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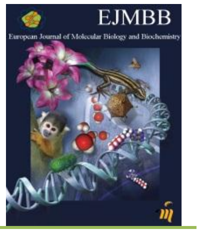

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MICROALGAE POTENTIALS IN BIOTECHNOLOGY

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ABSTRACT

Many useful biomolecules are being discovered, mapped and eventually utilised commercially as a consequence of the fast development of biotechnology and bioinformatics. This fact has brought about a great interest for seeking new products that can improve our health and wellbeing. Biotechnology is one of the key technologies of the present century that have changed and influenced industries, global problems, and bring great expectations for the future. Microalgae biotechnology is able to enhance the quality of life of humans and animal due to its chemical constituents, namely high protein content, with balanced amino acids pattern, carotenoids, fatty acids, vitamins, polysaccharides, sterols, phycobilins and other biologically active compounds, more efficiently than traditional crops. Polyunsaturated fatty acids, carotenoids and phycocolloids are examples for products used in the food industry, while other compounds serve as templates for the development of new pharmaceutical drugs. Generally, micro-algal biotechnology are being utilized in food, feed, fuel, fertilizer, and colourant, production of various secondary metabolites including vitamins, toxins, enzymes, pharmaceuticals, pharmacological probes and pollution abatement. The present work shows the results of a bibliographic revision done on the chemical composition of different microalgae together with a critical discussion about their potential in biotechnology.

INTRODUCTION

Biotechnology is based on the search for and discovery of exploitable biology. It starts with the assembly of appropriate biological material for screening after a desired attribute. It then moves through the selection of the best option among a few positive hits for further testing and purification, before culminating in the final development of a commercial product or process. It has become an important tool in modern human medicine, nutrition and increasingly in the industry as industrial processing (e.g. enzymes). It is a radical innovation that generates new industries, and its versatility is so great that existing industries that have previously not used biological systems are now exploring such options.

Microalgae are an enormous biological resource, representing one of the most promising sources for new products and applications [1]. They can be used to enhance the nutritional value of food and animal feed, due to their well balanced chemical constituents. Algae contain a broad spectrum of prophylactic and therapeutic factors that include vitamins, minerals, amino acids, essential fatty acids, the super anti-oxidants such as beta-carotene, vitamins E and C, and selenium, and a number of unexplored bioactive compounds. Antibiotics have been obtained from a wide range of algae and show great chemical diversity (fatty acids, bromophenols, tannins, terpenoids, polysaccharides, alcohols). The same holds for

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the neurotoxic and hepatotoxic compounds produced by algae. Since these many constituents stimulate numerous metabolic pathways, algae has been seen to promote antioxidant, anti-bacterial, antiviral, anticancer, anti-inflammatory, anti-allergic, and anti-diabetic actions, as well as vascular, mental, and intestinal health. Algae can be considered analogous to a modern-day vitamin supplement. Algae offer a more robust, natural, bioavailable, and inclusive blend of healthful compounds than supplements. Moreover, they are cultivated as a source of highly valuable molecules such as polyunsaturated fatty acids, pigments, antioxidants, pharmaceuticals and other biologically active compounds. The application of microalgal biomass and/or metabolites is an interesting and innovative approach for the development of healthier food products [2]. There are also some limitations in algae biotechnology as shown below.

