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Review

Efficient implementation of micro-algae for sustainable environmental and economic development

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ABSTRACT

Micro-algae constitute powerful unicellular factories with enormous potential for mitigation of miscellaneous pollutants from effluent gases and waste waters. Especially their outstanding capacity for photosynthetic CO_2 fixation underlines their potential for diminishing current ecological problems. Together with these contributions to beneficial environmental development, various micro-algae accumulate high-value marketable products as cell constituents. Low cell densities and moderate growth rates typical for algal cultivation are known as the major obstacles in industrial manufacturing of algal products: Here, high cell densities are required to obtain reasonable volumetric productivities. This raises the challenges for the process engineering design and novel nutrient supply regimes as needed for efficient algal cultivation. For some algal strains, mixotrophic cultivation by providing organic substrates together with CO_2 results in increased biomass concentration in a first cultivation step. For this purpose, numerous organic waste streams can be applied as substrate. In a second step, active algal biomass accumulates desired products via CO_2 fixation, e.g. from industrial effluent gases. This can be realized by two-stage, continuously operated photo-bioreactor systems. After optimized product recovery, the value-added conversion of residual algal biomass for energy recovery e.g. in biogas plants, constitutes another challenge.

KEYWORDS: two-stage continuous production, micro-algae, *Nannochloropsis oculata*, mixotrophic cultivation, removal of pollutants, biogas, eco-toxins

INTRODUCTION

Micro-algae constitute a versatile polyphyletic group of microbes with the common feature of photosynthetic fixation of CO_2 for generation of various algal cell components, energy and molecular oxygen. To underline the significance of these powerful microbes, one should consider that the global fixation of CO_2 by algae amounts to about the same quantity as the photosynthetic performance accomplished by terrestrial green plants.

From the microbiological point of view, micro-algae encompass eukaryotic and, if also including the cyanobacterial representatives (*Cyanophytae*; formerly also known as blue-green algae), prokaryotic microbial species [1, 2]. Figure 1 shows light microscopic pictures of the micro-algal strain *Spirulina* sp., a cyanobacterium, *Chlorella* sp.,

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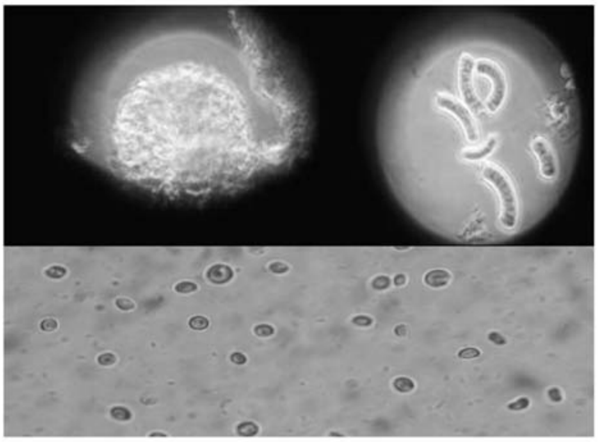

Figure 1. Light microscopic pictures of *Chlorella* sp. (left), *Spirulina* sp. (right) and *Nannochloropsis oculata* (bottom). Magnification: 1/1000.

a representative of the *Chlorophytae* (green algae), and the green alga *Nannochloropsis oculata*.

Biotechnological cultivation of micro-algae

General

The most decisive cultivation factors determining algal growth and product formation rates are quality and quantity of nutrients (encompassing the CO_2 import into the cultivation system), light supply (spectral range and photoperiod are crucial factors and have to be optimized for all micro-algal species) and light intensity. Regarding the light intensity, it is of importance to avoid as well light limitation that results in so-called "dark reactions" of the cells by utilization of molecular oxygen, as photo-inhibition by excessive irradiation with photons that might even cause cell damage. Further, salinity (ion strength and ionic composition of the cultivation medium), pH-value, turbulence and temperature are decisive for cellular growth and product formation. Typical values found in literature report temperature ranges of 16-27°C, pH-values of 4-11, salinities of 12-40 g L^{-1}, and light intensities of 1,000 to 10,000 lux [3, 4, 5].

During the last decades, different strategies have been developed for farming of micro-algae. Comparing the cultivation set-up, one can distinguish between in- and outdoor operated systems. In both cases, closed systems, so called photo-bioreactors, and open systems are available that have to ensure the sufficient supply of growing algal cells with light. This constitutes one of the most decisive factors for the apparatus design of the cultivation system. Light penetration is highly determined and often limited by the depth of the cultivation broth, cell density, the transparency of the photo-bioreactor material such as glass or plastic, and by the turbulence regime in the cultivation system [2, 3, 4].

In most cases, open cultivation systems like racing ponds are cheaper to install, but, as a major drawback, such simple systems do not provide the possibility to exclude microbial contamination; this is absolutely needed in order to operate the bioprocess under monoseptic conditions, excluding frequently occurring contamination by microbial competitors that can endanger whole cultivation batches. This problem might be overcome by selecting such specialized algal production strains that can be cultivated under extreme environmental conditions such as high salinity (described for *Dunaliella*), high substrate concentrations (*Chlorella*) or extreme pH-values (*Spirulina*) [2]. Such extreme conditions provide the micro-algal production strain with advantages during cultivation against competing microbial species.

