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The role of single cell protein in cellular agriculture

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More food needs to be produced for the growing human population, but the possibilities of expanding the area of arable land are limited. Cellular Agriculture is an emerging field of biotechnology, aimed at finding alternatives to agricultural production of various commodities. As a part of Cellular Agriculture, the use of microbes and microalgae as food and feed with high protein content, so-called single cell protein (SCP), is gaining renewed scientific and commercial interest. In this review, we give an introduction to SCP production by heterotrophic microbial species, phototrophs, methanotrophs and autotrophic hydrogen oxidizers, as well as highlight some challenges and the latest developments in the growing SCP industry.

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Introduction

Cellular Agriculture means production of agricultural goods with animal or plant cell cultures or microbes instead of using animals or crop plants. The term has sometimes been regarded as confusing, since Cellular Agriculture is not agriculture, but rather an alternative to agriculture. Production, which typically takes place in bioreactors, has been demonstrated for a wide variety of commodities ranging from potential large volume products such as food proteins (egg, milk, cultured meat) and materials (leather, fibers) to fine chemicals such as food ingredients and pharmaceuticals [1•,2]. The rationale of Cellular Agriculture relies in the limited possibility to expand traditional agriculture. It has been claimed that crop production needs to be doubled between 2005 and 2050 to meet the demand for food by the growing human population [3]. Yet, the possibility to increase the area of arable land is limited; in fact, it is being continuously lost [4]. Furthermore, there is a number of well-known problems associated with current agriculture, including soil erosion [5], eutrophication [6], excessive consumption of fresh water [7], and emission of greenhouse gases [8]. However, the reasons typically given for justifying Cellular Agriculture mostly apply only to bulk products. Agricultural production of small volume goods is not necessarily a significant environmental burden.

Although the term Cellular Agriculture, coined in 2015 [9], is relatively new, the idea of producing agricultural commodities without animals or plants has long roots in modern biotechnology. As pioneering work in this field, microbial protein production was developed already in the 1970's, when the first commercial microbial feed product, Pruteen (Imperial Chemical Industries, GB), derived from a methylotrophic bacterium, was launched [10].

Microbial protein produced for food or feed is commonly referred to as single cell protein (SCP), although microbes or microalgae do not always grow as isolated cells, but may also form multicellular complexes [11••]. A wide variety of microbes, including both autotrophic and heterotrophic bacteria, fungi and yeast as well as microalgae has been studied as the sources of SCPs. Depending on the species and growth conditions, microbial or microalgal mass contains high concentrations of proteins ranging from 30 to 80 % along with the other two basic macronutrients, lipids and carbohydrates [11••]. Microbial and microalgal cells proliferate considerably faster than higher, complex organisms with supportive tissues (bones, cartridge, cellulosic plant structures etc.), and the whole cell biomass is in principle edible. It is in many cases possible to utilize non-edible raw materials for cultivation, such as by-products and residues of agriculture and food processing, or abundantly available C1 compounds such as methane or carbon dioxide.

SCP has been cultivated for both food and feed. Although regulatory approval is also required for novel SCP products intended for animal nutrition, the process is simpler and a broader variety of growth substrates can be used. Even though production of SCP for feed is linked to animal farming and thereby again to agriculture, the utilization of non-edible raw materials still reduces arable land use and increases resource efficiency.

In this review, we give an introduction to SCP production as well as highlight some of the challenges and latest developments in this growing field. The classification of the microorganisms studied as SCP based on their carbon metabolism is presented in Figure 1 and the benefits and challenges of different microorganism classes in terms of SCP production in Table 1.

