doi:10.2533/chimia.2025.324 Chimia 79 (2025) 324–329

Microalgae as Key to a Land-free Circular On-farm Feed Production System

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Abstract: Currently, all feed and food come from plant- or animal-based production systems. The potential of the natural biodiversity of microorganisms for the highly efficient production of nutrients has yet to be leveraged. We present an overarching concept that ranges from the development of a national collection of regional microalgae to the decentralised production of nutrient-rich biomass for animal feed production. The focus is on the substitution of classic plant-based feed to reduce competition for arable land, to increase the nutritive value of animal-based food, and to reduce methane emissions from ruminants. The photoautotrophic and mixotrophic cultivation of microalgae are well aligned with the goals of CO_2 fixation and the later contribution to the valorisation of side streams from the food industry. We also present the initial results on strain adaptation to diverse cultivation conditions.

Keywords: Cultivation · Culture collection · Microalgae · Microbial biodiversity

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1. Introduction

All food and feed available today come from either plant or animal production systems. The growth of the global animal feed market is driven by sustainability factors, such as the increasing protein demand, environmental concerns, and awareness of animal health and welfare.[1] Producing around 1.3 billion tons in 2023, the animal feed market is worth around 600 billion USD with a forecasted growth of 4.3% until 2034,[2] putting great pressure on agriculture yields to support it. Despite the huge potential to address this issue, single cell-based nutrient production, based on the natural available microbial biodiversity, has not been fully leveraged thus far.

Single-cell production systems have huge advantages in terms of sustainability, efficiency, and rapid adaptation to changing climatic conditions.[3] In particular, microalgae, due to their exceptional diversity and photosynthetic efficiency, have potential to build nutritious biomass with excellent bioavailability using atmospheric CO_2 as a carbon source (1 kg of dry algal biomass requires about 1.8 kg CO_2). When compared to traditional crops, microalgae have numerous advantages: (i) achieve faster growth rates and higher areal productivity; (ii) being unicellular, all the biomass can be processed without tissue separation; (iii) does not require arable lands; (iv) despite growing in aquatic environments, require less water than terrestrial crops; (v) nutrients can be obtained from wastewaters and/or side streams; (vi) do not need herbicides or pesticides; among others.[4–6] This is a huge advantage in terms of the effective direct air capture of CO_2 and food security on a global scale, as the limited resources of arable land are not used. Moreover, several health benefits have been reported when microalgae were added to the feed of a wide range of animals.[7]

However, photoautotrophic production systems have a critical disadvantage in terms of low cell concentration in the cultivation processes used – that is, there is a high cost and energy requirement in the downstream dewatering processes.[8] To address these limitations, we designed a concept of a decentralised on-farm production system in which the feed is given to the animals in a liquid dosage form with minimal downstream processing. Thus, existing farms can produce nutrient-rich microalgae biomass on-farm in a continuous process.

2. AlgoScope: Unveiling the Swiss Native Microalgae Collection

The selection of suitable strains is the first step towards successful biotechnological exploitation of microalgae.[9] Therefore, Agroscope is currently working to develop the first indigenous Swiss microalgae culture collection, called AlgoScope. With the goal of producing microalgal biomass in on-farm cultivation systems, AlgoScope focuses on microalgal strains that grow naturally in an environment similar to the environment of future cultivation sites (*i.e.* farms). This approach ensures that the strains to be produced are well adapted to local climatic conditions, unlike commercial strains that may struggle in the Swiss environment or require a prolonged adaptation phase. Local microalgae thrive when nourished by natural fertilisers, including animal secretions such as saliva, sweat, and hair, as well as agricultural fertilisers. This process promotes the growth of particularly robust strains, which are then selected for potential biotechnological applications.

