

Chapter 5

The benefits and advantages of commercial algal biomass harvesting

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This chapter outlines the concept of integrated bioremediation and co-product development using microalgae. It ties potential products with taxonomically governed biochemical profiles, which are essential criteria for product-driven strain selection. It closes by briefly describing the current challenges to commercial cultivation and biomass harvesting.

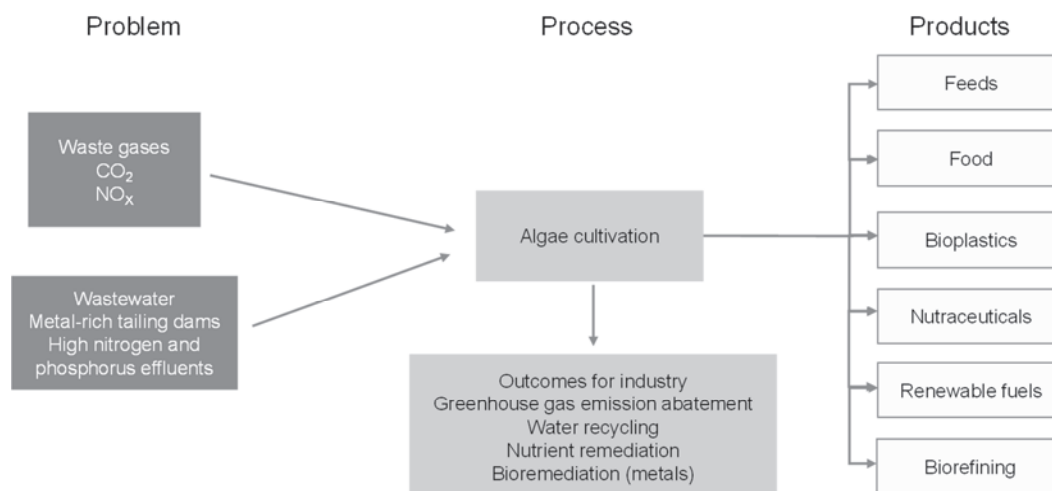
Concept of bioremediation using microalgae with value-adding co-product development

The unprecedented increase in greenhouse gas (GHG) emissions is predicted to lead to rapid environmental changes, such as, for example a general rise in global temperatures, more severe weather conditions and reduced freshwater availability, particularly in countries where freshwater is already a precious resource (Field et al., 2012). Global economies are under increasing pressure by governments and the general public to reduce their carbon emissions. For example, the global carbon dioxide equivalent (CO_2e) emissions for 2005 were 44.2 billion tonnes (Herzog, 2009). Many countries have introduced carbon taxes to force industries to rethink and actively work towards carbon reductions of their emissions (Ellis et al., 2010).

Global economies are not only pressured by GHG-induced proposed climate change scenarios, but are further challenged by the prediction of having reached or reaching peak oil and phosphorus in the foreseeable future (most likely in the next 15 years), which will negatively affect industries and agriculture (Cordell et al., 2009; Sorrell et al. 2009). It is possible that appreciable new fossil oil reserves exploration may be possible at greater depths; however, the quality of these so-called heavy oils is poorer, as the oil is more viscous, has a higher sulphur content and, hence, requires additional refining efforts. These efforts will be reflected in increased oil prices. Undeniably though and regardless, fossil oil reserves are not expected to be replenished within acceptable time frames to match the growing energy demands of the future world population (Owen et al., 2010). Peak oil also affects the agricultural sector, as farm machinery is oil driven and pesticides are oil-based products. The application of pesticides have led to sustained food supplies, which is directly linked to population growth (Pfeiffer, 2006). With regards to peak phosphorus, predicted population growth, limited arable land for food production, which is not predicted to increase substantially or in line with estimated population growth (United Nations, 2004), and scarcer freshwater resources as well as more unstable weather conditions and raised temperatures will challenge agriculture and aquaculture industries to meet future nutritional and food supply requirements.

Algae and the oxygenic photosynthetic cyanobacteria (chloroxybacteria) offer ideal solutions to the above-mentioned imminent problems, because they can be cultivated year round on non-arable land in various wastewater streams or brackish to marine waters, alleviating the pressure on arable land and freshwater resources. As algae are naturally high in protein and ω -3 polyunsaturated fatty acids and vitamins, which are essential in a balanced diet, they may well become a promising food supplement or food source to ensure a healthy diet for the growing population (Cribb, 2011), most likely not achievable with traditional terrestrial crops. In addition, malnutrition or lack of essential amino acids, fatty acids, minerals, antioxidants and vitamins are linked to numerous diseases, such as nutritional anaemia (iron and B12 deficiency), xerophthalmia (vitamin A deficiency) and endemic goitre (iodine deficiency), which are, according to the World Health Organisation, of growing concern (Edwards, 2010). Many algal strains are also suitable for producing renewable fuels (biodiesel, bioethanol and kerosene), restoring the carbon balance and fertility in weathered soils (biochar) (Bird et al. 2011; 2012), for the bioremediation of carbon dioxide (CO_2) (1 DT of biomass remediate 1.83 T of CO_2 (McGinn et al., 2011) and nitric oxide containing flue gasses (Nagase et al., 1997) and metal- and nutrient-rich wastewaters (Perales-Vela et al., 2006) (Figure 5.1).

Figure 5.1. **Concept of bioremediation using microalgae with value-adding co-product development**



Taxonomic affiliations: Implications for potential end product use

Just like the rather diverse habitat algae colonise, they also show an incredible taxonomic diversity (Table 5.1) and were formerly classified as belonging to the kingdom Protista, which became a collection bag for seemingly unrelated organisms. To make sense of the classification mess, a new classification scheme was proposed for the eukaryotic protists (Adl et al., 1995), excluding the oxygenic photosynthetic chloroxybacteria (formerly cyanobacteria or blue-green algae), which is shown in Table 5.1 as far as it is relevant to the algae. The algae are now distributed amongst four supergroups and grouped in regards to their relatedness with non-photosynthetic protists (Table 5.1). For example, the Euglenophyta are more closely related to the obligate parasitic Trypanosomes and Leishmania, as are the dinoflagellates to the obligate parasitic Apicomplexa, which cause for example malaria (*Plasmodium* spp.), and the ciliates (Table 5.1). It is hence not surprising that 50% of the euglenoids and dinoflagellates are actually not photosynthetic (see explanation below) (Zhang et al., 2000). Indeed, the parasitic Apicomplexa have retained a rudimentary plastid, termed the apicoplasts (McFadden, 2011), which no longer has a photosynthetic function, but is still the location for *de novo* lipid synthesis (Huerlimann and Heimann, 2012). The largest change to the former protistan classification scheme is that the green algae (Chlorophyta) are now classified together with the Embryophyta in the kingdom Viridiplantae and the red algae (Rhodophyta) and the glaucocystophytes in the kingdom Rhodophytae, which now form the supergroup Planta (Table 5.1) and, strictly speaking are no longer regarded to be protists.