Microbial species as a source of SCPs

Heterotrophic microbes

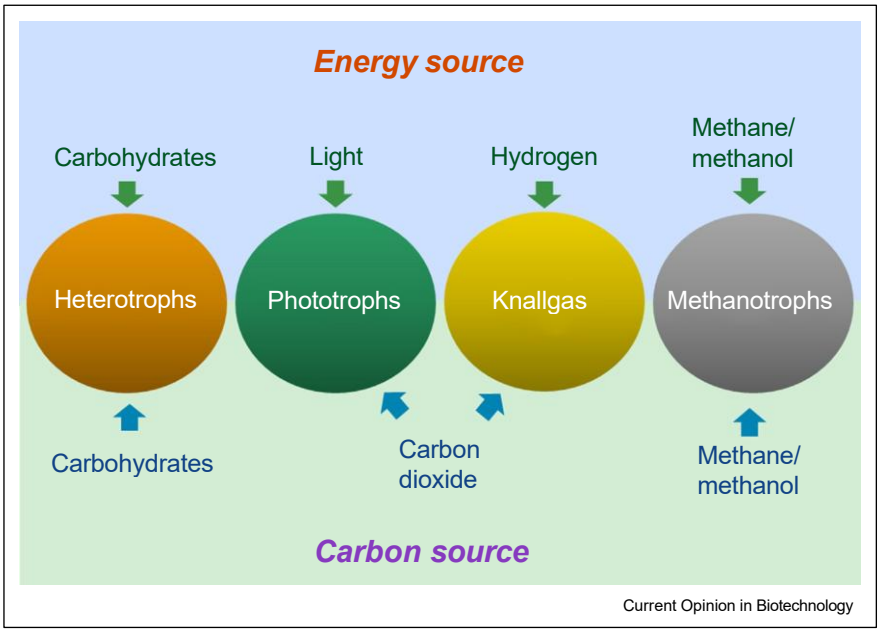
Fungi are a diverse group of eukaryotic heterotrophic microbes that consists of organisms from unicellular yeasts to filamentous fungi. Although a number of examples on SCP production by heterotrophic bacteria are given in the scientific literature [11••], commercial production of bacterial SCP has mainly focused on use of phototrophic and methanotrophic species, as described below. Industrial scale production of heterotrophic SCP has almost exclusively been carried out using yeast and fungi.

The fungal SCPs have nutritionally favorable composition of lipids, protein, and fiber (e.g. cell wall glucan and chitin). Despite the relatively low proportion of methionine, the amino acid composition of fungal SCP with high threonine and lysine contents generally meets the demands of FAO guidelines [11••,12,13]. Besides being an excellent protein-rich source for nutrition as such, fungal SCP can be utilized to improve the nutritional quality and the functional properties, such as texture and emulsifying and foaming capacity, of food products [14]. The potential of fungi has especially been noted for application as meat alternatives [15]. However, the possibility for mycotoxin production with certain species (such as *Fusarium* and *Aspergillus* species) during cultivation requires consideration [12,16].

One of the most well-known commercial fungal SCPs species is *Fusarium venenatum*, utilized for production of the meat alternative Quorn™, which was already launched in 1985 by Marlow Foods (GB). Brewer's yeast (*Saccharomyces cerevisiae*), which is a by-product of brewing industry, is a traditional source for the production of yeast extracts as well as for the salty yeast spreads Marmite1, Cenovis1 and Vegemite1. However, in these production processes the microbes are cultivated with starch-derived glucose as the substrate [11••], making the production ultimately dependent on agriculture. This type of heterotrophic production may therefore not be compatible with the rationale given for Cellular Agriculture.

As mentioned above, one of the beneficial characteristics of fungi is their ability to use various organic compounds for growth. Besides creating value-added products, this property can be applied to solving many challenges in industrial side stream management by establishing novel pathways for circular economy. Numerous studies using industrial side streams in fungal biomass production, mainly for feed use, such as cultivation on orange pulp, banana waste, brewer's spent grain, potato starch processing waste and dairy waste, have been reported [11••,17]. *Yarrowia lipolytica* is a commonly used yeast in industrial biotechnology sector not only due to its ability to use

Figure 1



Carbon and energy sources of SCP microorganisms. Knallgas refers to aerobic hydrogen oxidizing bacteria.

Table 1

Benefits and challenges of different classes of SCP microbes

Microbe	Examples of products and/or organisms	Benefits	Challenges
Yeast and fungi	● *Fusarium venenatum* (Quorn1) ◖ *Paecilomyces variotii* (Pekilo1) ◖ *Saccharomyces cerevisieae* (Marmite1, Cenovis1, Vegemite1)	◖ Possibility to use agricultural wastes and residues ● Fast growth ◖ High cell densities ◖ Long history of use ◖ Simple reactor design	● Glucose is an agricultural product ◖ Non-food carbon substrates should be found ◖ Possibility of containing mycotoxins
Microalgae	◖ *Arthrospira maxima* (Spirulina) ◖ *Arthrospira platensis* (Spirulina) ◖ *Chlorella* (Blonde Chlorella1, Golden Chlorella1)	◖ Sunlight is a free source of energy ◖ Long history of use ● Good tolerance towards water impurities	◖ Large surface areas are needed ◖ Relatively low cell densities ● Contamination risk in open pond cultivations
Methanotrophs	● *Methylophilus methylotrophus* (Pruteen1) ◖ *Methylococcus capsulatus* (Uni-Protein1, FeedKind1)	● Large amounts of methane are available as a side product from oil drilling ● Relatively fast growth ● Production is not dependent on arable land	● Poor solubility of methane ◖ Fossil feedstock (when natural gas is used) ● Need for special reactor designs ● Explosion risk
Knallgas-bacteria[a]	◖ Solein1 ◖ Air Protein™ ◖ Proton™	◖ Hydrogen and oxygen from water by renewable energy ◖ Possibility to use industrial CO_2 emissions ◖ Production is not dependent on arable land	● Poor solubility of the gases ◖ Water electrolysis and direct air capture are energy intensive processes ● Need for special reactor designs ● Explosion risk

