A., Kamzolova, S.V., Morgunov, I.G. and Vainshtein, M.B. Arachidonic acid synthesis from biodiesel-derived waste by *Mortierella alpina*. Eur J Lipid Sci Technol. 116 (2014) 429-437.

Figure captions

Figure 1. Properties and application of SCP.

Figure 2. Renewable resources for SCP production.

Figure 3. Conversion of cellulose and hemicellulose into monosaccharides.

Fig 1

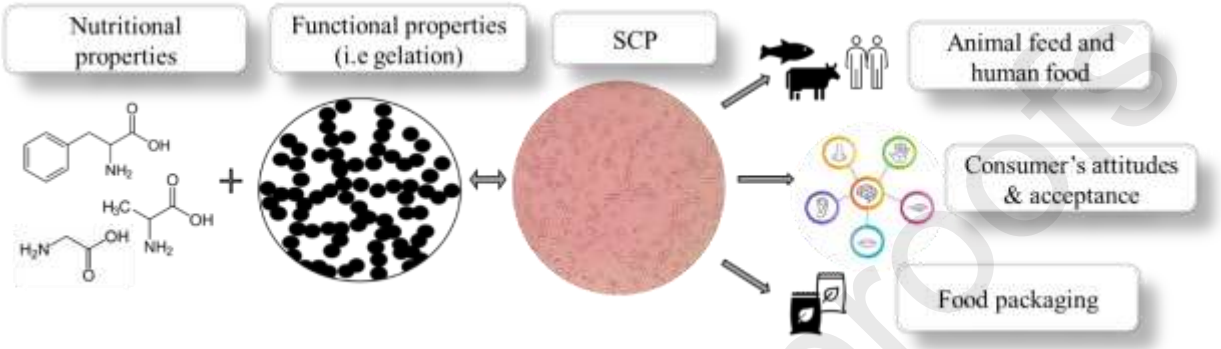
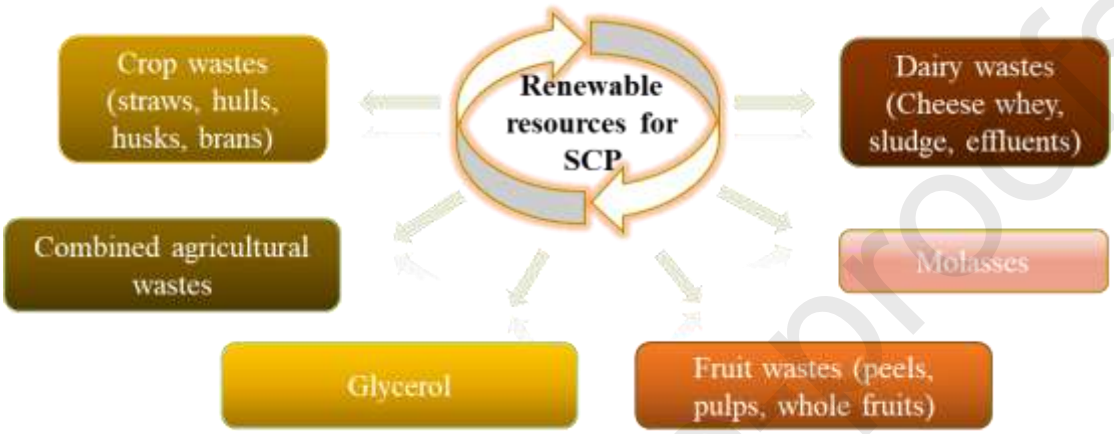