In order to understand algal diversity and classification schemes and their impact on end product suitability, it is necessary to understand the origin of the chloroplasts (plastids). The prokaryotic chloroxybacteria contain chlorophyll a only and water-soluble phycobilins as accessory pigments (Gould et al., 2008) and evolved oxygenic photosynthesis as a mechanism to convert solar energy into chemical energy and for carbon acquisition about 3.5 billion years ago. In essence, they created today's atmosphere, having evolved under essentially anaerobic conditions and high atmospheric CO₂ concentrations (Payne et al., 2011).

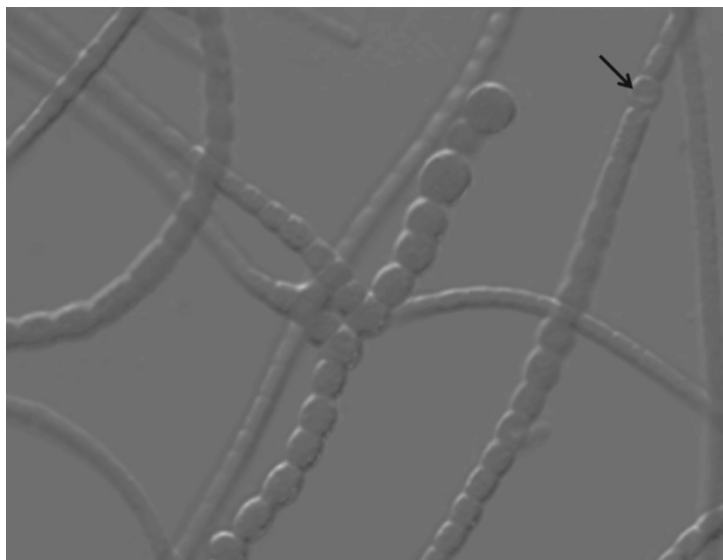
Table 5.1. **Taxonomic affiliation of algae**

Supergroup	Kingdom	Taxa (phyla)	Classes
Excavata ¹	Discicristatae	Euglenophyta, ² Trypanosomes, Leishmania	
Chromalveolata	Heterokontae	All heterokont algae also called <i>stramenopiles</i> or Ochrophyta	e.g. Bacillariophyceae, Phaeophyceae, Eustigmatophyceae
	Eukaryomonadae	Haptophyta, Cryptophyta	
	Alveolata ¹	Dinophyta, ² Ciliata, Apicomplexa	
Rhizaria ¹	Cercozoae	Chlorachniophyta, Radiolarians, Euglyphids	
Plantae	Viridiplantae	Chlorophyta, Embryophyta ³	
	Rhodophytae	Rhodophyta, Glaucophyta (sometimes also called Glaucocysto-phyta)	e.g. the Glaucophyte, <i>Cyanophora paradoxa</i>

Notes: 1. Supergroup and kingdoms containing heterotrophic zooplankton or obligate parasites. 2. Microalgal phyla containing heterotrophic and non-plastidial genera. 3. Embryophyta are the plants and do not have any algal representatives in this phylum.

Source: Based on Adl, S.M., et al. (1995), “Diversity, nomenclature, and taxonomy of protists”, *Systematic Biology*, No. 56, pp. 684-689.

Some chloroxybacteria are also capable of fixing atmospheric nitrogen. As the nitrogenase enzyme complex responsible for N₂ fixation is inhibited by oxygen, the process is either spatially separated into heterocysts (which only contain the non-water splitting and therefore not-oxygen evolving photosystem I) (Figure 5.2) or it is temporally segregated (occurring at night, when photosynthesis is not active and no oxygen is being produced) (Latysheva et al., 2012). This provides a growth advantage under nitrate-, nitrite-, ammonium- and/or urea-nitrogen-limiting conditions, which can be a large cost saver in commercial-scale production for various valuable end products.

Figure 5.2. **Micrograph of the branching filamentous cyanobacterium *Mastigocoleus* sp.**

Note: The arrow is pointing to the heterocyst.

These chloroxybacteria were taken up by a heterotrophic host, providing it with photosynthates and energy. Over time, this endosymbiotic relationship transformed into the chloroplast (plastid surrounded by two membranes) through gene transfer from the endosymbiont to the host. This primary endosymbiotic relationship, which gave rise to

organisms with a primary plastids (chloroplast surrounded by two membranes) resulted in the evolution of the first photosynthetic eukaryotes; for the algae, these are the green algae, characterised by having chlorophyll a and b, the red algae and the glaucophytes (chlorophyll a only and phycobilins; they also still contain the bacterial peptidoglycan cell wall between the inner and outer chloroplast membrane, hence the organelle is known as the cyanelle instead of chloroplast) and for plants it is the embryophytes (aquatic and terrestrial plants characterised by having chlorophyll a and b) (Archibald, 2008; Gould et al., 2008).

Then in a secondary endosymbiotic event, a heterotrophic eukaryote engulfed either a red or green lineage primary photosynthetic eukaryote (e.g. a red alga or perhaps glaucophyte for the red lineage and a green alga for the latter), giving rise to photosynthetic eukaryotes now containing plastids surrounded by three or four membranes (Archibald, 2008; Gould et al., 2008).

The origin of plastids, regarding red or green lineage, is still debated with one group hypothesising that the mechanisms required for the transformation of an endosymbiont into an organelle would be too complex to be derived from two separate events, hence claiming that all plastids were red lineage derived (Delwiche, 1999). Presumably, a green lineage endosymbiotic event gave rise to the Euglenoids and the Chlorarachniophytes as both groups contain chlorophyll a and b. In contrast, it is assumed that generally all other genera in the supergroup Alveolata (Table 5.1) arose from a secondary endosymbiotic event with a red alga. There are exceptions to the latter in the case of the dinophyta, which are believed to have entered tertiary endosymbiotic events with various other algae (Chlorophyta, Haptophyta, etc.) (Delwiche, 1999).