Embedding of the PHOTOCHEM project into the scientific field of micro-algal research

The project PHOTOCHEM is a currently ongoing cooperation by the Austrian research institutions ARENA, Graz University of Technology, University of Graz, and Joanneum Research. The project encompasses the scientific fields of microbiology, biotechnology, bioprocess engineering, analytical chemistry and chemical engineering for enhanced cultivation of selected micro-algal strains, aiming at high productivities both for the phase of algal growth (formation of catalytically active biomass) and for the phase of predominant formation of intracellular products, provoked by nutritional stress conditions. It is part of the research to focus the cultivation of the micro-algae on mixotrophic feeding strategies, using complex substrates for nutrient supply. This complex nutrients shall derive from various industrial waste streams that in future can be upgraded to substrates for formation of algal biomass able to catalyze the formation of high-value products (polyunsaturated fatty acids [PUFA] like 5,8,11,14,17-eicosapentaenoic acid [EPA] for dietary uses).

The engineering part of the project covers the development of a two-step - continuous process using two photo-bioreactors in series, were active algal biomass is produced under mixotrophic conditions in the first stage, whereas the intracellular product is subsequently produced under autotrophic conditions in the second stage. Further, the separation of algal biomass from the cultivation broth using novel techniques as well as the optimized recovery of the product EPA from the producing cells constitute central tasks within the project activities. Moreover, the project team assesses potential uses of residual algal biomass that remains after product recovery for their feasibility. Especially the fermentative conversion towards the green energy carrier methane in biogas plants is one of the envisaged targets. Hence, it is intended to minimize waste streams and to close as many material cycles as possible. In addition, all experimental results are assessed in respect to the economic feasibility of the process and the market potential of the final products, providing the needed sets of data to create a model of the entire process. This strategy shall finally create the required know-how enabling the design of a semi-industrial micro-algae production facility on pilot scale.

Cultivation strategy - batch *vs.* continuous

Concerning the cultivation mode, batch (discontinuous), fed-batch (semi continuous) and continuous set-ups are described to be applicable in biotechnological process design [6].

On a small laboratory scale, continuous strategies appear to be of special interest. This is due to the possibility of higher automation, a constant production of high-quality, catalytically active cells and it enables high volumetric productivities over extended time periods. Once the equilibrium for the kinetics of growth and product formation, the so called “steady state”, is reached, biomass and products are continuously produced in constant quantity and quality. This “steady state” conditions are characterized by unvarying concentrations of substrates and products, and by constant dilution rates and residence times in the cultivation system. In contrast to dis- and semi-continuous processes, no time is needed for preparation and post-processing of the reactor system that is required prior and after each fermentation batch.

As major drawback, continuous processes in biotechnology are rather complex to install and to run; hence, information on continuous biotechnological processes, especially multi-step continuous processes is still rather scarce in literature [7]. In addition, especially in the case of supplying waste streams as raw materials, the composition of the nutrient supply might to a high extent influence the growth and product formation kinetics as well as the product quality; therefore, a constant feedstock quality has to be ensured for reproducible cultivation set-ups. Furthermore, the instalment of a continuous production plant is more expensive, but - due to the higher volumetric productivities in comparison to discontinuous processes- these higher initial investment costs should be compensated within a rather short time frame. First literature reports already contain sophisticated attempts for continuous algal cultivation integrated in mollusc hatcheries, such as closed, artificially illuminated and external-loop airlift set-ups based on a succession of modules, each of them consisting of two transparent vertical interconnected columns [8].

Two-stage continuous cultivation of *Nannochloropsis oculata*

The viability of multistage-continuous processes for microbial formation of high-value intracellular products was recently demonstrated for poly-hydroxyalkanoate (PHA) production by the prokaryotic microorganism *Cupriavidus necator* by the biotechnological project partners of the PHOTOCHEM project [7]. Here, a five-stage bioreactor cascade was used, producing high densities of PHA-poor, catalytically active biomass in a first vessel under balanced nutritional conditions. This active biomass was transferred continuously into vessels 2 to 5, where carbon source was the only provided substrate. This provokes the enhanced accumulation of PHA by the bacterial cells due to the growth limiting nutrient conditions.

Both biomass- as well as PHA production was significantly enhanced in comparison to similar discontinuous processes in terms of volumetric and specific productivities. This is due to the fact that the characteristics of a multi-stage continuous process correspond exactly to the kinetics of biomass formation on the one hand and PHA accumulation on the other hand. Biomass growth is an autocatalytic process that, according to the chemical engineering theory, most favourably should be carried out in a stirred tank reactor. In contrast, PHA production constitutes a linear process of first order that, according to the theory, should be accomplished in a tubular plug-flow reactor. Vessel 2 - 5 of the multistage bioreactor cascade can be regarded as a process-engineering substitute for a tubular plug flow reactor (PFR). Considering the fact that micro-algal growth and intracellular product accumulation obey to the same kinetic characteristics, it was manifest to use this process mode also for the cultivation of and product formation by micro-algae.