Microalgae are photosynthetic organisms, which possess reproductive simple structures. These organisms constitute a total of twenty-five to thirty thousand species, with a great diversity of forms and sizes, and that can exist from unicellular microscopic organisms (microalgae) to multicellular of great size (macroalgae). In fact, some algae live in complex habitats submitted to extreme conditions (for example, changes of salinity, temperature, nutrients, UV-Vis irradiation, etc.) [3]. Microalgae adapt rapidly to new environmental conditions to survive, producing a great variety of secondary (biologically active) metabolites, which cannot be found in other organisms [4]. Due to their great taxonomic diversity, investigations related to the search of new biologically active compounds from algae can be seen as an almost unlimited field. Besides their natural diverse character, they are easily cultivated, have rapid growth (for many of the species) and as well there is the possibility of controlling the production of some bioactive compounds by manipulating the cultivation conditions (molecular farming). Microalgae content of proteins, carbohydrates, lipids, fibre, metabolites, etc. can be influenced by the growing parameters (water temperature, salinity, light and nutrients) [5], concluding that algae can be considered as a magnificent bioreactor able to provide different types of compounds at different quantities; in some cases, a good alternative to chemical synthesis for certain compounds [6].

The objectives of this paper are first to present the results obtained of a detailed bibliographical search about the composition of different microalgae and, secondly, to discuss their possibilities in biotechnological utilisation. The information provided on the different algae does not refer in many cases to the same constituents since it has been taken from different research papers with different objectives. Nevertheless, we believe the information provided can be useful to many research groups considering that biotechnology search strategies has hence opened new opportunities and new technology together with the realization of the recourse biodiversity represent, makes bioprospecting a very great present interest.

Sources of microalgae biomass

- Harvest from nature of active biological material.
- Cultivation of the bioactive organism.

Some Cultivated Species of microalgae

Some commercially cultivated microalgae include

- *Nannochloropsis*is attractive because of its high growth rates and the fact that the lipids in the algae are relatively high in omega-3 fatty acids. It grows in seawater, at average light levels. However it requires relatively low temperatures and low pH.
- *Tetraselmis*is high in carbohydrates and can be grown in variable environments.
- *Nannochloris*, an algae strain that grows in high temperatures and is high in carbohydrates, is capable of good growth in a range of light levels and at low pH.
- *Dunaliella*is attractive because of its relatively high levels of beta-carotene for the human market at attractive prices. It can also withstand high salinity, high light, and virtually all temperatures.
- *Diatoms* are also rich in omega-3 fatty acids and can be grown in a range of salinities at medium-low temperature and low light intensity.
- *Chlorococcum*, which has interesting properties in the area of pigments, can be grown in average temperatures and high light intensity.

Problems with Field Collected Material

- Very few bioactive microalgae biomass can be collected in large amounts [7].
- Collecting from the environment requires considerable time and effort [8].
- Harvesting of large quantities requires the consent of the country where the collection is made and must be care fully evaluated not to adversely impache collection site.
- Lack of reproducibility: Secondary metabolite production in field materials unpredictable:-many cyanobacteria prove to be non active on recollection;-bioactivity may vary within a few meters at the collection site [9].

Research and development of biomass from microalgae cannot rely on field collected material.

Advantages of Culturing

- Between 1and10 % of microalgae are cultivable by current techniques [10].
- Some active strains may be very rare in the field and thus their bioactivity is overlooked. We can find them through enrichment, isolation and cultivation [11].
- Synthesis of biomass is dependent on culture conditions (temperature, pH, light, nutrients).We can stabilize production of the active molecule by controlling culture conditions. Genetic manipulation is also possible [12,13].

Future Actions

- Screening programs must be extended to new areas
- Understand the physiology of biomass synthesis in mass cultures to stabilize and optimize production.
- Determine the chemical structure of as many biomass as possible and their mode of action (biochemical target).
- Develop efficient reactors for mass cultivation of bioactive strains and efficient harvesting and separation techniques.
- *Bioprocess intensification*: : optimisation of fermentation yields via media composition and feed strategies, control of physical conditions, induction, genetics, immobilisation and bioreactor engineering.