The accumulation of membranes surrounding the plastid obviously made nuclear-encoded plastidial protein import quite complex and this together with the physiological and cell biological phyla/class characteristics would explain why the different classes of algae are characterised by different carbon storage products (Huerlimann and Heimann, 2012), which is important when considering commercial production for specific end-product development.

Taxonomic biochemical characteristics regarding carbon storage have the following implications for end-product development. The primary carbon and energy store for members of the Chloroxybacteria, the supergroup Planta and the Cryptophyta belonging to the supergroup Alveolata is starch (Graham et al., 2008), but the chloroxy bacteria and algae lack the structural complexity of terrestrial plant cell walls, which contain lignin. Starch and simple cellulosic materials are easily fermented to bio-ethanol (Hirano et al., 1997) and also extracted and transesterified to biodiesel (Sivakumar et al., 2010) as a renewable fuel. In addition, the main fatty acid composition of the green algae belonging to the class Chlorophyceae, particularly freshwater species, is very similar to terrestrial plants (vegetable oil) with α -linolenic acid as the main ω -3 polyunsaturated fatty acid. This would render this group of organisms ideal for replacing terrestrial vegetable oil crops, such as canola, etc. with a green algal-derived vegetable oil produced on non-arable land using non-potable freshwater or nutrient-rich wastewater. To date, the marine chlorophyte *Dunaliella salina* and the freshwater chlorophyte *Haematococcus pluvalis* are used for the commercial production of β -carotene and astaxanthine, respectively, which are used for food colouring, as supplements in the aquaculture industry and as antioxidants in health foods, while biomass of the green microalga *Chlorella* spp and the chloroxybacterium *Arthrospira platensis* (formerly *Spirulina platensis*) are marketed unextracted as health food supplements based on the

content of Provitamin A and vitamins of the B-complex (Table 5.2). A 40-gramme supplement of *Arthrospira platensis* per 34 kilogramme feed per dairy cow changed the fatty acid profile of the milk from predominantly saturated fatty acid to the dominance of mono- and polyunsaturated fatty acids (sampled on days 15, 30 and 45 of a 7-week feed trial period), which will help to market such milk products as health foods and potentially achieve higher prices for the product due to the offset of negative health impacts associated with saturated fatty acid diets (Christaki et al., 2012).

Many members of the supergroup Alveolata (the diatoms, eustigmatophytes, cryptophytes and haptophytes, but not the brown algae Phaeophyceae) primarily store photosynthetic carbon as storage lipids (triacylglycerides [TAG]) instead of starch but also produce storage sugars, such as chrysolaminarin, a β -1,3-linked polysaccharide (Graham et al., 2008). Members of this supergroup are also characterised by having high content of ω -3 long chain polyunsaturated fatty acids, such as EPA (C20:5, eicosapentenoic acid) and DHA (C22:6, docosahexanoic acid), and the ω -6 long chain polyunsaturated fatty acid AA (C20:4, arachidonic acid), which are essential fatty acids in the diet of aquaculture organisms (Brown, 2002), but also humans (now usually provided as fish oil). In addition, it has been shown that a correct ω -6 to ω -3 ratio is critical for maintaining cardio vascular health (Simopoulos, 2002).

Given the biodiversity represented in this supergroup, there are very few species belonging to diverse genera (Bacillariophyceae: *Chaetoceros calcitrans* and *C. muelleri*, *Nitzschia* spp, *Phaeodactylum tricornutum*, *Skeletonema costatum*; Eustigmatophyceae: *Nannochloropsis oculata*; Haptophyta: *isochrysis* aff. *galbana*, *Pavlova salina* and *P. lutheri*; Cryptophyta: *Proteomonas sulcata*, *Rhodomonas salina*, Dinophyta: *Akashiwo sanguinea* (formerly *Gymnodinium sanguineum* [Heimann, 2012], *Cryptothecodinium cohnii*) that are currently primarily cultivated for aquaculture feed purposes, where they serve as a primary food source for crustaceans, filter-feeding molluscs and fish larvae, with the latter being fed either directly on the algae, or being fed microalgae-reared *Artemia* and rotifers, if larger food particles are required (Brown, 2002; Harwood and Guschina, 2009; Heimann, 2012) (Table 5.2). Given the growing importance of aquaculture-reared seafood in maintaining a healthy diet for the growing population whilst protecting naturally oil-rich wild fish populations and crustaceans, the natural affiliation of aquaculture with microalgal cultivation and the nutrient-rich wastewaters this industry sector creates, it would make perfect sense to add algal commercial-scale cultivation and biomass-derived co-products to this industry's commodities, whilst bioremediation of the nutrient-rich wastewater would allow for efficient water recycling, reducing environmental impact and thereby allowing the industry to expand.

Even though the macroalgal food market is well established and lucrative, fetching USD 2 billion for Nori (*Porphyra* sp., Rhodophyta), USD 600 million for Wakame (*Undaria pinnatifida*, Phaeophyceae) and Kombu (*Laminaria japonica*, Phaeophyceae) and a global market potential ranging from 20 000 to 40 000 t (Jensen, 1993; Radmer, 1996), most of these materials are harvested from the wild, which might not be sustainable in the long term. The market potential for microalgae ranges from lucrative health food products (e.g. *Arthrospira* sp. sells at USD 100 kg⁻¹, (Radmer, 1996)