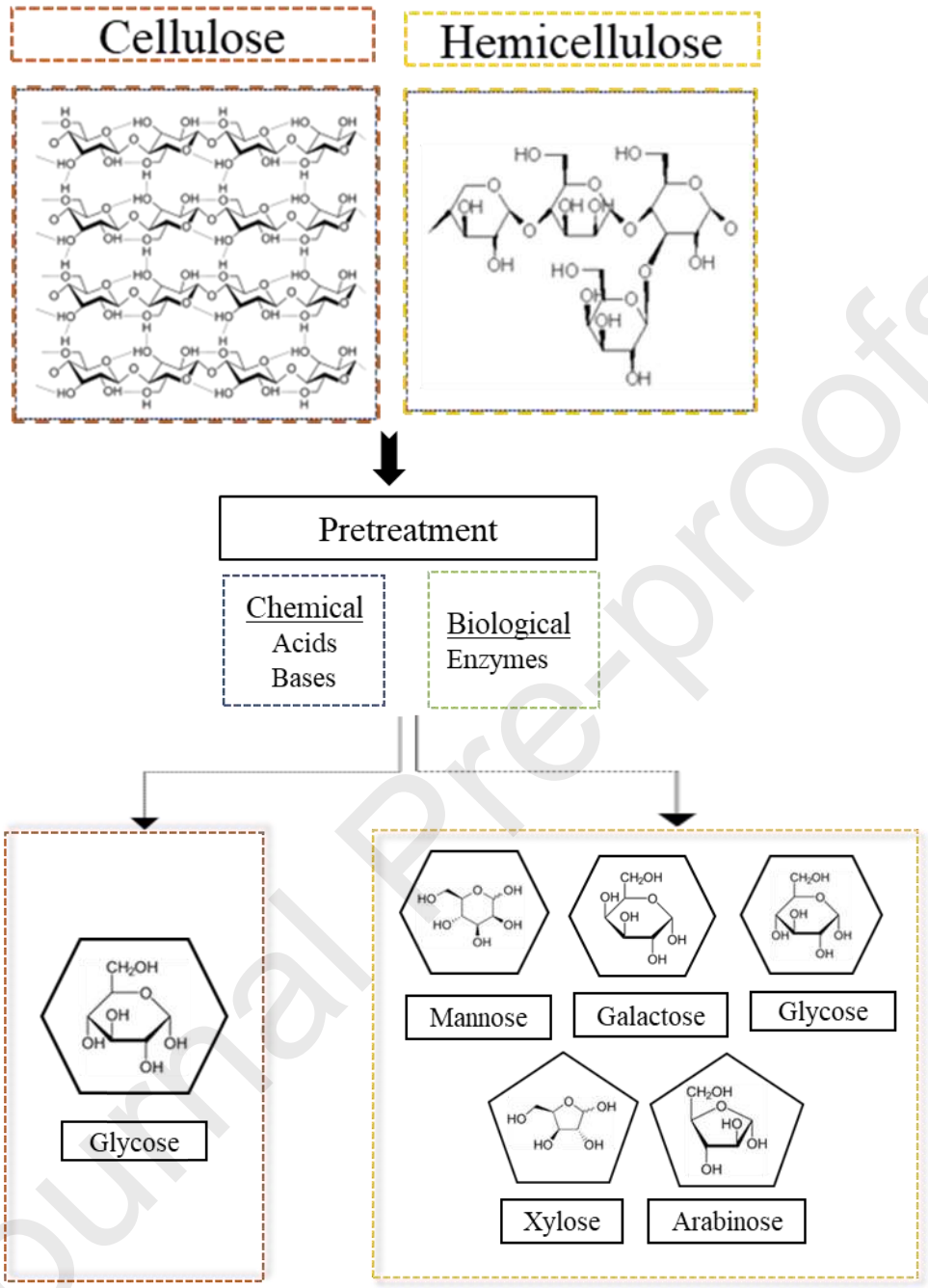
Fig 2



(a)

(b)

Fig. 3



1 **Table 1**. Production of SCP by various yeast species.

Yeasts	Renewable feedstock	Fermentation mode	Biomass (g/L)	Protein Content (% w/w)	Reference
K. marxianus	Cheese whey	Batch, bioreactor	6.0 (normal cell density inoculum) 12.4 (medium-cell-density inoculum followed by changing to continuous fermentation) 15.9 (high-cell inoculum concentration)	42.0	[41]
K. marxianus *C. krusei* (mono and mixed culture)	Cheese whey (diluted)	Batch, bioreactor	4.7 (mono culture) 6.1 (mixed culture)	43.4	[74]
S. cerevisiae	Date juice	Batch, bioreactor	42.8	67.8	[126]
Y. lipolytica	Waste cooking oil	Batch, flasks	57.3	12.6	[25]
C. utilis	Deproteinized potato wastewater & glycerol	Batch, flasks	>30.0	40.7	[42]