During the research accomplished for the PHOTOCHEM project, it was already possible to demonstrate the long-term stability of a two-stage continuous process for micro-algal cultivation. Stable steady-state conditions were already maintained for several weeks. The investigated production strain, *Nannochloropsis oculata*, was cultivated continuously in a first stirred-tank photobioreactor using a mixotrophic feeding regime (see also next section), providing the cells all nutrients required for growth (proteinacious hydrolysate containing carbon, phosphate and nitrogen; mineral salts; CO_2 for maintenance of the chloroplasts). Cell rich cultivation broth was continuously transferred into a second stirred photobioreactor, were the only provided nutrient was CO_2. Under these autotrophic conditions without availability of nitrogen source and phosphates, the carbon flux stemming from CO_2 was redirected towards predominant lipid accumulation that was the desired metabolic reaction of the strain in order to obtain high concentrations of PUFA like EPA (structure see Figure 2). This product is of significance for dietary purposes as a food additive, and, due to its considerable market value, was selected as the main product for the PHOTOCHEM project. The continuous two-stage operation mode provided higher volumetric productivities both for the growth of catalytically active biomass and for the subsequent accumulation of EPA-rich lipids.

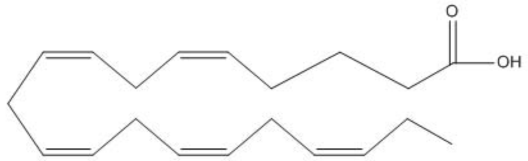

Figure 2. Chemical structure of 5,8,11,14,17-eicosapentaenoic acid (EPA).

Figure 3 shows the experimental set-up for the two-stage continuous cultivation of *Nannochloropsis oculata*.

Cultivation conditions in dependence on the desired end products

For a commercially viable production of algal biomass and its products, high cell densities are absolutely required in order to obtain reasonable volumetric productivities and to make the downstream processing economically feasible. For some algal strains, the heterotrophic cultivation, i.e. the provision of organic materials as carbon source instead of the photosynthetic fixation of CO_2 (autotrophic cultivation) features a viable strategy to obtain high concentrations of catalytically active biomass. Heterotrophic feeding regimes and the combination with additional supply of CO_2 (mixotrophic cultivation) are described in literature for the genera *Chlorella, Crypthecodinium, Galdieria* and even for cyanobacteria (*Arthrospira*) [6, 9, 10]. In this case, numerous waste materials can be applied as substrate.

The algal biomass itself is of interest as health food, as protein source for fish farming and feeding of cattle, pigs and poultry, for cosmetic purposes (especially anti-aging skin preparates; extracts from *Chlorella vulgaris* support collagen repair mechanisms), pharmaceutical purposes (immunity response, weight control) [10] and for production of biogas [1] (see below). It must be emphasized that under such heterotrophic cultivation conditions the production of photosynthesis-based products, especially pigments, tremendously slows down or is totally hampered. In principal, three major groups of pigments are found in micro-algae, being responsible for the light harvesting, CO_2 fixation, and colour of the algae, as summarized in Table 1:

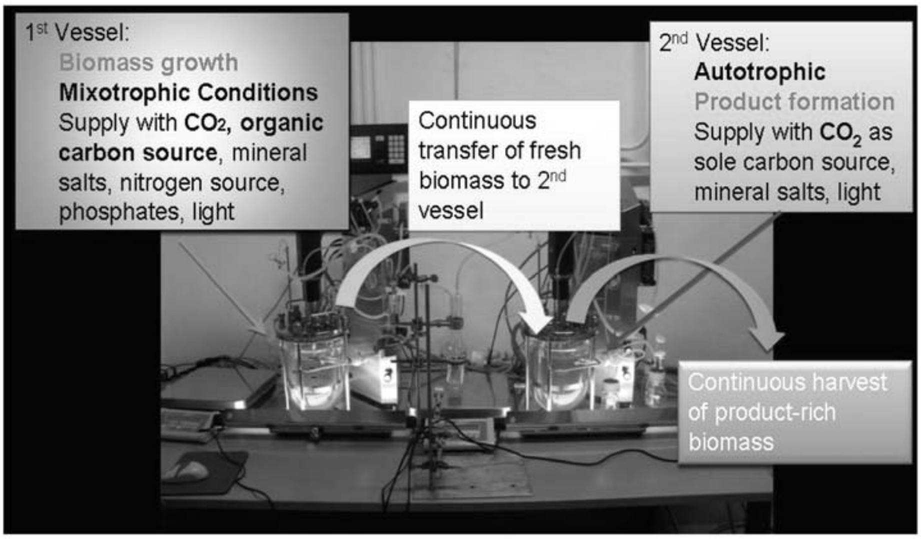

Figure 3. Experimental set-up for the two-stage continuous cultivation of *Nannochloropsis oculata*.