Table 5.2. Use of algal products and current market prices

Product	Use	Organism	Market price (USD)	Global market	References
Biodiesel	Renewable fuel		0.73 L ⁻¹		Harun et al. (2010); Subhadra and Edwards (2011)
Bio-ethanol	Renewable fuel	<i>Arthrospira</i> sp. (Chloroxybacteria), <i>Scenedesmus dimorphus</i> (Chlorophyta), <i>Porphyridium cruentum</i> (Rhodophyta)			Harun et al. (2010)
Bio-methane	Renewable fuel	<i>Ulva</i> sp. (Chlorophyta), <i>Gracilaria</i> sp. (Rhodophyta), <i>Laminaria</i> sp. and <i>Macrocystis</i> sp. (Phaeophyceae)			Harun et al. (2010)
Bio-butanol	Renewable fuel, fine chemical	<i>Dunaliella</i> sp. (Chlorophyta) or any of the above that are also suitable for bio-ethanol production			Harun et al. (2010)
Acetone	Fine chemical	<i>Dunaliella</i> sp. (Chlorophyta) or any of the above that are also suitable for bio-ethanol production			Harun et al. (2010)
Glycerine	Pharmaceuticals, paints, industry bulk chemical	By-product of bio-diesel production	320-500 t ⁻¹		Subhadra and Edwards (2011)
ω-3 fatty acids	Neutraceuticals animal feed	<i>Nannochloropsis</i> sp. (Eustigmatophyceae), <i>Nitzschia</i> sp. and <i>Phaeodactylum tricornutum</i> (Bacillariophyceae), <i>Pavlova viridis</i> (Haptophyta)	50 000-135 000 t ⁻¹	USD 1 billion ¹ USD 7 billion ²	Subhadra and Edwards (2011)
EPA	Neutraceuticals, animal and aquaculture feeds	<i>Nannochloropsis</i> sp. (Eustigmatophyceae), <i>Nitzschia</i> sp. and <i>Phaeodactylum tricornutum</i> (Bacillariophyceae), <i>Pavlova viridis</i> (Haptophyta)	508 000 g ⁻¹	954-1219 g ⁻¹	Harun et al. (2010); Harwood and Guschina (2009); Spolaore et al. (2006)
DHA ³	Neutraceuticals, animal and aquaculture feeds	<i>Cryptocodinium cohnii</i> (Dinophyta), <i>Ulkenia</i> sp., <i>Schizochytrium mangrovei</i> and <i>Thraustochytrium aureum</i> (Heterokontae, Thraustochytriaceae), <i>Amphidinium carterae</i> (Dinophyta), <i>Isochrysis galbana</i> (Haptophyta)		689-901 g ⁻¹	Harun et al. (2010); Harwood and Guschina (2009); Spolaore et al. (2006)
AA (Arachidonic acid)	Neutraceuticals	<i>Porphyridium</i> sp. (Rhodophyta)		636-795 g ⁻¹	Harwood and Guschina (2009); Spolaore et al. (2006)
γ-linolenic acid	Neutraceuticals	<i>Arthrospira</i> sp. (Chloroxybacteria)		445-636 g ⁻¹	Harwood and Guschina (2009); Spolaore et al. (2006)
Chlorophylls	Food colouring, functional food, pharmaceuticals, cosmetics	<i>Chlorella</i> sp. (Chlorophyta)	184 000-268 000 g ⁻¹		Harun et al. (2010)
Accessory non-polar pigments	Food colouring, functional food, pharmaceuticals, cosmetics				

Table 5.2. Use of algal products and current market prices (*cont.*)

Product	Use	Organism	Market price (USD)	Global market	References
Phycobiliproteins	Biomedical uses (fluorescent markers)	<i>Arthrospira</i> sp. (Chloroxybacteria), <i>Porphyra</i> sp. and <i>Rhodella</i> sp. (Rhodophyta), potentially also Cryptophyta and Glaucophyta	3 000-25 000 g ⁻¹	USD 50 million	Milledge (2011); Radmer (1996)
	Food colouring				
	Pharmaceuticals				
	Cosmetics				
Astaxanthin	Antioxidant	<i>Haematococcus pluvalis</i> (Chlorophyta)	2 500 000 t ⁻¹	USD 200 million	Milledge (2011)
	Food colouring				
β-carotene ³	Food colouring, functional food, pharmaceuticals, cosmetics	<i>Dunaliella salina</i> (Chlorophyta)	300 000-3 000 000 t ⁻¹		Ben-Amotz (2007); Milledge (2011)
Vitamin B ₁₂ ³	Neutraceuticals, health food	<i>Chlorella</i> sp. (Chlorophyta), <i>Arthrospira platensis</i> ⁴ (Chloroxybacteria)			Harun et al. (2010)
Provitamin A ³	Neutraceuticals, health food	<i>Chlorella</i> sp. (Chlorophyta), <i>Arthrospira platensis</i> ⁴ (Chloroxybacteria)			Harun et al. (2010)
Algal meal	Animal feed	<i>Arthrospira</i> sp. (Chloroxybacteria), <i>Chlorella</i> sp. (Chlorophyta), <i>Laminaria</i> sp. (Phaeophyceae)	1 200-1 800 t ⁻¹	635 million t ⁶ 5-10 million t ⁶	Milledge (2011); Subhadra and Edwards (2011)
Stable isotopic amino acids			5 900 g ⁻¹		Milledge (2011); Spolaore et al. (2006)
Stable isotopic nucleic acids			28 000 g ⁻¹		Milledge (2011); Spolaore et al. (2006)
Agar ³	Food, biomedical	<i>Gracilaria</i> sp., <i>Gelidium</i> sp., <i>Pterocladia</i> sp. (Rhodophyta)			Radmer (1996)
Agarose ³	Biotechnological applications	Rhodophyta			Radmer (1996)
Alginates ³	Food, paper, biomedical uses	<i>Ascophyllum</i> sp. <i>Laminaria</i> sp. and <i>Macrocystis</i> sp. (Phaeophyceae)		Up to 25 000 kg ⁻¹	Radmer (1996)
Carageenans	Food products (gelling, thickening, stabilising)	<i>Eucheuma cottonii</i> , <i>Chondrus crispus</i> and <i>Eucheuma spinosum</i> (Rhodophyta)			Radmer (1996)
Fertilisers	Soil conditioners	All algae, but easier with macroalgae			

Notes: 1. 2008. 2. 2011. 3. Current commercial production. 4. Formerly *Spirulina platensis*. 5. Animal feed. 6. Replacement with algae.

to relatively low-value products (e.g. biodiesel USD 0.73 L⁻¹; Subhadra and Edwards, 2011), but there is also an enormous potential to replace existing animal feeds with microalgal meal, as the former need to be raised in substantial quantities on arable land and in some countries (i.e. Australia) require irrigation and expensive import, which, considering carbon and energy budgets, may not be sustainable in the long run. For example, soy-, copra- and fish meal fetch USD 320-1 200 t⁻¹, when sold as animal feed (Subhadra and Edwards, 2011), yet microalgae contain a similar biochemical profile compared to soy (Table 5.3), but do not require arable land and can be cultivated in nutrient-rich wastewaters, thereby taking the ammunition out of the feed versus food debate. The same argument can be expanded to fish oil, currently produced from caught wild population, which is environmentally unsustainable in the long term and will not cover the growing needs of the future human population. The current market price of fish oil is USD 800-1 000 t⁻¹ (Subhadra and Edwards, 2011), which is used in aquaculture and for supplementation of ω -3 long chain polyunsaturated fatty acids in human nutrition. Thus, the higher value markets for microalgal products (Table 5.2) exist, making economical production of a variety of microalgal products possible, if commercial production adopts a mixed product approach where production of high-value commodities offsets production costs for low-value market goods, such as biofuels. Ultimately, it will be necessary to guarantee food, feed and fuel security for future generations.