Established in 2021, AlgoScope launched the Swiss microalgae collection to identify, catalogue, characterise, and conserve native Swiss microalgae with biotechnological potential. Strains are collected throughout the year, targeting those acclimated to the high temperatures of summer or the low temperatures of early spring and late autumn. This enables the creation of cultures, in analogy to crops, that are adapted to different temperature ranges, allowing crop rotation according to the time of the year and thus biomass production over a wider temporal spectrum. Filtered environmental samples containing a wide array of microorganisms are plated and single colonies are serially subcultured until monoalgal cultures are obtained. After isolation from environmental samples and purification of individual strains (Fig. 1), microalgae are identified through microscopy and genetic sequencing of a ribosomal DNA region frequently used for species identification called the internal transcribed spacer (ITS). The purified strains are stored on agar plates for medium-term preservation and in cryotubes for long-term storage.

The species isolated during our isolation campaigns were mainly freshwater algae, with one third belonging to the *Chlorellaceae* family (*e.g. Chlorella*, *Auxenochlorella*) and another third belonging to the *Scenedesmaceae* family (*e.g. Tetradesmus*, *Scenedesmus*, *Desmodesmus*). The remaining species were distributed among other families, including *Chlamydomonadaceae* (*e.g. Chlamydomonas*), *Watanabeaceae* (*e.g. Chloroidium*, *Jaagichlorella*), and *Mychonastaceae* (*e.g. Mychonastes*), which together accounted for 20% of the total. Approximately 10% of the strains could not be successfully identified using our current methods. All relevant data about the strains are recorded in Agroscope's internal database DataRepo[10] (Fig. 2). The basic characterisation of strains consists of their biochemical composition (*i.e.* proteins, carbohydrates, lipids, and pigment contents), amino acids and fatty acids profiles, and growth parameters under standardized conditions. When a strain is not generally recognized as safe,[11] safety assessments are made case-by-case.

Regular sampling campaigns take advantage of Switzerland's rich biodiversity and have led to the isolation of approximately 120 strains. Although most strains currently originate from the Swiss Plateau (see Fig. 3), future campaigns will extend to other regions, such as the Alps. Given that the isolation process was

Fig. 1. (a) add (b) Typical isolation sites, (c) sampling, (d) purification procedure, and (e) medium-term strain storage and preservation.

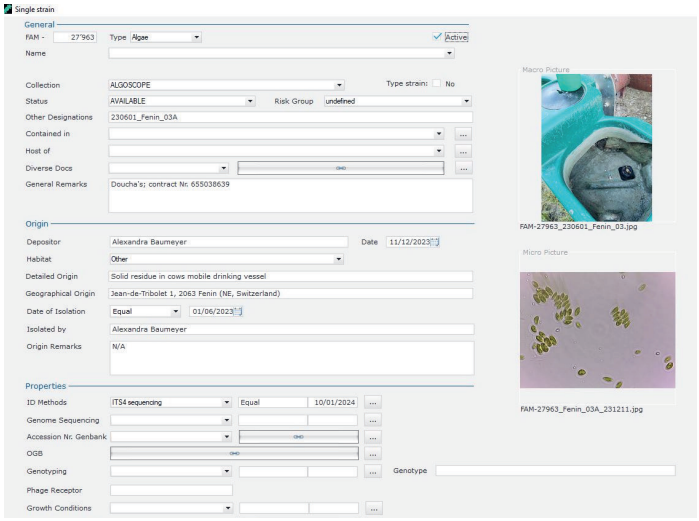


Fig. 2. Agroscope's internal data repository BCRepo.

carried out under defined conditions to select strains that could be easily reproduced in future decentralised production facilities, some more robust strains may have been favoured over more sensitive ones. This probably resulted in the exclusion of fragile species that could not be preserved and therefore could not be catalogued.

Fig. 3. AlgoScope microalgae isolation sites (blue crosses) in Switzerland.

3. How to Produce Microalgae in Larger Scales?

Microalgae cultivation can serve numerous purposes. Research laboratories, for example, mostly carry out small-scale cultivation (in the millilitre to few litres range) in flasks with rigorous control of physicochemical conditions to avoid variability in the results. In demonstration facilities, pilot-scale cultivation systems (in the dozens to hundreds of litres range) mimic commercial photobioreactors and are equipped with monitoring devices to assist scaling up and optimising the operation. Finally, an industrial microalgae production structure can employ huge photobioreactors (several hundred cubic meters) equipped with automated systems for up- and down-stream processes, optimised to maximise productivity and minimise costs.[12] Clearly, the ideal microalgae cultivation system to be used strongly depends on the aims and scope of each case.