[a] Aerobic hydrogen oxidizing bacteria.

various carbon sources, but also produce lipids [18,19]. In 2019, the dried and inactivated biomass of *Y. lipolytica* was accepted as novel food granted by European Food Safety Authority (EFSA) [20]. In addition, the PEKILO1 process, which uses *Paecilomyces varioti* biomass for animal feed, was developed in Finland by the forest industry already in 1974. The process has now been awakened and updated by the start-up company eniferBio, which uses various industrial by-products to produce the biomass [21••].

Although fungal SCPs are mostly produced in submerged fermentations [22] there is an increasing interest in solid-state fermentation [23,24]. Solid-state fermentation means fermentation in the presence of solids, which typically provide both nutrients and physical support for the culture. It has some advantages over submerged production, such as lower energy requirements and production of less wastewater. Solid-state fermentation has been used for upgrading the nutritional value and protein content of agricultural residues to be used as feed [25]. However, a potential risk in using such complex waste materials is that the microbes may form unexpected toxic secondary metabolites.

New production and cultivation methods have been developed to enhance the side-stream utilization as well as to exploit more complex waste streams. Submerged and solid-state fermentations have been combined and waste-based and solid-free volatile fatty acids, recovered from membrane bioreactors, have been investigated as growth substrates [26•,27]. In addition, the interest towards the use of co-cultures for SCP production has increased [24,28]. One benefit of this approach is the possibility to broaden and complement the range of hydrolytic activities needed for substrate utilization. For instance, it is well-known that a synergistically acting cocktail of enzymes is required for efficient usage of lignocellolosic raw materials. A metabolic product of one species may also be the substrate for another, or the species may complement each other by catabolizing different substrates. Co-cultures have been suggested for enhancing the nutritional content, for instance by balancing the distribution of essential amino acids or supplementing the product with vitamins or lipids. There are also challenges: negative interactions such as production of inhibitors or generation of antagonistic environments may take place, and process control may be more complicated. Furthermore, as in the case of solid-state fermentations, otherwise hidden routes for secondary toxic metabolites may emerge, which can compromise the safety of the SCP product [29,30].

Microalgae and cyanobacteria

Microalgae are unicellular organisms that grow individually or in groups or chains, the size of the cells varying

from few micrometers to couple of hundreds. Being capable for photosynthesis and carbon dioxide fixation, microalgae are important oxygen generators as well as a great asset for SCP production. Microalgal SCP production that utilizes various water streams (e.g. fresh, marine or wastewater) as the nutrient source aims particularly to replacement of soy as feed. Furthermore, for example, *Scenedesmus obliquus* has shown potential in fixing carbon dioxide at high concentrations typical for flue gases, at measured maximum biomass productivities from 590 to 810 mg l^{-1} d^{-1} in the presence of 4.1% carbon dioxide [31•].

Cyanobacteria, in turn, are not true algae, but because of their phenotype and photosynthetic capacity, they are commonly referred to as blue-green algae and classified together with microalgae. *Spirulina*, belonging to cyanobacteria, and eukaryotic *Chlorella* species, both currently marketed as functional foods, are the most important ones in the cyanobcteria and microalgae category having protein contents of up to 50–70%. In addition to high protein content microalgae and cyanobacteria contain other favorable components such as lipids, pigments, small peptides, and vitamins thus bringing added-value to food and feed applications [32].