Table 1. Summary of micro-algal pigment groups.

Pigment group	Predominant colour of the algal culture	Micro-algal representatives	Examples of the pigments
Carotinoids	Brown	*Phaeophyta, Chrysophyta*	ß-Carotin, Bixin; Xanthophylls like Violaxanthin, Astaxanthin, Lutein, Zeaxanthin Fucoxanthin
Phycobillins	Red	*Rhodophyta*	Phycocyanin, Phycoerytrin
Chlorophylls	Green	*Chlorophyta*	Chlorophyll a (present in all photosynthetic eurcaryotic life forms)

Those pigments are considered as the algal products of highest potential for commercial success [11, 12, 13, 14]. They can be applied as nutrient supply (pro-vitamin A, vitamin E) [13], other pharmaceutical and medical purposes (anti-inflammatory effects, antioxidative effect, cancer prevention) [9], in cosmetic industry and also for food industry. In food industry, algal pigments posses high importance as food colorants [9]; an important example is the production of astaxanthin that is used to give salmon the typical reddish color by *Haematococcus pluvialis*; the global market potential of astaxanthin was estimated with 200 million US-$ [15, 16, 17]. Additionally, ß-carotene is needed for the yellow coloration of egg yolk. For many dairy products such as cheese, butter or margarine, the food additive bixin (E160b) provides a yellowish to peach-color shade. Violaxanthin features an orange colour and can technically be used as a food colorant (E161e). Figure 4 presents the chemical structures of some of the most important algal pigments.

Hence, the combination of heterotrophic and autotrophic cultivation appears to be a powerful strategy to produce high concentrations of active biomass using cheap substrates (e.g. starch hydrolysate) as first step and as second step, to switch to the production of high value compounds such as pigments or valuable lipids like EPA as investigated in the PHOTOCHEM project by the photosynthetic fixation of CO_2. In this second phase, the removal of CO_2 from effluent gases by algal fixation provides the combination of mitigating surplus CO_2 with value creation by the formation of marketable products.

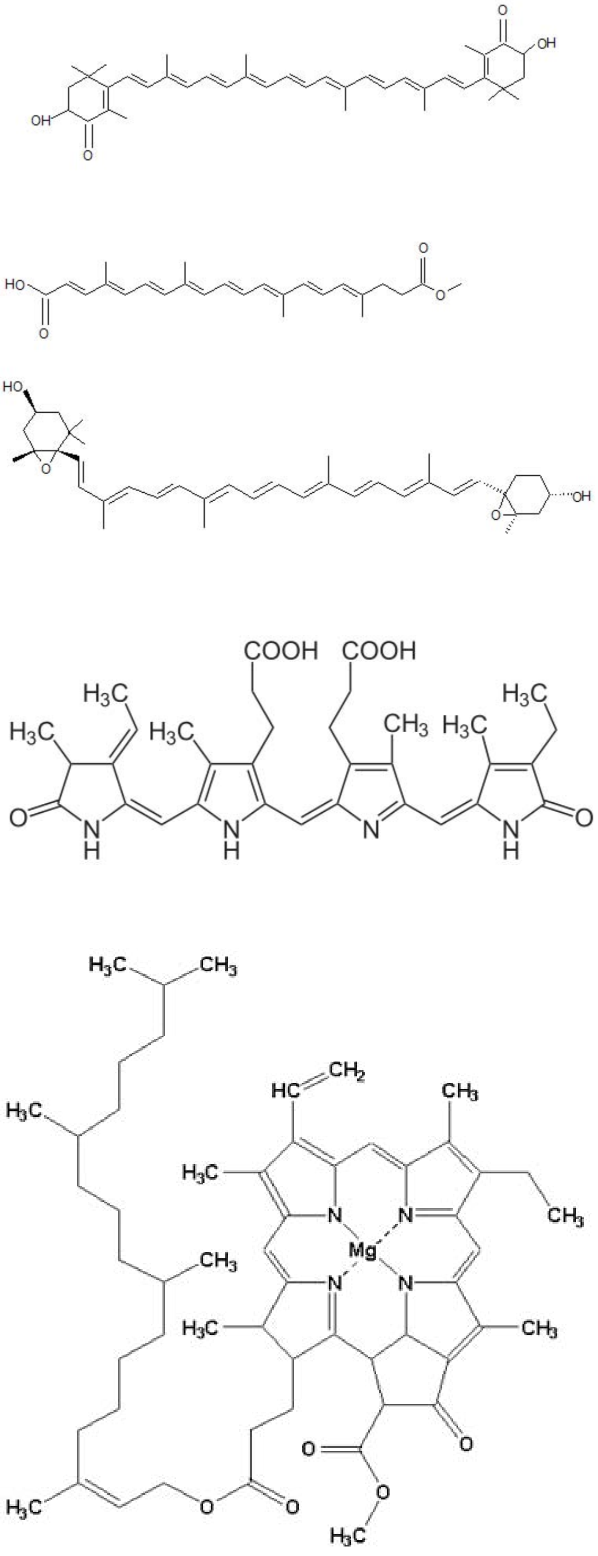

Figure 4. Important micro-algal pigments: (from top to bottom): *Astaxanthin*, *Bixin*, *Violaxanthin*, *Phycocyanin*, *Chlorophyll a*

It must be emphasized that many algae are able to adapt to a broad range of salinity conditions. In this case, the extents of production of biomass, lipids, carotenoids and carbohydrates can vary to a high extend depending on the salinity of the cultivation medium [12]. Therefore, the applied salinity conditions have a major impact on the productivity of a desired end-product. For the selection of the appropriate production parameters, the decision is made from case to case to which final product the nutrient flux should be directed.