A photobioreactor is defined as any device or system that supports the culture of photosynthetic organisms using light. In practice, however, the selection of a given photobioreactor design, material, and/or technology affects the success or failure of the microalgae production endeavour.[13] A wide array of reactors are available on the market. Yet, reports in the scientific literature are heterogenous and unclear about the ideal photobioreactor for many cases.[14] Despite the market availability of different reactors, as well as current developments in the field, in principle, the design of photobioreactors for microalgae production should consider three main factors: (i) light penetration in the cell suspension, (iii) gas (O_2/CO_2) exchange, and (iii) mass transfer for homogeneous distribution of suspended cells.

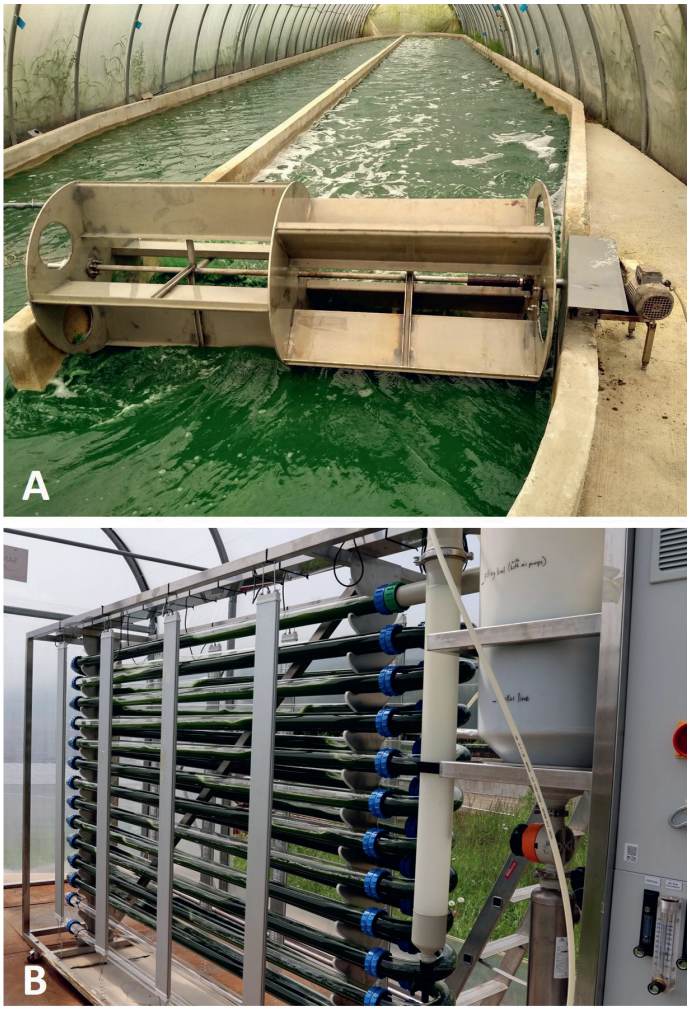


Fig. 4. Examples of photobioreactors used for large scale microalgae production. An open raceway pond (A), and a horizontal glass tubular photobioreactor (B).

Open photobioreactors are systems in which the cell suspension is in direct contact with unfiltered or untreated atmospheric air. They are the most widely used systems for large-scale microalgae production due to their simplicity and low cost compared to closed systems.[15] In open raceway ponds, for example, the water column is shallow to allow light penetration, resulting in a low surface-to-volume ratio that promotes gas exchange, and mass transfer is conducted with a paddlewheel (Fig. 4A). Thin-layers, another type of open photobioreactor, are able to achieve high volumetric productivities by exposing a very thin layer of microalga culture to light (hence, its name) in a cyclic way with the use of pumps on a slightly inclined surface. Still, open systems occupy large land areas and are notably vulnerable to unfavourable weather conditions (*e.g.* rain and evaporation) and contamination, which limits their use to low-quality applications.[16]