C. lipolytica	Olive fruit wastes (Alkali hydrolysis treatment)	Batch, flasks	15.0 17.5 (supplemented with 0.4 g/L peptone)	70.0 64.0	[29]
S. cerevisiae	Fruit wastes from Mango, Prickly Custard Apple, Papaya, Pineapple, Banana, Mangosteen, Cashew apple, Cacao, Jackfruit, Pomegranate	Batch, flasks	0.4[1]	48.3 (Pineapple media)	[28]
Y. lipolytica	Sugarcane molasses, Waste cooking oil, Crude glycerol	Batch, bioreactor	39.0 (molasses-based media) 17.9 (waste cooking oil- based media) 19.7 (crude glycerol) 151.2 (molasses- based media in 10L bioreactor)	45- 54	[24]
S. cerevisiae (CCMA 0186	Vinasse & molasses	Fed- batch, bioreactor	8.8 (*C. parapsilosis CCMA 0544*)	22.0	[43]

+ CCMA 0188) *C. parapsilosis* *C. glabrata* *M. caribbica*			(50:50 vinasse: molasses)		
P. kudriavzevii *P. jadinii* *G. candidum* *C. tropicalis* (MO- M5 & CGMCC 2.587) *S. cerevisiae* (XJU-2 & JJ)	Biogas slurry (derived from chicken manery)	Bottles	6.8	39.4	[15]
N. rattus	Biogas slurry (derived from chicken manery)	Batch, flasks	12.6	35.9	[14]
[1] grams					

Table 2. Literature results regarding biomass (dry cell weight; DCW in g/L) production by microorganisms cultivated on solid/ semi solid and liquid waste types.

Solid and semi solid waste types				
Waste type	**Microorganism**	**Culture configuration**	**DCW (g/L)**	**Reference**
Waste breads	*Rhizopus delemar* CBS 145940	Fed- batch, bioreactor	12.4	[127]
Pomegranate & orange fruit peels	*S.cerevisiae*	Batch, flasks	9.4	[128]
Stearin (industrial derivative of tallow composed of solid free fatty acids)	*Yarrowia lipolytica* ACA-DC 50109	Batch, bioreactor	30.5	[129]
Animal fat	*Sporobolomyces pararoseus* CCY 19-9-6	Batch, Flasks	8.0	[130]
Coffee husks	*Rhodotorula mucilaginosa* CCMA 0156	Batch, Flasks	8.69	[131]
Liquid waste types				
Waste cooking oil	*Y. lipolytica* YLY	Batch, bioreactor	17.9	[24]
Cheese whey	*K. marxianus strain* CHY1612	Batch, bioreactor	4.7	[74]

Coffee wastewaters	*Candida sorboxylosa*	Batch, Flasks	3.4	[132]
OMWs	*Yarrowia lipolytica* ACA-YC 5033	Batch, bioreactor	4.8	[133]

Table 3. Literature results regarding biomass (dry cell weight; DCW in g/L) production by yeasts and fungi cultivated on (enriched with salts or lactose) cheese-whey substrate.

Microorganism	**Culture configuration**	**DCW (g/L)**	**Reference**
Candida curvata D	Batch bioreactor	13.8	[134]
Candida curvata D	Continuous bioreactor	14.2	»
Cryptococcus curvatus ATCC 20509	Batch bioreactor	23.2	[135]
Cryptococcus curvatus ATCC 20509	Fed-batch bioreactor	85.0	»
Cryptococcus curvatus ATCC 20509	Continuous bioreactor	21.0	»
Cryptococcus curvatus ATCC 20509	Continuous bioreactor with recycling	91.4	»
Mortierella isabellina ATHUM 2935	Batch, shake flasks	42.3	[136]
Thamnidium elegans CCF 1465	Batch, shake flasks	29.5	»
Mucor sp. LGAM 366	Batch, shake flasks	28.5	»
Cryptococcus curvatus KCTC 27583	Batch, shake flasks	7.2	[137]
Cryptococcus curvatus NRRL Y-1511	Batch, shake flasks	38.5	[138]

Cryptococcus curvatus NRRL Y-1511	Batch, shake flasks	10.8	[139]
Cryptococcus laurentii UCD 68-201	Batch bioreactor	14.4	»
Cystobasidium oligophagum JRC1	Batch, shake flasks	21.0	[140]
Papiliotrema laurentii NRRL Y-2536	Batch, shake flasks	22.0	[65]
Papiliotrema laurentii NRRL YB-3594	Batch, shake flasks	14.7	»
Cryptococcus curvatus ATCC 20509	Fed-batch, shake flasks	38.1	»

Table 4. Experimental results concerning biomass (dry cell weight, DCW g/L) production of microbial strains cultivated on pure or crude glycerol-based media. Variable quantities of cellular lipids and polysaccharides were reported.