Combining CO_2-mitigation from effluent gases by micro-algae to the production of eco-benign “green” energy carriers

Biofuels stemming from photosynthetic generation of algal lipids

Caused by prevailing ecological concerns affecting the entire planet, biological sequestering of CO_2 by living algal cells for abatement of green house gases has become a research field of increasing global significance [1, 18]. Using autotrophic micro-algae for fixation of CO_2 deriving from the exhaust gases of industrial power plants, some issues have to be considered. It is of major importance to adapt the CO_2 supply to the demands of the applied production strain. Values for ideal CO_2 concentration ranges vary considerably between different algal species. Regarding the solubility of CO_2 in the cultivation medium it is clear that this is influenced to a very high extend by the pH-value that is decisive for the CO_2/ HCO_3^- balance. If effluent gases are introduced into algal cultures without prior processing as desired for cost effectiveness of the entire process, one has to consider that the high temperature of such exhaust gases could elevate the temperature of the cultivation system to a too high extend. This would require special technical pre-requisites for sufficient cooling capacities. In this case, thermophilic specialists among the broad variety of algal strains might also be a solution to overcome this aspect.

The combination of fixating of CO_2 stemming from combustion of fossil resources by micro-algae together with the conversion of this effluent gas component towards biofuels constitutes the most efficient strategy in abating green house gases. In principle, there is no fundamental difference in the capture of CO_2 from air or from industrial effluent gases. The real progress in lowering the amounts of green house gases is the substitution of fossil fuels by biofuels produced by the micro-algae from CO_2; hence, the amount of new fossil based fuel is diminished by the quantity that is substituted by the algal biofuel [18].

Different strains are potential candidates for micro-algal biofuel production. Especially among the genera *Botryococcus*, *Chlorella*, *Nannochloropsis*, *Neochloris*, *Nitzschia*, *Scenedesmus*, *Dunaliella* and *Schizochtyrium*, several species are described to show exceptionally high amounts of lipids in their cell mass under optimal cultivation conditions [17, 18]. In the case of *Botryococcus braunii*, 75% (w/w) of hydrocarbons in cell mass were reached. The type of hydrocarbons produced by *Botryococcus braunii* depend on the race of this species (race A, B, L). A large number of monounsaturated and polyunsaturated and even branched hydrocarbons is produced by *Botryococcus braunii*; these compounds can be converted by cracking them to fuels with properties similar to those of gasoline [12]. Other species produce high amounts of typical “vegetable oils” with high contents of mono-unsaturated, di-unsaturated and threefold unsaturated fatty acids. These oils can be classically converted to biodiesel by the well-known alkaline transesterification with alcohols like methanol [19, 20, 21]. As a by-product of this transesterification process, the glycerol phase can be digested anaerobically in biogas plants, or can be thermally converted, or can be applied as an efficient carbon source for numerous biotechnological applications [20]; in addition, glycerol can be commercialized for manufacturing of e.g. cosmetic products or be applied in food industry.

It has to be emphasized that many algal lipids contain special fatty acids with high market values, such as EPA. These fatty acids can be commercialized yielding much higher prices than utilizing these lipids as biofuels for combustion. Therefore, the project PHOTOCHEM does not aim at the production of algal biofuels, but intends to enhance the production of lipids containing special fatty acids such as EPA for e.g. for application as food additive.

Beside the CO_2 supply, additional nutrients like nitrogen (as ammonia, nitrate, and organic nitrogen), phosphates, sulphur as well as different minor elements have to be provided to the algae. Regarding nitrogen and sulphur, effluent gases typically contain considerable amounts of NO_x and SO_x depending on the type of the combusted material. After being dissolved and neutralized in the aqueous cultivation medium, these gases can be converted by the algae as substrates [18].

Biogas from algal biomass

After cell harvest and isolation of lipids and high-value pigments from the micro-algal cells by means of extraction or mechanical disruption methods, residual biomass is generated that can be converted in different directions. Attempts have been made to generate bio ethanol from algal biomass. This can be accomplished by the fermentation of starch-rich algal biomass by the action of yeasts [Reference!]. Starch and starch-like polysaccharides constitute algal reserve materials typically produced by several species among the genera of *Chlorophytae*, *Rhodophytae*, *Cryptophytae*, and *Pyrrophytae*. Due to the low yields that characterize the anaerobic ethanol production by yeasts, the large-scale application of this strategy appears rather doubtful. In addition, a biotechnological production strategy using two types of micro-organisms in two separated processes (starch accumulation by micro-algae followed by the anaerobic conversion of starch to ethanol) is rather complex to install and demands a rather big number of intermediary process steps.