Unlike open systems, closed photobioreactors do not allow the cell suspension to come into direct contact with the atmosphere, overcoming some of the issues associated with open reactors. Closed systems, such as bubble-column, tubular, and flat-panel photobioreactors, minimise contamination and allow monospecific cultivation over long periods of time (Fig. 4B). Moreover, these reactors are relatively compact for their working volume capacities, and controlling cultivation parameters is possible, making them less dependent on environmental conditions. Due to their higher maintenance and operational costs when compared to open photobioreactors, closed systems are preferentially used to produce high-value products[15] (*e.g.* food, feed, cosmetics, and pharmaceuticals). Table 1 presents a multifactorial comparison of open and closed cultivation systems.

Table 1. Parametric comparison of open and closed microalgal cultivation systems.

Parameters	Open systems	Closed systems
Construction and operational costs	Low	High
Land area requirement	High (nonarable)	Lower (nonarable)
Water consumption	High	Low
Energy consumption	Low	Medium to high
Risk of contamination	Very high	Medium to low
Process control	Limited	Possible
Dependency on climate	Very high	Low to none
Scaling up	Easy	Laborious
Maintenance	Easy and cheap	Hard and expensive
Productivity	Medium to low	Higher

As with the photobioreactors, all up- and downstream processes for microalgae production are readily scalable. They often use similar machinery as from large sectors such as the chemical and pharmaceutical industries (*e.g.* industrial centrifuges, membrane filtration, freeze- and spray drying). In fact, the cost of the final microalgae-based product strongly depends on the production scale.[17] Still, it should be noted that the global production of microalgae biomass is in the order of 10,000 tons per year.[18] Therefore the proposition of using the existing microalgae market as an alternative raw material for a giant sector such as the animal feed industry must be interpreted with caution. The decentralised production concept proposed here also addresses this problem by not relying on the existing algae market, which is dominated by very few strains.

4. Sustainable Microalgae Cultivation Strategies in the Agri-food Sector

Microalgae can be grown either heterotrophically, with sugar as a carbon source, or photoautotrophically, with carbon assimilated from CO_2 through photosynthesis. A third mode of growth is possible with certain microalgae species that can combine both trophic growths, known as mixotrophy. The cultivation of mixotrophic microalgae can take advantage of the carbon-rich side streams regionally available in the agri-food sector, allowing a local transformation into biomass. This strategy not only avoids the use of chemical fertilisers, as the streams usually contain minerals, but also the use of water.[19] Water conservation in agricultural practices is critical because global climate change threatens the availability of hydric resources. Furthermore, it is well known that in traditional agriculture only half of the nutrients are used by crops.[20] When applying mixotrophic growth, the biomass volumetric productivity greatly surpasses the autotrophic productivity, optimising the use of water in microalgae cultivation systems.[21,22] Additionally, the use of closed systems for microalgae cultivation completely recovers nutrients from liquid streams. Some studies have claimed that mixotrophic growth of microalgae: (i) decreases the sensitivity of microalgae cells to excessive light – photoinhibition, (ii) achieves higher growth rates than autotrophy and heterotrophy, (iii) reduces biomass losses during the night, and (iv) reduces photo-oxidative damage during the cultivation.[23–25] Hence, mixotrophic microalgae production is a breakthrough technology at a lower maturity level than phototrophic production, but with huge potential.[26]

When organic compounds are used in mixotrophic cultivation, microalgal growth is not entirely dependent on photosynthesis, and light is no longer the limiting factor. This advantage means that more biomass can be produced at the same light intensity, increasing the overall efficiency of the system.[27] However, light remains essential in mixotrophy without being the most important factor. The combination of sunlight and artificial light or the use of only artificial light is the common practice in northern countries. Artificial light allows stable production, leads to higher biomass yield, and guarantees supply and quality,[26] but it increases the production costs.[28] Optimal selection of artificial light must consider the photoperiod, light intensity and quality (*i.e.* wavelength spectrum), and is highly case-dependent.[29]