CAPTURING CO_2 WITH MICROALGAE

Global warming is a serious threat to both humans and nature. The increase in Earth's surface temperature bring about extreme weather occurrences, rise in sea levels, extinction of species, retreat of glaciers and many other calamities. The rise in global temperature is attributed to the high amount of carbon dioxide (CO_2) gases in the atmosphere. CO_2 is emitted from the burning of fossil fuels for electricity, transport and industrial processes. Due to the serious threat of global warming, the Kyoto Protocol in 1997 proposed a reduction of greenhouse gases by 5.2% based on the emissions in 1990. Since then, many CO_2 mitigation options have been considered to meet the proposed target. The various strategies suggested can be classified into two main categories: chemical reaction-based approaches and biological mitigation. Chemical reaction-based strategy captures CO_2 by reaction with other chemical compounds before the CO_2 is released to the atmosphere. The cost of the chemical reactions as well as the disposal of both CO_2 and the wasted chemical compounds discourages the use of this technique. On the other hand, biological mitigation is deemed to be more favourable as it not only captures CO_2 but also generates energy through photosynthesis [14]. This method will be much simpler than physical CO_2 sequestration (Pienkos and Darzins, 2009). Even though the use of plants to capture CO_2 is viable, it is by no means efficient owing to its slow growth rate. On the other hand, microalgae as photosynthetic microorganisms are able to capture solar energy and CO_2 with an efficiency of 10 to 50 times greater than that of higher plants. In addition, microalgae have rapid growth rates and higher productivities than any other plant systems. Microalgae can also grow in variable environmental conditions [15]. Apart from CO_2 and sunlight, microalgae also need nutrients, trace metals and water to grow. In short, biomass from microalgae cultivation is produced based on the following reaction:

$CO_2 + H_2O + \text{nutrients} + \text{light energy} \longrightarrow \text{biomass} + O_2$ (1)

Microalgae can capture CO_2 from the atmosphere, from industrial gases (i.e., power plant flue gases) and in the form of soluble carbonates (i.e., Na_2CO_3 and $NaHCO_3$). Generally, CO_2 concentration from power plants is higher than the atmosphere. The low CO_2 concentration in the atmosphere can slow down the growth of microalgae due to mass transfer limitation. Efficiency of CO_2 capture by microalgae increases with power plant flue gases that have up to 15 % of CO_2 and studies have shown that microalgal species (such as *Scenedesmus spp*. and *Chlorella spp.*) can tolerate 10 % to 30 % CO_2 in its gas supply. Therefore microalgae cultivation can be synergistically located adjacent to a power plant where the CO_2 emitted from such a plant can be conveniently sequestered by the algae as a source of their nutrients [16].

BIOENERGY TECHNOLOGY

Global warming and dwindling fossil fuel supplies have led to a need to develop clean and sustainable energy supplies. Micro-algae may prove to be a source of fuels for the future owing to their ability to capture solar energy and store it as chemical energy in a range of substrates for the production of biodiesel, biomethane, and biohydrogen.

Algal biomass can be converted into biofuel by a variety of different methods. Thermochemical methods such as gasification, pyrolysis or liquefaction or even direct combustion convert the stored energy into gases such as hydrogen or methane, oils, charcoal, electricity, heat and mechanical power [17]. Biological methods include fermentation of the biomass to produce energy carriers like bioethanol, biomethane, biohydrogen, or extraction of lipids and hydrocarbons to produce biodiesel [18,19]. Advantages of producing biofuels from algae compared to biofuels from energy crops include production possibilities on non-arable land, low water consumption, no competition with food production, and high biomass per area ratio [20]. However, using the algal biomass produced in this multidisciplinary approach for biofuel production will most likely bring less income to the process than many of the other products mentioned above. Micro algal biotechnology appears to possess high potential for biodiesel production because a significant increase in lipid content of microalgae is now possible through genetic engineering approaches [21]. Microalgae are photosynthetic microorganism which converts sunlight, water and CO_2 to sugars, from which macromolecules such as lipids and triacylglycerides (TAGs) can be obtained [22]. The TAGs are the promising and sustainable feedstock for biodiesel production. Many micro algae have the ability to produce substantial amounts (20-50%) of triacylglycerols (TAGs) as a storage lipid under photooxidative stress or other adverse environmental conditions.