By degradation in biogas plants, the residual biomass can be used for the generation of biogas, a more or less carbon neutral energy carrier. The generated biogas typically contains comparable amounts of the energy carrier methane and CO_2. If compared to the production of hydrocarbons or biodiesel by algae, biogas generation from algal biomass is technically simple to realize. The so called “digestate” remains as residue from the biogas production. This material is rich in nutrients such as potassium or phosphates and constitutes a precious green fertilizer in agriculture. In addition, it appears reasonable to apply the digestate as nutrient supply to later algal cultivations. This recycling strategy should allow additional production of algal biomass and act as a supplement to the nutrient supply obtained from the waste water input.

Alternatively, residual algal biomass can be thermally converted to generate energy and ash; this constitutes the simplest method for energy recovery from algal biomass. The remaining ash can further act as a valuable agricultural fertilizer or as mineral nutrient supply for subsequent algal cultivations.

In a recent study, the efficiency of abating CO_2 by using biogas stemming from algal biomass is compared to the utilization of natural gas. It was calculated that the production of 1 ton of algal biomass results in avoiding 0, 5 tons of CO_2. It can be estimated that this value can be doubled if natural gas was replaced by coal fired energy generation, saving energy of conventional waste water treatment and replacing the energy demanding production of fertilizers by digestate [18]. Within the PHOTOCHEM project, the optimization of conversion of algal biomass after product isolation is a central point of research.

Bio-hydrogen production by micro-algae

Bio-hydrogen production provides a novel field of algal research. Bio-hydrogen that can be applied in fuel cells is generally considered a future-oriented green energy carrier that is of interest for many industrial branches. Also in the case of bio-hydrogen production, photosynthesis is the fundamental driving force that supports the synthetic processes [22, 23, 24]. Biotechnological production of molecular hydrogen (H_2) is advantageous compared to electrolysis of water due to the high energy demand of this well-known technique. Under anaerobic conditions, hydrogen is produced as a by-product during conversion of a variety of organic wastes into organic acids which are then used for methane generation. The acidogenic phase of anaerobic digestion of wastes can be optimized to improve hydrogen production. Photosynthetic processes include algae which use CO_2 and H_2O for hydrogen gas production. Bio-hydrogen production by microalgae requires several minutes to some hours of anaerobic incubation in the dark to induce the synthesis and/or activation of enzymes involved in the hydrogen metabolism, especially a reversible hydrogenase enzyme. The hydrogenase enzyme responsible for production of H_2 is highly sensitive to O_2, photosynthetic production of H_2 and O_2 must be temporally and/or spatially separated. In a two-phase process, CO_2 is first fixed into hydrogen-rich substrates such as carbohydrates during the well-investigated steps of photosynthesis (Phase 1), followed by light-mediated generation of molecular H_2 when the microalgae are incubated under anaerobic conditions (Phase 2).

It has to be emphasized that bio-hydrogen formation by micro-algae provides a field with a huge variety of needed improvements; still the productivities for bio-hydrogen are rather low. Apart from required process engineering improvements, genetic modification of micro-algae actually is regarded as the most promising strategy to efficiently generate the “green energy source” bio-hydrogen by the action of photosynthetic microbes, also in this case starting from various carbon-rich waste streams [25].

Removal of various toxins from water by microalgae

Removal of toxic heavy metals

Several heavy metals cause severe concern because of the manifold possibilities of being exposed to them; this exposure may be environmental, occupational or residential. Small amounts of these elements are common in our environment and diet and are actually necessary for the human metabolism, but large amounts of them may cause acute or chronic toxicity, resulting in health problems. In the case of environmental pollution by heavy metals, micro-algae were identified as potential candidates to remove them from various environments, especially from industrial waste water [26].

Already in 1988, the green alga *Chlorella fusca* was described to produce a cadmium-binding complex, composed of phytochelating peptides. In addition, it was demonstrated that among the ten classes of Phycophyta, six revealed phytochelatin synthesis after exposure to Cd(II) ions. Phytochelatin was also induced by other heavy metal ions like lead, zinc, silver, copper and mercury. These experiments demonstrated that algae sequester heavy metals by an identical mechanism as higher plants via phytochelatins which form stable complexes with the metal cations, thus removing these pollutants from the aqueous environment [27].

The potential of micro-algae for bio-mediated removal of toxic Cr(VI) ions from aqueous solutions was demonstrated using *Scenedesmus incrassatulus*. Strategies to avoid Cr(VI) exposure is of special significance due to the fact that this ion is suspected to cause cancer. Among all other investigated algal species, this organism turned out to be exceptionally robust against Cr(VI) ions. The experimental set-up consisted of a split-cylinder internal-loop airlift photobioreactor that was operated continuously. 1 mg per litre of the toxin heavily affected the pigment formation of the algae, but did not negatively influence biomass growth [28].