If biomass productivity is doubled in mixotrophy, the land area requirement is reduced by half. This advanced bioprocess holds big promise for growing more algae with less land. Heterotrophy could potentially achieve significantly higher productivity but at the cost of CO_2 release and lower carbon yields,[30] which are not aligned with the new climatic targets in agriculture. A promising possibility using mixotrophy is running a reactor without any gas exchange during daylight hours.[12] This is possible in a new type of microalgal process called oxygen-balanced mixotrophy, which combines autotrophic and heterotrophic growth in a balanced manner through careful regulation of the organic carbon substrate supply.[19,21] Dosing the organic carbon guarantees the stability of dissolved oxygen in the medium by establishing a cyclic alternance of heterotrophic oxygen consumption and photosynthetic oxygen production, in perfect intracellular gas recycling.[31]

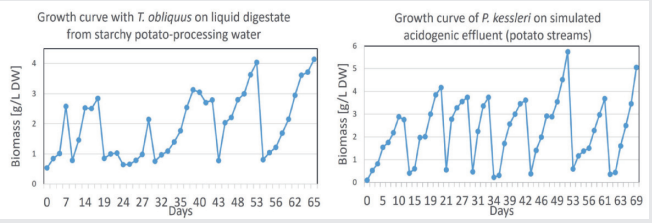
Still, mixotrophy has to overcome certain barriers. A high risk of bacterial contamination is one of the major concerns in mixotrophic cultivation, but different strategies exist to circumvent this issue. Examples of contamination mitigation are choosing fast growing species and/or extremophiles and adapting nutrition to change the internal nitrogen quota in microalgae, which is known as the feast and famine strategy to limit heterotrophic bacteria.[32–34] According to the European Technical Report for 'Algae – Food and feed applications' (CEN/TR 17559:2022 – standardisation document[35]), the total aerobic counts of 107 CFU/g or higher are frequent and tolerated in algae and algae products as soon as the risk of pathogens is discarded.

Another critical aspect in mixotrophy is that the sugars needed as primary organic substrates in biotechnological fermentation of microalgae are typically derived from terrestrial crops, in competition with the food industry,[26] and increase production costs. It is therefore fundamental to explore cheaper and more sustainable organic substrates, such as clean agrifood side streams. High-quality streams are typically found in the food and feed industries, and a careful selection of streams is crucial for preventing global fertiliser shortage and meeting the opportunity to deploy microalgae

mixotrophic technology. A recent project named '*A'propos: Algal proteins on potato and other streams*' demonstrated, for the first time, the production of high-quality microalgae biomass in a 200 L pilot tubular photobioreactor directly installed in the food industry, using starch-rich processing waters as a substrate for microalgae growth (Box 1). Agri-industries that produce side streams with potential for application in microalgae cultivation include dairy, vegetable production/transformation, swine, poultry, aquaculture and many others.

Box 1: The A'Propos Project

The recently developed A'propos project (Algal proteins on potato and other stream; BAFU-087.2- 64764/1/1) has considered the upcycling of agri- food waste into microalgal biomass. Tetradesmus obliquus and Parachlorella kessleri were selected for the cold and warm seasons, respectively, after strain screening in agri-food mixtures. Liquid digestate from the conversion of starch-rich effluents (potato bleaching process) into biogas was selected as growth medium after supplementation with pure ammonium sulfate and acetate. The maximum concentration obtained with the cold-adapted strain was 4.2 g L^{-1} of algal biomass. When expressed as average areal productivity, ~17 g m^{-2} d^{-1} of algal biomass was attained. Average protein productivity was ~10 g m^{-2} d^{-1}. The average productivity of the warm- adapted strain was >31 g m^{-2} d^{-1}, with an average protein productivity of 12 g m^{-2} d^{-1}. A gradual acclimation was evident with productivity gains observed cycle after cycle. The digestible amino- acid profile suggested that both strains could serve as a good protein source for monogastrics and even for dairy cows (unpublished data, in preparation). The microbiological analysis on wet biomass was compliant with quality and safety reference values for a mixed feed in pig nutrition.