The major challenge in cultivation of microalgae and cyanobacteria, whether in bioreactors or in open ponds is the requirement of high intensity light exposure. Since light penetration is inversely proportional to cell density, the cell yields per volume tend to remain low (typically between 0.5 and 6 g/L), which also complicates cell recovery [33,34]. Most of the cultivations take place in simple open pond systems, which have major contamination issues as well as low productivity. Production of *Spirulina* species has been most successful, and *Spirulina* is used in dietary supplements as well as a SCP source in food products [35,36]. Unfortunately, the amino acid composition of *Spirulina* protein is not optimal as, for example, methionine, lysine and cysteine contents are low. This is compensated to some extent by the presence other beneficial compounds for food solutions such as B_{12}, B_2, B_1, B_3 and E-vitamin. However, the best use is perhaps in the feed industry, where *Spirulina* brings advantageous features to aquaculture by coloring fish flesh and to poultry by giving rise to healthy yolk composition [37]. Among the top five cyanobacteria companies only Spira Inc. (USA) produces nutritious *Spirulina* powder for food and beverage products, while others focus on cyanobacteria management and are cultivation technology providers [37,38].

Chemoautotrophs and methylotrophs

The bacterial species using gaseous one-carbon substrates for growth include aerobic and anaerobic hydrogen oxidizing bacteria, and methanotrophs. Autotrophically growing hydrogen oxidizing bacteria energize their metabolism using hydrogen as the electron donor. The group of hydrogen oxidizing bacteria consists of aerobic and anaerobic (acetogenic) species. Aerobic hydrogen oxidizing bacteria, or Knallgas bacteria, as they are also called, use oxygen as the electron acceptor and assimilate carbon dioxide through the reverse tricarboxylic acid cycle or the Calvin-Benson-Bassham cycle. Anaerobic hydrogen oxidizing acetogens, on the other hand, fix carbon dioxide (or monoxide) via the reductive acetyl-CoA (Wood-Ljungdahl) pathway, by which the carbon is mainly directed to organic co-products such as acetate and ethanol instead of cell mass [21••,39,40]. So far industrial activity on the utilization of acetogens has focused on the production of chemicals such as ethanol and the resulting cell mass has been mostly been considered as a by-product [41].

Because of the limited potential of acetogens to directly convert carbon dioxide to cell mass and protein, a two-step process utilizing acetogens has been suggested for SCP production. Carbon dioxide is first reduced by acetogens to acetate, which is then supplied as the carbon source for an edible heterotrophic yeast or fungal species in a second cultivation step [42,43]. Calculation of the theoretical yield of biomass from input energy suggests that a two-step process combining anaerobic autotrophic and heterotrophic production would in fact be more favorable than either anaerobic or aerobic one-step production [44]. However, the main focus of industrial one-step production has been on Knallgas bacteria, presumably due to the higher yield of cell mass.

Hydrogen, needed for reduction of carbon dioxide, can be produced by water electrolysis that is powered by renewable wind, solar, nuclear or geothermal energy. Production is therefore not connected to the use of arable land, but is in principle possible anywhere where energy and water are available [45]. Carbon dioxide can be sourced from industrial exhaust gases or isolated from ambient air by direct air capture techniques. Both water electrolysis and direct air capture are energy intensive processes and special equipment is needed [43]. Nevertheless, it has been shown recently that photovoltaics energized production of SCP from carbon dioxide captured from air is superior to crop cultivation in terms of protein yield per land area [46].

Utilization of hydrogen and methane is hampered by their poor solubility to aqueous solutions. Reactor configurations (U-loop reactors, reactors with special stirring systems etc.) alternative to the conventional stirred tank bioreactors have therefore been designed to improve the mass transfer of the gases to the growth medium [47–49].

Although several companies are developing SCP processes based on Knallgas bacteria (e.g. Solar Foods, Finland and Kiverdi, USA for food; Deep Branch Biotechnology, UK for feed) [41], scientific reports on their

cultivation for food are rather scarce. Three strains of gram-negative Knallgas bacteria were shown to produce high concentrations of protein with a balanced amino-acid profile, limited only by the somewhat low contents of the cysteine-methionine pool and isoleucine [50]. Gram-negative strains are, however, not at first hand the best choices for production organisms, because lipopolysaccharide endotoxins are typically present in their cell walls. The cultivation of gram-positive species has been studied in a cultivation vessel with internal electrolysis unit for supply of hydrogen and oxygen. Except for the low tryptophan content, the essential amino acid profiles of the cell proteins were nutritionally balanced [51]. The cultivations described in these reports were carried out in shake flasks and prototypical bioreactor systems, which do not reveal the full potential of Knallgas bacteria. For the most studied Knallgas bacterium, the Gram-negative, endotoxin producing [52] species *Cupriavidus necator*, cell doubling times of approximately 1 hour [53] (comparable to Baker's yeast) and final cell densities of 90 g/L dry weight [49] have been reached in bioreactor cultivations.