Gloeothece magna, a non-toxic cyanobacterium (*Cyanophyta*; formerly blue-green alga) classically found in freshwater, is reported to adsorb Cd(II) and Mn(II) ions from polluted water samples. The authors of the study explicitly suggest that *G. magna* could be cultivated in water bodies contaminated by these heavy metals to decrease their toxicity. Also dry material of this cyanobacterium obtained after cell harvest and further processing, e.g. *via* lyophilization or thermal drying, could be used as an efficient bio-filter system for heavy metal removal from drinking water [29].

The removal of Ni(II), Fe(II/III), Hg(II), Cd(II), Cr(IV), Zn(II) or Au(II) ions from waste water by different immobilized *Chlorella* and *Scenedesmus* strains was also investigated and reported in literature. Here, some results indicate that immobilization of the algal cells makes them more tolerant to heavy metal ions in comparison to free cells. Hence, this strategy might imply great potential for future waste water treatment plants. In addition, this might also be a viable technological strategy for a novel bioleaching process for enrichment of e.g. gold and other valuable metals [30]. This approach appears of special significance for such metals which occur only at very low concentrations in the respective aqueous matrices, making their isolation *via* classical techniques economical not feasible.

Removal of formaldehyde

Biodegradation of formaldehyde (methanal), a compound severely toxic for skin, eyes and the respiratory system, was demonstrated by the marine micro-alga *Nannochloropsis oculata* ST-3, a representative of the *Chlorophyta*. Formaldehyde is often released to marine environment via waste water from different industrial branches like paper, resin, and glue producing companies. During the reported experiments accomplished with *N. oculata* ST-3, formaldehyde concentration in the medium decreased in parallel with the increase of the micro-algal biomass due to the potential of this micro-alga to oxidize formaldehyde to formiate. Within three weeks of cultivation, a nearby complete degradation of the toxic aldehyde in the test medium was observed. After step-wise adaptation to formaldehyde-containing cultivation media, the strain was even able to tolerate up to 20 ppm of the toxin [31].

Additional nutrient from waste water for algal cultivation

If waste water is used for nutrient supply, naturally occurring bacteria accomplish the breakdown of the organic waste materials to such nitrogen and phosphate sources that can subsequently easily be converted by micro-algae. Hence, symbiotic interactions exist between the metabolism of the bacteria and the algae. Many different waste waters from agriculture, municipal origin or different industrial branches provide the suitable ingredients for algal nutrient supply. Additionally, the carbon present in waste water can also be converted by the algae during the heterotrophic phases of cultivation. Regarding waste water treatment plants, the disposal of activated sludge into landfills or by incineration also contributes to the formation of green house gases. Nevertheless, the major effect of classical waste water treatment on the formation of green house gases is due to often highly energy demanding treatment strategies, e.g. nitrogen removal via the so called “tertiary treatment” [18].

In order to get deeper understanding for the on-goings during the removal of nitrogen, phosphorus, and metal ions from waste water by the action of micro-algae, a comprehensive study was carried out using a wide range of immobilized