Table 5.3. **Microalgal biochemical profiles in comparison to soy**

Species	Lipid 100g ⁻¹	Protein 100g ⁻¹	Carbohydrate 100g ⁻¹
<i>Nannochloropsis oculata</i> (Eustigmatophyceae)	16.4-29.7 ¹	29.7	38.2
<i>Picochlorum atomus</i> (Chlorophyta) ²	9.7-40 ^{1,3}	50	32
Soy meal	19.9	36.5	30.2

Notes: 1. Higher lipid content is achieved under nitrogen-limiting conditions. 2. Three times the growth rate of *Nannochloropsis oculata*. 3. Highest lipid yields under nitrogen starvation.

Considerations for strain selection for commercial-scale algal production

Strain selection must consider the quality of the water source, the environmental conditions, cultivation system, fertilisation regimes and integrated cultivation-harvest cycles, because they influence biomass productivity, product quality and hence marketability. Market potential and commercial viability in terms of required CAPEX (capital expenditure) and OPEX (operational expenditure) also need to be modelled to ensure successful commercial production. It is often best to invest in desk studies in order to create at least preliminary business plans. These should consist of a good knowledge of endemic strains to evaluate their use, particularly if water remediation (e.g. metal remediation from industrial tailing dams or secondary sewage) is the goal, as these organisms are likely already adapted to local conditions (water quality and environmental conditions) (Park et al., 2011).

Strain selection is intimately linked with product selection, especially when targeting fine chemicals or nutraceuticals, because biochemical composition is, as explained before, often class specific (e.g. the ω -3 long chain polyunsaturated fatty acids EPA, DHA, AA will only be produced in certain strains [Brown, 2002], while quantities of the desired product are often influenced by fertilisation regimes and environmental conditions [Huerlimann et al., 2010]). For example, growth of the green microalga

Picochlorum atomus was not affected by salinity levels ranging from 2 ppt to 36 ppt (Figure. 5.3A) (Alvensleben, 2010), neither was lipid content, while nitrogen limitation and starvation significantly increased total lipid content (Figure 5.3B). This makes *Picochlorum atomus* an ideal organism for wastewater remediation with vastly differing salinities, while the end product can range from health food supplements (e.g. like *Chlorella* tablets) and animal feeds (Table 5.3) to biodiesel (Table 5.4). Biodiesel quality parameters calculated from fatty acid methyl ester (FAME) profiles and compared to available standards and common plant oils used for biodiesel production showed that the green microalga *Tetraselmis* sp. and the eustigmatophyte *Nannochloropsis oculata* had the most suitable FAME profile for biodiesel production with regards to cetane number, iodine value and cold filter plugging point, followed by *Picochlorum atomus* with similar cetane and iodine values but less desirable cold filter plugging point and the haptophyte *Isochrysis* aff. *galbana*, which had the least suitable profile regarding cetane number and iodine value, but an exceptional low cold filter plugging point due to the high amounts of long chain polyunsaturated fatty acids (EPA and DHA) generally produced in haptophytes (Table 5.4). The large variation in quality parameters show that fatty acid profiles, and hence biodiesel quality, is strongly influenced by nutrient status (growth phase) and fertilisation regime.

Figure 5.3. Salinity tolerance of *Picochlorum atomus*

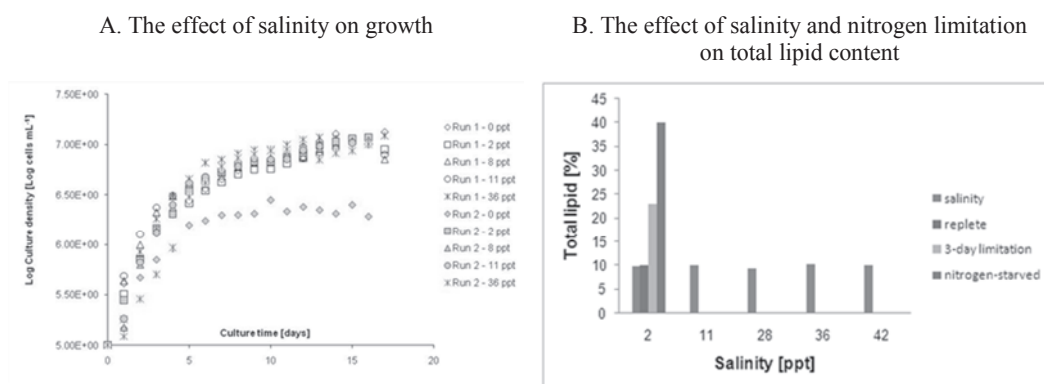


Table 5.4. Biodiesel properties of select microalgae calculated from FAME profiles

Standards	Cetane number	Iodine value	Long chain saturated fatty acids	Cold filter plugging point	Degree of unsaturation
ASTM D675	47 minutes	n.d.	n.d.	n.d.	n.d
EN 14214, AU 255	51 minutes	120	n.d.	n.d.	n.d
NPA Brazil	45 minutes	n.d.	n.d.	n.d.	n.d
Palm	55.1	73.9	3.6	-5.2	86
Soy	53.2	112.9	1.1	-13.0	131
<i>Tetraselmis</i> sp.	42.8-56.6 (51.5)	78.7-140.6	3.2-4.4	-2.8/-6.3	83.7-118.1
<i>Nannochloropsis oculata</i> ¹	30.9-54.8 (50.5)	83.4-163.2	3.0-6.5	-0.4/-8.5	74.1-118.5
<i>Picochlorum atomus</i> ¹	43.7-61.5 (42.2)	52.5-137.9	6.2-9.3	2.9/12.8	51.2-135.5
<i>Isochrysis</i> aff. <i>galbana</i>	28.1-40.7 (32.7)	149.1-205.5	1.7-7.0	-3.1/-11	104-128

Notes: 1. Strongly influenced by nutrient and growth status. Numbers in brackets denote average values of all FAME profiles obtained.