Aerobic methanotrophs oxidize methane via methanol to formaldehyde, which is then metabolized further through ribulose monophosphate or serine pathways [54]. The methanotrophic species *Methylophilus methylotrophus* and *Methylococcus capsulatus* were the first bacteria cultivated in commercial scale for SCP production from late 1970s to 1990s, but production was discontinued due to poor economics. However, there has been a renewed interest in utilization of methane and methanol for production of microbial feed and several products, such as UniProtein1 (Unibio, Denmark) and FeedKind1 (Calysta, USA) are on the market [55••]. *Methylobacterium extorquens* has been engineered by KnipBio (USA) for production of taurine and fish color enhancing carotenoids in aquafeed. The product, KnipBio meal is apparently the first genetically modified SCP product that has gained a GRAS (Generally Recognized As Safe) status [11••,55••] from The United States Food and Drug Administration. However, agricultural outputs instead of methanol or methane are apparently used for cultivation of *M. extorquens* for KnipBio meal [56].

The source of methane is typically natural gas, but other feedstocks have also been suggested. For instance, a co-culture of microalgae and a methanotrophs has been cultivated on a carbon dioxide — methane mixture, mimicking biogas [57]. It has been suggested that by using carbon dioxide, hydrogen and/or methane generated by anaerobic digestion of biomass the raw-material base could be broadened to organic wastes such as sewage sludge [43].

Nitrogen source

In the above presentation, the SCP microbes were grouped into different categories based on their carbon metabolism (Figure 1). The other essential component needed for protein synthesis is obviously nitrogen. Although cultivation in bioreactors ensures that nutrients such as ammonium salts are efficiently used by the cells and not lost to the environment, reducing elemental nitrogen to ammonia by the current Haber-Bosch process is nevertheless a considerable source of carbon dioxide emissions [58]. Therefore, production of SCPs by microbes capable of nitrogen fixation has been of recent interest. For instance, a consortium of gaseous nitrogen assimilating Knallgas bacteria has been cultivated for SCPs, but with lower growth rate and cell yield than what was achieved with ammonium as the nitrogen source [59•]. Use of fertilizers in agriculture has led to an excess of reactive nitrogen in the environment, which is a major cause of eutrophication. It has therefore been suggested that recycling the reactive nitrogen available should be preferred to fixing even more nitrogen from air [43]. There is a growing interest in development of techniques for recovering ammonia from waste waters (e.g. by gas stripping, ion exchange, electrodialysis) [60], which could be used in conjunction with SCP production [58].

Outlook

An important Cellular Agriculture related trend in SCP development is the transition from the use of edible, agriculturally produced raw materials to non-edible ones, such as one-carbon compounds (carbon dioxide and methane) and different organic side-streams and wastes. However, regulatory aspects and safety of the feedstock used for SCP production should also be taken into account. As has been pointed out, safety records for raw materials are required in many countries [11••]. Not every waste qualifies as a substrate for microbial food, or even feed production.

Furthermore, safety and the general suitability of the microbial and microalgal species as food have not always received adequate attention. Complex mixed populations [61], endotoxin-producing bacteria, and even potentially pathogenic species [62] have been studied as sources of SCPs. Toxicological concerns include the presence of exotoxins, cytotoxins and genotoxins in the microbial masses that should be examined before considering food or feed use. A known obstacle of direct use of microbes as food is their relatively high content of nucleic acids, which are metabolized to uric acid, a cause of gout. However, there are well-established methods for reduction of nucleic acid concentrations of the SCP products [63] and because of the presence of the uricase enzyme in most animals this is not always a concern for feed applications [46].

An essential characteristic that is hardly ever touched upon in the literature pertains specifically to the use of SCP for human consumption. The flavor and aroma profile of the SCP product intended for food should

ideally be pleasant or at least neutral. For instance, the fishy smell of microalgal SCP may limit its use in many food applications, if further processing is not carried out [64]. Furthermore, different waste materials are in principle suited for feed SCP production, but consumers may not be in all cases ready to accept them for manufacturing food.

The nutritional aspects of the microbial and microalgal biomass should also be given more emphasis, not only for the balanced distribution of the essential amino acids, but also for the digestibility of the protein and other components. Besides proteins that are usually highlighted, the microbial and microalgal cell mass provides also a rich source of other important nutrients such as vitamins, carotenoids, fiber, essential fatty acids and so on [11••].

Conflict of interest statement

Concerning the manuscript: The role of single cell protein in cellular agriculture by Antti Nyysso¨ la¨, Anniina Suhonen, Anneli Ritala, and Kirsi-Marja Oksman-Caldentey

We have no conflict of interest to declare.

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