Strain	**Culture configuration**	**DCW (g/L)**	**Reference**
1) Yeasts			
Cryptococcus curvatus ATCC 20509	Fed-batch bioreactor	118.0	[93]
Yarrowia lipolytica ACA-DC 50109	Single stage continuous	8.1	[141]
Yarrowia lipolytica ACA-DC 50109	Fed Batch bioreactor	4.7	[142]
Cryptococcus curvatus ATCC 20509	Fed Batch bioreactor	32.9	[143]
Rhodotorula glutinis TISTR 5159	Shake flasks	5.5	[144]
Cryptococcus curvatus ATCC 20509	Fed Batch bioreactor	22.0	[145]
Yarrowia lipolytica MUCL 28849	Fed Batch bioreactor	42.2	[146]
Yarrowia lipolytica MUCL 28849 b	Fed Batch bioreactor	41.0	»
Rhodosporidium toruloides AS2.1389	Shake flasks	19.2	[147]
Rhodosporidium toruloides AS2.1389	Batch bioreactor	26.7	»
Yarrowia lipolytica A10	Fed Batch bioreactor	23.0	[148]
Candida sp. LEB-M3	Shake flasks	19.7	[149]
Kodamaea ohmeri BY4-523	Shake flasks	10.3	[150]
Trichosporanoides spathulata JU4-57	Shake flasks	17.1	[151]
Trichosporanoides spathulata JU4-57	Fed Batch bioreactor	13.8	»
Yarrowia lipolytica TISTR 5151	Batch bioreactor	5.5	[152]
Cryptococcus curvatus ATCC 20509	Shake flasks	50.4	[153]
Rhodosporidium toruloides Y4	Batch bioreactor	35.3	[154]
Yarrowia lipolytica Q21	Shake flasks	3.85	[155]
Yarrowia lipolytica ATCC 20460	Shake flasks	11.6	[156]
Rhodosporidium toruloides Y4	Shake flasks	24.9	[157]
Rhodosporidium toruloides AS 2.1389	Batch flasks	18.9	[158]
Debaryomyces prosopidis FMCC Y69	Batch flasks	31.9	[91]

2) Fungi and micro-algae			
Mortierella isabellina ATHUM 2935	Shake flasks	8.5	[159]
Cunninghamella echinulata ATHUM 4411	Shake flasks	7.8	[160]
Aspergillus niger LFMB 1	Shake flasks	5.4	[161]
Aspergillus niger NRRL 364	Shake flasks	8.2	»
Schizochytrium limacinum SR21	Shake flasks	13.1	[162]
Schizochytrium limacinum SR21	Single stage continuous	≈11	[163]
Mortierella ramanniana MUCL 9235	Shake flasks	7.0	[164]
Mortierella ramanniana MUCL 9235	Batch bioreactor	9.7	»
Cunninghamella echinulata ATHUM 4411	Shake flasks	6.9	»
Cunninghamella echinulata ATHUM 4411	Batch bioreactor	4.2	»
Mortierella alpina LPM 301	Shake flasks	28.6	[165]
Mortierella alpina NRRL-A-10995	Shake flasks	26.7	»
Schizochytrium sp. S31	Batch bioreactor	≈40	[166]
Mortierella alpina LPM 301	Shake flasks	15.6	[167]
Mortierella alpina NRRL-A-10995	Shake flasks	20.5	»

Highlights

- Single Cell Protein (SCP) as a promising protein alternative for multiple applications.
- Production of SCP by a plethora of microorganisms, including fungi, yeasts, bacteria, and algae.
- The production of SCP and process sustainability could be enhanced via the valorization of agricultural waste.
- Potential application as packaging material.

- Sensorial-, and safety-aspects as well as consumers perception issues of SCP incorporation into food-related matrices.