With regards to water remediation or raising aquaculture species with unknown dietary requirements, a green aquaculture approach may be beneficial, where a body of water is fertilised to allow the local microalgal flora to bloom (Neori, 2011; Park et al., 2011). However, there is little control over species composition, which can adversely affect product development, particularly when the microalgal community consists of taxa belonging to different phyla with markedly different biochemical profiles, as it will be difficult, if not impossible, to guarantee product quality. In addition, quite a number of microalgae, most notably cyanobacteria, which often contaminate microalgal cultures, can produce potent toxins which would render the biomass unsuitable for feed or food. A green aquaculture approach is nonetheless beneficial if the microalgal community to be cultivated belong to the same class or family, because this negates large biochemical profile changes due to dominance shifts or if a distinctive biochemical profile is less important, e.g. biochar applications (Atkinson et al., 2010; Bird et al., 2011). Experiments with chlorophyte consortia dominated by *Scenedesmus* spp showed that this group of organisms is capable of remediating up to 40-60 mg of nitrite $\text{L}^{-1} \text{ day}^{-1}$, which is an expected conversion product of nitric oxide (NO_x) in water, and more than 350 mg of nitrate L^{-1} over seven days (data not shown), making them ideal for NO_x remediation from flue gas from coal-fired power stations and for nutrient-rich wastewater remediation (Park et al., 2011).

Tailing dams of coal-fired power stations also contain heavy metals, which is of concern when considering the biomass for use in animal feed applications. Experiments using the *Scenedesmus* spp-dominated consortia and supplemented with the average tailing dam concentrations of boron, molybdenum, vanadium and zinc, the metals identified as of concern with regards to animal feeds, and grown under low and high nitrogen and phosphorus, showed that green freshwater strains remediate 100% of these metals, with slightly higher uptake observed for boron and significantly increased uptake for zinc under high nutrient conditions (Table 5.5). These data suggest that *Scenedesmus* spp chlorophytic freshwater consortia are suitable for metal remediation from industrial tailing dams, but care must be taken when considering end product use, as biomass generated in such applications would need to be mixed with other uncontaminated feeds to avoid potential metal poisoning (Alvarez Roa, 2012). The same experiment also showed that metal treatment had no effect on growth or fatty acid composition, but the fertilisation regime had a significant impact on the amounts of polyunsaturated fatty acids (PUFA), which were nearly twice as high under high nutrient conditions compared to low nutrient conditions (Table 5.5). This has significant implications with regards to product choice for this group of organisms, suggesting that low nutrient conditions would render a biomass suitable for biodiesel production, while a high nutrient regime would allow use of the biomass in animal feed applications (Alvarez Roa, 2012).

For products where the biochemical composition is critical and must be guaranteed with regards to minimum and maximum content, e.g. animal feeds, health food supplements, etc., this is extremely important to know as is in which way fertilisation regimes and environmental conditions affect biochemical composition of the biomass. For example, photosynthetically acquired carbon can either be used for growth for incorporation into membrane lipids, DNA and RNA and proteins or diverted to storage as either storage oils (triacylglycerides, TAGs) or sugars, such as starch. Algal culture growth typically continues until carrying capacity of a particular cultivation system and maximum cell densities for a particular strain are reached, as long as none of the nutrients (nitrogen, phosphorus, minerals – such as iron) are limiting. This entails that rapidly

growing cultures deposit less carbon into storage, which is undesirable if products development relies on either high starch or TAG content such as bio-ethanol or biodiesel production, respectively. For example, it has been shown that nitrogen limitation (growth phase) and culture medium composition affect lipid content and fatty acid profiles of microalgae, but that the extent of the effect is strain dependent, affecting some species more than others (Huerlimann et al., 2010 and references therein). Thus, integration of fertilisation regimes with harvest cycles, e.g. allowing for a period of nutrient limitation prior to harvest to optimise lipid content, becomes an important consideration, which is, in an economical and environmental sense, at least as important as strain selection.

Table 5.5. **Metal bioaccumulation ($\mu\text{g L}^{-1}$) and effect of nutrient regime on fatty acid classes (%) in a chlorophyte community dominated by *Scenedesmus* spp**

Metal	Low nitrogen and phosphorus	High nitrogen and phosphorus
Boron	0.15 – 0.225	0.25 – 0.3
Molybenium	0.055 – 0.06	0.06 – 0.7
Vanadium	0.1 – 0.14	0.1 – 0.14
Zinc	0.19 – 0.28	0.35 – 0.45

Fatty acid class	Control, treatment	Control, treatment
Saturated fatty acid	30, 30	19, 22
Mono-unsaturated fatty acid	30, 32	19, 19
Polyunsaturated fatty acid	40, 38	38, 59

Source: Alvarez Roa, C. (2012), “Microalgae bioremediation of trace metals commonly found in ash-dam water from Tarong power station: A coal-fired power plant in Qld”, School of Marine and Tropical Biology, Vol. Master of Applied Science, James Cook University, Townsville, Australia.

Cultivation considerations

Generally, three types of microalgal cultivation systems can be distinguished:

- open systems such as ponds, raceways and high rate algal ponds (HIRAPs) traditionally used in aquaculture and for the commercial production of microalgae
- closed systems: tube or plate photobioreactors, where the algal biomass is generally cycled through a solar compartment and a mixing compartment, which allows for degassing and nutrient addition
- hybrid systems: which are essentially open systems but operate under positive air pressure compared to the outside, making it less likely for contaminants to invade the system (da Rosa et al., 2011; Henrard et al., 2011; see also Chapter 4).