Because of their high productivity and the accumulation of oils in their biomass, algae can be utilized as an alternative, renewable energy source that can reduce fossil fuel depletion, increasing air pollution, and global warming [23]. One way is to use the biomass of algae to produce methane. Another most promising way is to use algae to produce hydrogen. Hydrogen yields energy when it is either combusted or used with fuel cell technologies, leaves nothing behind but water [24]. Many photosynthetic prokaryotic and eukaryotic microorganisms evolved the ability to reduce protons to hydrogen during light absorption by the photosynthetic apparatus [25]. Table below highlights some limitations associated with algal bio-energy technology and conventional feedstock.

Micro algae can be degraded into carbondioxide, methane and water by micro biological process. Liquefaction of Spirulina in various organic solvents or water under a hydrogen, a nitrogen or a carbonmonoxide atmosphere in the temperature range of 300-425^{0}C using Fe(CO)s-S catalyst developed for the liquefaction of coal

BIOFERTILIZER

Microalgae can be utilized to produce fertilizers and soil conditioners, especially for the horticultural industry. Algal biomass used as a fertilizer has water binding properties and may improve the soil's mineral composition [26]. This may be a potential use of the algal biomass either directly, or after extraction of metabolites. The cyano bacterial symbiont *Anabaena-azollae* fixes atmospheric nitrogen estimated between 120 and 312 kg N_2 per hectare. *Azolla* supplies 150–300 tons per hectare per year of green manure, which supports growth of soil microorganisms including heterotrophic N_2 fixers. The use of algae and cyanobacteria in waste treatment is beneficial in different ways since they can bring about oxygenation and mineralization, in addition to serving as food source for aquatic species.

MOLECULAR FARMING

The idea of molecular farming (also called molecular pharming, biopharming or gene pharming) in (micro) algae is to generate biomolecules valuable to medicine or industry that are difficult or even impossible to produce in another way, or which require prohibitively high production costs in other systems. Successful expression and assembly of a recombinant human monoclonal IgA antibody has already been demonstrated for *Chlamydomonas reinhardtii* [28], while stable expression of the hepatitis B surface antigen gene has been shown in *Dunaliella salina* [29-31]. In this way, antibody and vaccine production can become not only much more convenient, but also much cheaper than expression in other systems. Expression in an organism without an immune system allows expression of antibodies that would otherwise interfere with the immune system of the host animal used in conventional antibody production. Since *Dunaliella*is otherwise used for nutrition, there is no need for purification of the antigen, so the intact algae could be used to deliver a vaccine. Microalgae have also been shown to be useful for expressing insecticidal proteins. Because the green alga *Chlorella is* one possible food for mosquito larvae, the mosquito hormone trypsin-modulating oostatic factor (TMOF) was heterologously expressed in *Chlorella*. TMOF causes termination of trypsin biosynthesis in the mosquito gut. After feeding mosquito larvae with these recombinant *Chlorella* cells the larvae died within 72 h [32]. Because diseases such as malaria, dengue and west Nile fever are transmitted via mosquitoes, mosquito abatement is an expensive requirement in tropical countries. Use of such transgenic algae might be a much cheaper alternative. The utilization of algae as an expression system is not restricted to antibodies, antigens, or insecticidal proteins.

BIOREMEDIATION OF WATER AND SOIL

The huge amount of agricultural waste that is generated from confined animal feeding operations provides opportunities for both potential economic gain and also for benefits from an environmental perspective, if the nutrients in the wastes can be recycled appropriately, such as through the use of growing lipid-rich algae. The method is much less energy intensive for treating waste water compared to the conventional activated sludge (AS) process, which requires a lot of energy input for aeration and later sludge disposal. The micro algae also assimilate CO_2autotrophically or mixotrophically, removing at least some of the greenhouse gas that contributes to global warming [33].