	Spoilage-indicating bacteria	Streptomycetes	Product typical moulds	Spoilage-indicating moulds	Mucorales	Yeasts	*Salmonella*	*E. coli*	*EB*	*Staph.*
	cfu/g	cfu/g	cfu/g	cfu/g	cfu/g	cfu/g	cfu/g	cfu/g	cfu/g	cfu/g
Raw cake *To*	<10000	<10000	<100	8.41E+02	<100	<100	Not detected	Not detected	1.00E+05	Not detected
Ref Values mixed feed for swine [1]	1.00E+06	1.00E+05	5.00E+04	5.00E+04	5.00E+03	8.00E+04	Not detected	Not detected	$10^4 - 10^{5*}$	Not detected

1 *VDLUFA 2017, Methodenbuch III, 28.1.4; *Cullen et al., 2021*

Only recently have the related regulations started to clarify important aspects of side-stream valorisation using microalgae. This matter has been discussed in the EU workshop on legal aspects of algae grown in wastewater/waste gas/manure and food waste by the EU sustainable phosphorous platform.[36] In the document of support, the concept of 'End-of-Waste' is provided, representing a viable classification for a new non-waste algae product. The 'End-of-Waste' criteria help define when waste-derived materials have been sufficiently processed so that they can lose their 'waste' status. Two important conclusions were drawn: (i) when industrial waters are not discharged (subject to complete wastewater treatment) but simply discarded, they are under the scope of the Waste Framework Directive, which is necessary to qualify for the 'End-of-Waste' test; and (ii) the use of industrial waters for other production applications without further treatment for extracting valuable nutrients (for microalgae production in our case) is a good way to apply for a by-product test or End-of-Waste test and make the process compliant for a non-waste product as feed or food.

Finally, sustainability goals in agriculture are also driven towards the reduction of methane emissions by livestock, and microalgae have the potential to contribute in this direction. In one of our recent studies,[37] we showed that microalgal polyunsaturated fatty acids (PUFAs) were effective in CH_4 mitigation, although their bioavailability could be an issue. The biohydrogenation of dietary PUFAs is one of the key factors in CH_4 mitigation, and it is favoured when the cell walls of microalgae are disrupted by the rumen microbiota, releasing free fatty acids.

5. Conclusions and Perspectives

Applied microalgae biotechnology for the production of nutrient-rich biomass can address many current challenges in the agri-food ecosystem. By leveraging the existing but poorly characterised Swiss microalgal biodiversity, local production systems can be tailored for each specific producer. Moreover, strains can adapt to a wide range of environments, improve their tolerance to specific conditions, and optimise their metabolic machinery to thrive in new substrates and accumulate high-value biomolecules. In addition, closed photobioreactors minimise contamination, land requirements, and dependency on the natural environment while maximising productivity, quality of the final biomass, and the robustness of the overall process. Mixotrophic conditions reduce energy, water, and land area demands compared to photoautotrophy. When combined, these factors lead to the optimised concept of decentralised on-farm production systems, where microalgal biomass is continuously produced and applied to agricultural processes (*e.g.* as a feed ingredient).

Further research and development are needed to overcome the main limitations of the proposed system. In the regulatory frame, the term 'waste' is not adapted for clean side streams/agri-food residues with great potential to be valorised as resources. For food and feed applications, the resulting biomass must be objectively proven safe to consume. Furthermore, the digestibility, palatability, and general acceptability of microalgae-based products are relatively unexplored, considering the huge diversity of microalgae. These topics should be a matter of future research.

Acknowledgements

The authors would like to thank MSc Rodrigo Cardeiras for technical support in the projects mentioned here. We kindly acknowledge financial support from Lidl Switzerland. We thank also the Swiss Federal Office of the Environment, Frigemo SA / Fenaco and Ava Altenrhein for their financial and technical support in the frame of the A'propos project and the scientific partner, the Applied Science University of Wadenswil – ZHAW.

Author Contributions

Conceptualization: F. W., A. B. B., M. B-V.; Writing - Original Draft: F. W., A. B. B., M. B-V., D. K.; Writing – Review & Editing: D. K.

Received: February 4, 2025

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