All cultivation systems have their advantages and disadvantages. Disadvantages of open systems are: prone to invasions, shallow, making mixing and gas solubilisation difficult, high water loss due to evaporation, large land requirements, low biomass productivities and often poor temperature control. Open systems also have significant advantages. The shallow depth allows for effective degassing of the photosynthetically produced oxygen, which can inhibit photosynthesis if it accumulates in the system, evaporative water loss provides a means of non-energy derived cooling, most microalgal species investigated can be grown in these systems and they are inexpensive in terms of CAPEX (Christenson and Sims, 2011; Weissman and Goebel, 1987). However, evaporative water loss and the large area requirement, particularly for biomass use for biofuel production, are of environmental concern considering future freshwater resources

(Murphy and Allen, 2011). To avoid these negative impacts, it would be mandatory that evaporative water loss is compensated for using non-potable wastewater and that system operation must occur on non-arable land. Currently, open systems are used for the commercial production of β -carotene mainly using the chlorophyte *Dunaliella salina*, production of the chloroxybacterium *Arthrospira platensis* and the chlorophyte *Chlorella* sp. as a health food supplements (Table 5.2). Reported long-term operation averages for the eustigmatophyte *Nannochloropsis oculata* are 20 g dry weight $\text{m}^{-2} \text{day}^{-1}$, which still significantly exceeds productivities of even the most productive terrestrial oil crops (CSIRO, 2011), make such systems potentially useful to also secure high-quality aviation fuel, an area the aviation industry is actively pursuing. With reference to the sustainability of aviation fuel, it is noteworthy that the CSIRO considers bio-derived jet fuels the only sustainable replacement for fossil oil-derived aviation fuels, which will not interfere with arable land use for human food production and can be generated in sufficient quantities to make this a possibility (CSIRO, 2011).

Closed systems are believed to have significant advantages over open cultivation systems in that they are considered to be less prone to contamination, do not suffer from evaporative water loss, show higher productivities on a volume and area basis due to improved light penetration and biomass resuspension (Carvalho et al., 2006). Disadvantages of these systems are that current systems are relatively small scale, only very few organisms can be successfully cultivated, mixing and degassing (build up of photoinhibitory concentrations of photosynthesis-derived oxygen) is still problematic and energy-intensive, require extensive ground preparations for their set up and cooling due to the small volumes in tubular and thin plate solar compartments, are highly technical and very expensive requiring highly trained personnel, which almost prohibits operating them in less developed countries.

In general, improved productivities are typically not large enough to offset the higher costs of CAPEX and OPEX (energy requirements), making it energetically and economically unattractive to use them for the production of low-value end products, such as fuels (Xu et al., 2009). Volumetric daily productivities of closed photobioreactor systems are being advertised as 4-6 g dry weight $\text{L}^{-1} \text{day}^{-1}$; however, long-term multi-year production records are lacking, which makes it unclear whether these productivities could be maintained year round. Regardless, as volumes in closed production systems are typically 10-20 times smaller than open systems, but costs are 10 times higher, it is questionable if this increased productivity would actually stand out compared to the reported long-term year-round productivities of open systems' 0.5-1 g dry weight $\text{L}^{-1} \text{day}^{-1}$, which for lower value products is most likely not the case. In terms of cost and volumes, closed photobioreactors are attractive for the cultivation of microalgal biomass for the high to very high value product market where much smaller biomass or compound quantities are required to strike economical success. As such, to date, commercial-scale cultivation is restricted to the freshwater chlorophyte *Haematococcus pluvialis* for the production of the antioxidant astaxanthin (Li et al., 2011).

Given the economical and energetic drawbacks of closed systems, current research also focuses on developing hybrid systems, which are essentially a semi-closed cultivation system where a positive air displacement between the system and the outside should restrict air-borne contamination. Another definition of hybrid system exists where the term describes a closed photobioreactor tasked with maintaining biomass for the inoculation of open systems for short-term cultivation in order to curb contamination (Singh and Dhar, 2011). Regardless of the definition used for hybrid systems, they are likely to be similarly expensive with regards to energy used for culture resuspension and

will also suffer from similar rates of evaporative water loss, displaying approximately twice the price tag of commercial-scale open systems. In essence, however, these systems have inherited the positive sides of the open cultivation systems and more of the advantages of the closed system. This makes these systems economically attractive for the mid-price range product market, as contamination is one of the major economic losses associated with open cultivation systems. Whether these systems display appropriate productivities remains to be shown, but initial results show that horizontal systems, which are comparable in depth and volumes to commercial raceways, show similar productivities and that these can be increased fivefold and more if cultivation occurs in vertically oriented systems (data not shown). The latter systems, however, are of much lower volume, thus it remains to be demonstrated whether vertical hybrid systems of similar volumes to horizontal ones and raceways would maintain this aerial productivity advantage.

It is also possible to grow many microalgal species (e.g. the chlorophytes *Chlamydomonas reinhardtii* or *Chlorella protothecoides*) heterotrophically in fermenter-style cultivation systems on glucose or acetate in the absence of light, which increased lipid productivity around 24-fold (Xiong et al., 2008) compared to photosynthetically grown microalgae with high lipid productivities, such as the green alga *Tetraselmis* sp., a marine species belonging to the class Prasinophyceae (Huerlimann et al., 2010). While this approach shows immense promise for the production of low-value end products such as biodiesel, there are no ecological advantages to promote this to a commercial scale considering rising atmospheric CO₂ concentrations and the competition for arable land and irrigation-derived sugar, as heterotrophic growth generates CO₂ and the approach would enter the food versus fuel debate if conducted on a large enough scale to substantially contribute to renewable biofuels to meet growing future demands in industry and for general transport. In addition, the approach requires axenic (bacteria-free) cultures, which will be challenging to maintain on an industrial scale. Furthermore, the beneficial allelopathic interactions between the microalgae and their bacterial flora are lost in axenic cultivation, which leads to the cultivation of strains that are tolerant to this loss, thereby restricting strain choice. In addition, the demand for organic carbon would, at the required scales, negatively impact on sugar prices and arable land committed to carbohydrate production for fuel rather than human food, which has already been criticised with regards to the use of corn for bioethanol production (Liao et al., 2011). Even if life cycle and economic analysis were favourable, at this stage, the negative aspects outweigh the positive aspect of fuel security.