Lead, cadmium, and mercury are the most frequent water and soil polluting heavy metals. Industrial processes, including plastic manufacturing, electroplating, Ni-Cd battery production, mining and smelting industries, continuously release substantial amounts of these heavy metals into the environment. Algae readily take up heavy metals like cadmium from the environment and then induce a heavy metal stress response, which includes production of heavy metal binding factors and proteins [34]. However, higher heavy metal levels obstruct other main processes (e.g. photosynthesis, growth) and finally kill the cells. The wild-type green alga *Chlamydomonas reinhardtii* tolerates high amounts of cadmium during its rapid reproduction, but a genetically altered *Chlamydomonas*, heterologously expressing the mothbean*P5CS* gene, grows in the presence of much higher heavy metal concentrations. Expression of the *P5CS* gene, which catalyzes the first dedicated step in proline synthesis, in the genetically engineered cells results in an 80% higher free proline level and a four-fold increase in cadmium binding capacity relative to wild-type cells. Moreover, expression of this gene results in rapid growth at otherwise deadly cadmium concentrations. Generation of this transgenic *Chlamydomonas* is a significant step toward the use of algae for remediation of contaminated sites and waters.

Algae can be used in wastewater treatment to reduce the content of nitrogen and phosphorus in sewage and certain agricultural wastes. Algae that are applicable to wastewater treatment must tolerate a wide variation in medium conditions (e.g. salinity) [35]. It is known that the biological method is considered the most effective and economically efficient method for the purification of industrial wastewater by using the microbiological active slime and alga. However, bacteria of the active slime have low stability to high concentration of organic and mineral components, when considering big water flow volumes. This method also requires further destruction of superfluous quantity of active slime. Microalgae however, have higher stability, which enables working in more concentrated and toxic environments.

It is believed that the removal of wastes through biological cleaning will help raise the availability of microalgae biomass for food, agriculture, medicinal and biofuel [36]. Therefore including microalgae production by using waste and wastewater in production cycles opens new ways for environment friendly manufacturing and nature preservation. It is possible to expect that in the near future the above mentioned problems will be better perceived, thus leading to global reorientation of priorities for waste and wastewater management.

WATER QUALITY MANAGEMENT

All phytoplankton groups contain the chlorophyll molecule, which is the primary molecule used for photosynthesis. The optical characteristics of chlorophyll allow for easily detecting and quantification of phytoplankton. Understanding phytoplankton population and distribution enables researchers as well as resource managers to draw conclusions about a water body’s health, composition and ecological status. For in-situ monitoring, the measured parameter is the chlorophyll contained within the phytoplankton [37]. Chlorophyll is essential to the existence of phytoplankton. Phytoplankton can be used as an indicator organism for the health of a particular body of water. Monitoring chlorophyll levels is a direct way of tracking algal growth [38]. Surface waters that have high chlorophyll conditions are typically high in nutrients, generally phosphorus and nitrogen. These nutrients cause the algae to grow or bloom. When algae populations bloom, then crash and die in response to changing environmental conditions, they deplete dissolved oxygen levels - a primary cause of most fish kills. High levels of nitrogen and phosphorus can be indicators of pollution from man-made sources, such as septic system leakage, poorly functioning wastewater treatment plants, or fertilizer runoff. Thus, chlorophyll measurement can be utilized as an indirect indicator of nutrient levels.

Lake and Reservoir Management

The most frequent water quality problem in lakes and reservoirs is the excessive growth of phytoplankton due to high concentrations of plant nutrients. Water bodies with high nutrient concentrations and low dissolved oxygen levels are classified as eutrophic waters. Excessive phytoplankton and frequent algal blooms, caused by eutrophication, result in water with an unpleasant taste, odour and appearance. These problems adversely affect drinking water quality and diminish the water's recreational utility. Also of concern is the production of blue-green algal toxins and clogged drinking water filter systems. Thus, monitoring the algal population and distribution in lakes and reservoirs is extremely important for resource preservation, public health and safety, and overall economics.