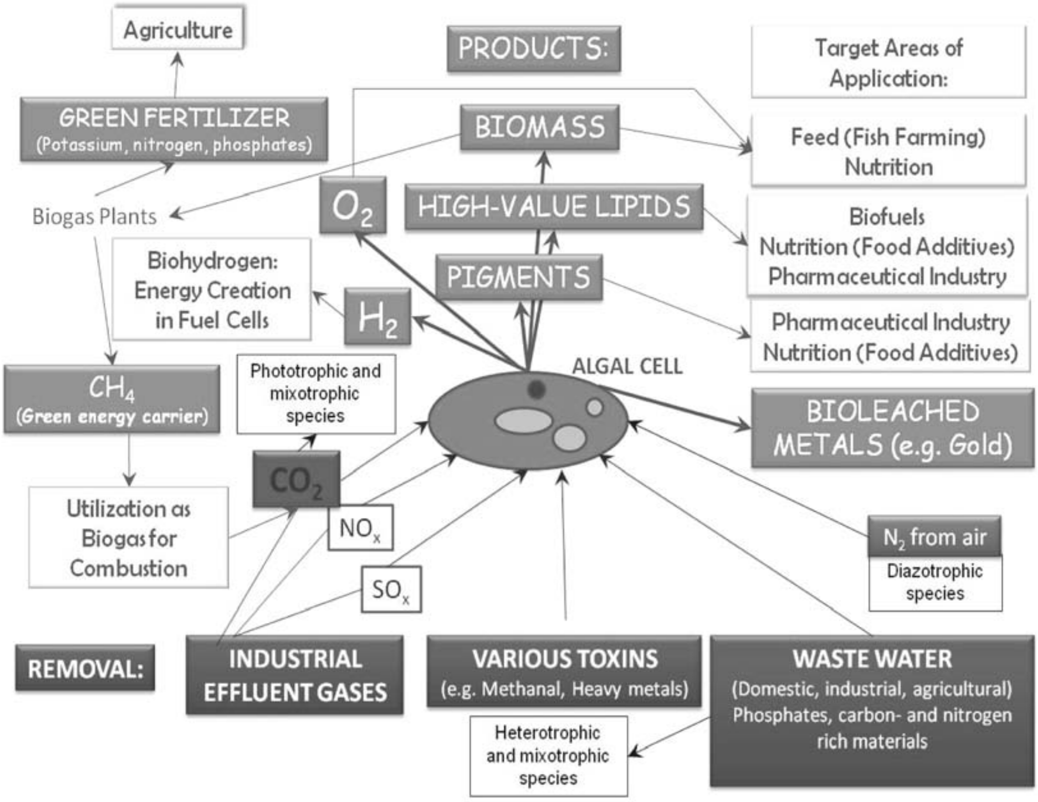

Figure 5. Combining the removal of pollutants with the production of high-value products by algal strains (based on [2]).

algal strains by Mallick [30]. The algal species …were used for the experiments. The study concludes that the application of micro-algae has high potential for efficient waste water treatment.

Synopsis of micro-algal product formation from various waste streams

Figure 5 provides a graphic schematic encompassing the potential micro-algal products like lipids, pigments, biomass, molecular oxygen or bio-hydrogen that can be obtained by converting the huge variety of discussed pollution streams of different origin like waste water or industrial effluent gases. In addition, the areas of final application of the algal products, such as agriculture, energy generation, or pharmaceutical and nutritional purposes are indicated.

CONCLUSION AND OUTLOOK

Up to date, the authors of the work at hand are not aware of any literature on the heterotrophic or mixotrophic large-scale fed-batch or even continuous cultivation of micro-algae. This shortcoming in micro-algal research is in contrast to exhaustive reports on low-productive batch cultivations.

Combining the application of available “liquid waste streams” like waste waters or solutions of hydrolysis products of organic waste with the utilization of CO_2 stemming from industrial effluent gases, suitable raw materials are available for both phases of the formation of high value products by selected micro-algal species. Both micro-algal growth and product formation have to be improved regarding the productivities. During the heterotrophic or mixotrophic phase, characterized by the formation of high densities of catalytically active algal biomass, carbon, - nitrogen- and phosphate-rich waste streams of various origins can be supplied. The second phase of the cultivation, characterized by the generation of vendible products like pigments or special lipids provides a possibility of CO_2 mitigation for numerous involved industrial branches. This abating of CO_2 might contribute world wide to achieve the agreed global goals for climate protection as they are defined in the frequently discussed Kyoto Protocol to the United Nations Framework Convention on Climate Change (2005) or the well-known Rio Declaration of The United Nations Conference on Environmental Protection (1992). The two-stage - continuous cultivation mode provides a novel, powerful process engineering tool for high-efficient production of intracellular algal products. Closing all the material cycles in algae production, the application of those micro-organisms constitutes a powerful and sustainable strategy towards a real "White Biotechnology". In addition, the value-added conversion of residual algal biomass after product isolation for production of biogas gives novel impulses for the area of "green energy".

The two-stage continuous cultivation set-up could also be applied to novel micro-algal strains improved by genetic engineering approaches. Fundamental knowledge enabling tailor-made strain design may be derived from advanced metabolic flux analyses. Considerable progress in this direction is very likely within a rather short time frame due to the successful research accomplished during the past 10 to 20 years, which resulted in the complete sequencing of the first micro-algal genomes [9].

In addition, the continuous formation of biomass under balanced nutritional conditions in a first stage followed by increased, continuous product formation provoked by nutritional stress in a second stage could also be applied to other microbial "cell factories". Here, the accumulation of oils by oleaginous yeasts by nitrogen limitation in the second stage appears reasonable to be investigated. These oils obtained from yeasts might be used as a novel raw material for biodiesel production.

In future, genetic engineering and technological optimization of production facilities might open the route for the efficient micro-algal production of bio-hydrogen as an additional sustainable energy carrier. Further, the removal of various pollutants in typically aqueous environments, such as eco-toxins like heavy metals is a seminal field for application of micro-algae in the coming years.

The comprehensive implementation of already highly advanced techniques of photovoltaic for generation of electrical power needed for running the cultivation system und the downstream processing can provide a sustainable strategy for an autarkic energy supply of the entire algal-based production plant. Following this strategy, one takes direct profit from solar energy firstly for the photosynthetic fixation of CO_2 by the algal cells, and, secondly, for energy generation to run the production facilities.

Uniting the possible enhancements of each process step, one can definitely make substantial progress towards a cost-efficient algal-based technology. In any case, the development of really efficient processes for manufacturing of algal products starting from diverse waste streams needs the narrow cooperation of experts from different scientific fields. Chemical engineers, microbiologists, genetic engineers and experts in the fields of Life Cycle Assessment and Cleaner Production Studies have to concentrate their special expertises and know-how in order to close the existing gaps between promising data from the laboratory scale to industrial realization.

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