Harvest and process considerations

Following CAPEX and OPEX expenditures for commercial-scale cultivation systems, harvesting of microalgae grown in suspension cultures is the single largest CAPEX and energy expenditure often responsible for unfavourable economics and energy budgets for low-value commodities such as biofuels (Ghasemi et al., 2012). Harvest capacity is immediately important to closing the production cycle between biomass generation and biomass processing and the effectiveness of the harvesting system chosen will impact on cultivation regimes and will allow for the design of production cycles and biochemical optimisation of the biomass produced (e.g. incorporation of nutrient limitation phases prior to harvest and the ability to harvest on scale with biomass production). For example, different microalgal strains can show very different harvest requirements for growth phase and nutrient status in order to optimise biomass and lipid productivity (Table 5.6)

(Huerlimann et al., 2010). For example, scheduling of the harvest of the chlorophyte *Tetraselmis* sp. should be for the logarithmic phase, as total lipid content does not increase in the nitrogen-limited stationary phase (Table 5.6). In contrast, total lipid content of the eustigmatophyte *Nannochloropsis oculata* increases significantly during nitrogen limitation in the stationary phase and hence harvest for this species should be timed to coincide with this growth phase (Table 5.6) (Huerlimann et al., 2010). Implications of harvest integration with the culture growth phase are less critical for the haptophyte *Isochrysis* aff. *galbana* and the cryptophyte *Rhodomonas* sp.; however, as both biomass and lipid productivity are substantially reduced in the stationary phase without the offset of improved lipid content, harvest schedules should aim for harvests in logarithmic growth phase (Table 5.6). The harvesting methodology applied is also critically linked to cultivated microalgal strains, as differently sized and shaped cells will affect the harvesting process, which will necessitate optimising harvesting strategies for strain-dependent energy and economic efficiencies. Furthermore, different downstream biomass process technologies and end products will require different moisture levels of the biomass.

Table 5.6. Growth phase-dependent total lipid content, biomass productivity and lipid productivity of four tropical microalgal species

Species	Logarithmic phase			Stationary phase		
	Total lipid content	Biomass productivity	Lipid productivity	Total lipid content	Biomass productivity	Lipid productivity
	% of dm	g m ⁻² day ⁻¹	g m ⁻² day ⁻¹	% of dm	g m ⁻² day ⁻¹	g m ⁻² day ⁻¹
<i>Nannochloropsis</i> sp.	21.3	13.4	4.2	32.7	2.2	0.6
<i>Isochrysis</i> aff. <i>galbana</i>	23.5	18.8	4.4	28.6	3.2	1
<i>Tetraselmis</i> sp.	10.6	45.0	4.8	10.1	5.1	0.5
<i>Rhodomonas</i> sp.	9.5	13.4	1.3	12.5	4	0.5

Source: Huerlimann, R., R. de Nys and K. Heimann (2010), "Growth, lipid content, productivity, and fatty acid composition of tropical microalgae for scale-up production", *Biotechnology and Bioengineering*, No. 107, pp. 245-257.

Total suspended solid content (number of cells per unit volume) of commercial-scale cultivation systems normally does not exceed 1%. This means that 99% of the water-based cultivation medium needs to be separated from the 1% solids, which becomes harder and less economically and energetically feasible on scales required for sufficient microalgal biofuel production. The scaling aspect of harvesting of microalgal biomass is far less important for the production of lower volume and high-value end products and is therefore not often critically assessed, as centrifugation techniques (traditionally disc flow through centrifugation) are adequate in this context and initial CAPEX and energy costs are offset by the value generated by the end product (e.g. astaxanthine, β -carotene, and *Chlorella* production).

The harvesting process is essentially a dewatering process that can draw on different strategies, dependent on the microalgal strain, biomass productivities, daily culture volumes that require processing, the size of the production facility (m³) and the level of water content required for further processing of the biomass. The larger the facility with regards to culture volumes, the more important an integrated harvest-biomass production process becomes (Alabi et al., 2009). This makes the harvesting/dewatering process the biggest bottleneck to the commercial production of microalgae for sustainable renewable fuel generation.

Centrifugation is by far the most effective and most versatile harvesting technique; it is, unfortunately, also the least economical and energy efficient process with regards to large-scale applications (Alabi et al., 2009). Therefore, preconcentration of the dilute suspension culture is desirable. Current preconcentration techniques typically used are flocculation, either achieved through bio-flocculation (often self-aggregation) or chemical flocculation (using either inorganic or organic flocculants), a process that relies on neutralising the negative surface charges of microalgal biomass (Alabi et al., 2009). Chemical flocculation is not desirable as it can render the biomass unsuitable for nutraceutical products. Filtration is another dewatering process; however, costs at biofuel production scales are typically prohibitive. By far the cheapest way is gravity settling, which is achievable with appropriate microalgal strains, but time and land requirements still need modelling to scale with culture volumes required for renewable fuel production. Gravity settling is also not possible, with many of the small-sized microalgae that show promise for biofuel, bioplastic and higher value nutraceuticals or health food products (e.g. *Nannochloropsis occulata*, *Picochlorum atomus*, etc.). Dissolved air floatation, a dewatering process used in the paper industry, shows promise for dewatering, but consistent results would be highly strain and prior treatment (e.g. electro-coagulation) dependent. In short, with regards to harvest strategy, there will be no one size fits all, due to strain dependence and the amount of dewatering required for further processing of the biomass into desired end products.

Various process technologies exist for different end products. For biodiesel production, unless oil can be mechanically extracted from the biomass, hexane extraction followed by transesterification will be required, which potentially leaves the high protein and vitamin-rich microalgal meal unusable for animal feed production. This process technology also requires complete drying of the biomass, an energy expense that is hardly affordable given the energy requirements for cultivation and dewatering/harvesting. Several other process technologies show real promise, particularly for renewable fuel production such as subcritical hydrothermal liquefaction, as a certain amount of water is required, thereby avoiding the CAPEX and energy-intensive complete dewatering and drying requirements. This process has already been used successfully on dilute microalgal growth medium generated in HIRAPs for wastewater treatment, but complete lifecycle analyses will be required to assess economical and environmental sustainability of the process, which shows immense promise for biofuel production from microalgae, as the water content is beneficial rather than a hindrance in the conversion of the biomass (Lam and Lee, 2012).

Conclusion

Microalgal commercial-scale cultivation is achievable and superior with regards to biomass productivities to terrestrial crops, showing tremendous potential for the bioremediation of gaseous wastes and polluted waters, whilst affording cost recovery through value-adding co-product development. Simple commercially viable systems exist to produce sufficient biomass today, but a more integrated approach and complete lifecycle analyses still need to be conducted to evaluate large-scale potential environmental implications. The most promising approach to renewable energy and fuel production from microalgae lies in designing an integrated approach for cheap and environmentally/energetically cultivation, dewatering and applying new technologies for the conversion of the complete biomass, such as hydrothermal liquefaction, particularly for the generation of renewable aviation fuel.

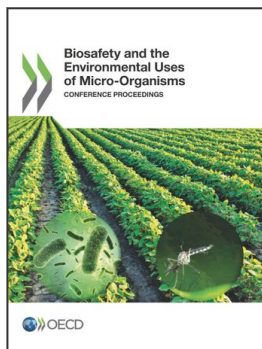
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