Water Treatment

Water resource managers use chlorophyll determinations to monitor drinking water directly at the water source [39,40]. However, water monitoring just prior to the treatment process holds many economic advantages. Immediate pre-treatment monitoring enables the facility operator to optimize the amount of treatment chemical added and therefore minimizes the downtime and expense of plugged filters. Measuring chlorophyll concentration is also a step in the process of screening/monitoring for nuisance algal blooms that may influence the taste and odour of drinking water sources. These blooms may actually create conditions that are toxic to fish, wildlife, livestock, and humans. Blooms of benthic or planktonic macroalgae can have major ecological impacts such as the displacement of indigenous species, habitat destruction, oxygen depletion and even alteration of biogeochemical cycles. Bodies of water used as drinking water sources are also monitored for phytoplankton concentrations for the early detection of algal blooms to minimize filtration system clogs.

Aquaculture

Chlorophyll measurements are used in fish and shellfish hatcheries to estimate changes in the quantity of the phytoplankton food source [41]. Hatchery managers use this information to optimize the amount of phytoplankton present in the larval tanks.

A SOURCE OF NATURAL DYE [42]

In Clothing: Chlorophyll Derivatives are used for dyeing of fabrics such as wool, acetate derivatives and cotton.

In Paint Additives: Diatoms are also used in paint additives, other than algal pigments, due to the iridescent nature of their silica shells. Diatoms are a group of algae which have a unique cell wall made of Silica, known as frustule. The hard silica shells exhibit iridescence and they behave like crystals. The configuration of holes in these shells affects the color exhibited and this phenomenon has found application in dyeing fabrics without chemically altering their composition. Diatoms have also found to be efficient additives to Dye Sensitized Solar Cells. Their nanometer pores trap light that enters the solar cell. This increases interaction and improves efficiency of capture of solar energy. Diatoms sensitized Solar cells have the advantage of easy fabrication at room temperature.

In Paper Industry: The paper products used generally are not recyclable because of the chemical inks they use. The paper industry is shifting their focus to algae because the inks derived from them are easy to break down and hence easily recyclable.

A SOURCE OF AGROCHEMICALS

Microalgae are potential source of algicides, herbicides, fungicides and insecticides [43-45]. These compounds such as allelochemicals inhibit growth of sympatric algae and microbes and inhibitors of photosystem II [46-48]. They are likely to be short lived in environment compared to synthetic agrochemicals. Some examples are shown below.

A RAW MATERIAL FOR THE FORMATION OF BIOCHAR

Biochar is a carbon-rich solid materials produced from the pyrolysis (heating in the absence of oxygen) of biomass [49]. Biochar is a fine-grained and porous stable substance that cannot be decomposed by soil microorganisms nor be oxidised that returns carbon to the atmosphere in the form of CO_2 [50]. This technique increases the amount of carbon in agricultural soils although very often the efforts aimed to achieve carbon sequestration in soils are offset by other greenhouse gas emissions since soils generally show low potential to accumulate carbon. Transforming algae biomass to carbon-rich materials with potential mega-scale application is a material option to sequester carbon from plant material, taking it out of the short-term carbon cycle and therefore binding CO_2 efficiently and even in a useful, productive, way into longer term non-atmospheric carbon pools [51].

Feedstock used in making biochar include wood chip and wood pellets, tree bark, crop residues (including straw, nut shells and rice hulls), switch grass, organic wastes including distiller's grain, bagasse from the sugarcane industry and olive waste, chicken litter, dairy manure, sewage sludge and paper sludge [52,53].

Conversion of algae in ponds, bioreactors and off-shore systems to valuable products like biochar requires processing steps such as harvesting and dewatering. Live microalgae are tiny particles (1 to 30 *m*m) suspended in the culture media. Therefore separating and collecting these fine particles with low specific gravity from the bulk liquid is challenging and costly. The isolation of algae from their culture medium is challenging for two main reasons: their small size and the low concentrations in which they can be grown (typically less than 2 g algae/L water). Difficulties arise when attempting to separate and recover algae grown due to the fact that microalgae are typically very small and they form stable suspensions due to their negatively charged surfaces. Suspensions tend to be relatively dilute, adding to the difficulty in harvesting algae.

Biochar Application in Agriculture

Carbon sequestration is the primary driver for considering the application of biochar to soil. Biochar may also help in controlling the levels of greenhouse gas in the atmosphere. Biochar can be used as a fuel or as a soil amendment. When used as a soil amendment, biochar can boost soil fertility, prevent soil erosion, and improve soil quality by raising soil pH, trapping moisture, attracting more beneficial fungi and microbes, improving cation exchange capacity, and helping the soil hold nutrient [54]. Moreover, biochar is a more stable nutrient source than compost and manure. Therefore, biochar as a soil amendment can increase crop yields, reduce the need for chemical fertilizers, and minimize the adverse environmental effects of agrochemicals on the environment [55]. Another potentially enormous environmental benefit associated with biochar used in soil is that it can sequester atmospheric carbon. In the natural carbon cycle, plants take up CO_2 from the atmosphere as they grow, and subsequently CO_2 is emitted when the plant matter decomposes rapidly after the plants die. Thus, the overall natural cycle is carbon neutral. In contrast, pyrolysis can lock up this atmospheric carbon as biochar for long periods. When CO_2 is pulled from air to make biochar, the net process is carbon negative. Therefore, the biochar approach is an attractive solution to alleviating global warming concerns.

Use of biochar to manage water quality

Biochar may offer benefits in reducing diffuse pollution originating from agriculture through deployment in soils from which polluting elements arise. It may also be possible to utilise its sorptive capacity to remove contamination in the water treatment process [56]. However, whilst biochar may loosely hold nutrient elements in a plant-available form, the by-product of water treatment could also be contamination by toxic organic compounds in wastewater [57]. This could confound use of the post-treatment biochar product on land; the economic and overall carbon and environmental gain to be achieved from centralised versus diffuse deployment for management of water quality have yet to be assessed. The precedent for a centralised approach is the current use of activated carbon for the removal of chlorine and organic chemicals such as phenols, polychlorinated biphenyls, trihalomethanes, pesticides and halogenated hydrocarbons, heavy metals, and organic contaminants. It is not clear whether the higher surface area and sorptive capacity resulting from activation of biochar from agricultural crop wastes results in significant differences compared to biochar.

Table 1. Limitations Encountered In the Field of Algal Biotechnology

Limitations	**Remedy**
Commercial application of wild-type algae is still limited and that of transgenic algae is in its infancy.	Optimization of algal biotechnology for extensive commercial use [58]
Light is a limiting factor for algal growth	The development of special bioreactors [59]. The use of stirred tanks, or

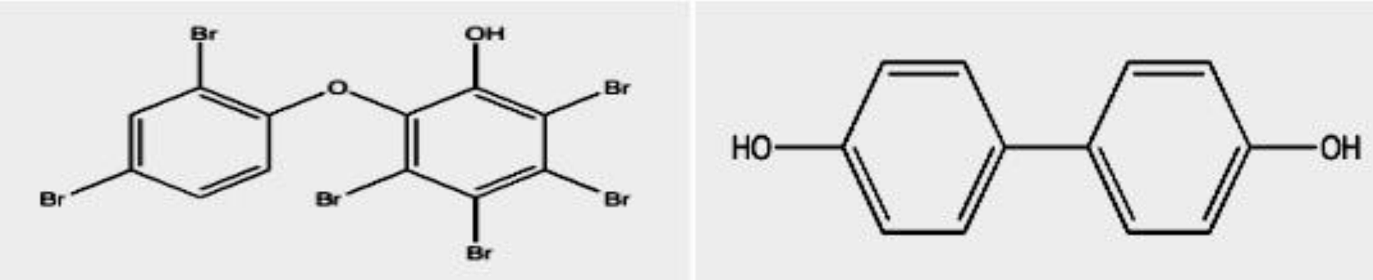
especially in dense cultures beyond the first few centimeters and this restricts cell growth.	shallow ponds. The use of heterotrophic algae and addition of the required organic substrate. Transformation of photoautotrophic algae into heterotrophic algae by introducing a gene for a sugar transporter into their genome by genetic engineering [60].
Harvesting of algae from an open pond is inefficient and expensive, so also is the extraction and purification of bioproducts from such cultures [61].	Development of large closed culture systems although very expensive Development of strategies that reduce the probability and impact of gene flow between transgenic algae and their wild relatives Starter cultures grown in closed photo-bioreactors in order to avoid overrun of the species of interest by other species in the unsterile open pond system.

Table 2. Comparison of Algae and Conventional Feedstock for Power Generation

Algae	**Conventional Feedstock**
Fundamental limitations of photosynthetic algal culture Cultures restricted to low cell densities due to light limitation as the concentration of cells increases Dependence on local weather/climate Require large open ponds or costly equipment like photo bioreactors Certain strains have high oil content Many species grow in brackish water--low impact on freshwater resources	**Fundamental limitations of conventional feedstock** Sustainability issues Displacement of food crops
Environmental impacts of commercial algal culture Biomass grown on marginal or abandoned lands Utilization of waste materials Does not require prime agricultural land--will not displace food crops Algae have much higher growth rates than terrestrial plants--higher yields of oil per acre	**Environmental impacts of commercial agriculture** Ecological damage from clearing natural lands (*e.g.*, forests, grasslands) to create new croplands Water consumption Application of fertilizers and pesticides
Processing algal biomass Drying harvested algal biomassPulverizing / lysing algal cellsExtraction and purification of bio-oils. Solvent extraction isolates bio-oils from algal biomass Transesterification of bio-oils to methyl esters	**Pre-treatment steps potentially required for feedstocks** Oilseed crushing/pressing DegummingBleachingDeodorizing Removal of free fatty acids (< 2% FFA for base-catalyzed transesterification
Solid catalysts used for biodiesel production Examples of solid catalyst include zeolites, acidic ion exchange resins (e.g Nafion and Amberlyst), mixed metaloxides (*e.g.*,Esterfip-H), Sulphated zirconia, Sulphonated, partially carbonized sugarsSolid catalysts can be recovered and recycled after reactionReduce/eliminate water washing of crude biodiesel productHigher quality glycerin byproductAbility to process high fatty acid feedstocks without additional pre-treatment stepsSolid catalysts could significantly reduce the price of biodiesel production	**liquid catalysts used** (*e.g.*, sodium methoxide in methanol)

Figure 1. Microalgae cultivation systems [62]

Figure 2. Structure of Polybrominateddiphenyl ethers (photosynthesis inhibiting, antimitotic) and 4, 4'-dihydroxybiphenyl



CONCLUSION

In this work a bibliographical revision has been carried out on the bioactive molecular constituents and biotechnological applications of microalgae. Thus, it is possible to conclude that microalgae show a high potential as natural sources of bioactive ingredients with many different biological applications. However, the development of algae biotechnology depends in particular on the identification of more high value products in microalgae. Before algal biofuels can become economically feasible on the commercial scale, significant advances must be made in the understanding of algal biology and the mechanisms of growth, lipid production pathways, and photosynthesis in algae which could be instructive for the manipulation of biolipid productivity in addition to increasing biomass. It is foreseeable that the search for microalgae biotechnological potentials will be one of the hot challenges that, basically, try to give response to the social demand of new products with scientifically demonstrated economic properties.

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