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Algae Culture Conditions and Process Parameters for Phycoremediation and Biomaterials Production

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Abstract **– Climate change and increasing world population call for careful utilization of water and energy sources. Microalgae to treat wastewater in a coupled process to produce biofuels and other value-added products for human consumption are promising solutions. An analysis of culture parameters and cultivation processes is presented as essential to achieve economical sustainability from the algae. Results of the activity of microalgal strains in detoxification of wastewater are compared and discussed, particularly in remediation of nitrogen and phosphorous compounds, heavy metal, pharmaceuticals and personal care products. Phycoremediation mechanisms and culture conditions to obtain optimal microalgal growth are discussed. Finally, valuable products that can be produced by microalgae and ecological problems of untreated wastewater are presented.**

Keywords **– Algal growth; biofuels; biodegradation; biomass harvesting; bioreactor; detoxification; microalgae; pond; wastewater treatment (WWT)**

Nomenclature		
WWT	Wastewater Treatment	–
PPCPs	Pharmaceuticals and Personal Care Products	–
PUFAs	Poly Unsaturated Fatty Acids	–
ROS	Reactive Oxygen Species	–
EPA	Eicosapentaenoic Acid	–
DHA	Docosahexaenoic acid	–

1. INTRODUCTION

According to UN estimates, 7.7 billion humans lived on the planet in 2019 and it is expected that world population will reach 9.7 billion people by 2050 and 10.9 billion by 2100 [1]. The constant increase in population will cause several difficulties like increased wastewater production and fuels demands.

Wastewater is the broad term indicating any water that has been "used" and thus contaminated by human activities [2]. The definition implies that wastewater is generated by households, agriculture, industries and any human practice. Consequently, the composition of wastewater is strongly related to the type of human activity, period of the year, geographic area, etc. Main problematic components in wastewater are nitrogen and phosphorus derivatives, organic compounds, heavy metals, chlorinated organic compounds, mineral oils,

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phenols, sulfates, mineral acids and salts. In many cases wastewaters contain also antibiotics and other pharmaceutical compounds, which enhance the serious problem of antibiotic resistance [3]. Recently, also the effects of polyvinyl chloride microplastics on wastewater and sewage sludge are object of investigation [4].

It was estimated that global wastewater production in 2020 was around 359 billion m^3 yr^{-1} of which only 63 % was collected and only 188 billion m^3 yr^{-1} (52 %) was treated [5]. Wastewater treatment (WWT) is essential not only to improve water quality and environmental impact of human activities but also to guarantee availability of usable freshwaters and to reduce the competition for traditional water sources.

Microalgae can be a valuable solution in WWT not only because they can remove various hazardous compounds for the environment and for the human health [6], but also because some of these compounds can be used to enhance microalgal growth [7]. This is the general concept called phycoremediation, from the ancient Greek *phykos* (seaweed or algae) and the Latin *remedium* (restoring balance). Phycoremediation implants are a viable option for increased wastewater production and fuels demands [8]. Currently, microalgae-based technologies are also used for the removal of pharmaceuticals and personal care products from the wastewater [9]. In conclusion, in a circular bioeconomy perspective, the microalgae-based approaches are encouraging solutions for resource recovery from wastewaters [10].

In this paper, phycoremediation mechanisms, culture parameters and conditions to obtain optimal microalgal growth are discussed. Moreover, valuable products that can be produced by microalgae and ecological problems of untreated wastewater are presented.

2. PHYCOREMEDIATION

Phycoremediation process was firstly used in 1960 and consists in using microalgae to treat wastewater and in exploiting the algae metabolism to convert harmful compounds into safe molecules thus increasing water safety [11]. The use of microalgae compared to traditional WWT or the utilization of other microorganisms in bioremediation processes presents various advantages [12]. Microalgae:

- Have higher photosynthetic and growth rates if compared to higher plants [13];
- Can use CO_2 from flue gasses as carbon source: both reducing energy demands and greenhouse gas emissions [14];
- Can use N and P compounds present in wastewaters as nutrients to produce protein, lipids, nucleic acids and other essential compounds for cellular growth [15];
- Can degrade numerous organic materials, antibiotics, pharmaceuticals and eliminate heavy metals from wastewaters [16];
- Can tolerate very broad climatic conditions without competing for agricultural land use [17];
- Can grow both in mixotrophic and heterotrophic conditions guaranteeing higher growth rate and productivity and also increasing ammonium uptake up to 4 times in mixotrophic conditions compared to autotrophic conditions [11];
- Can have bactericidal activities and can lower the chances of pathogens proliferation as conditions favoring microalgal growth are detrimental for coliform bacteria [18];
- Can be used as source of value-added products such as biogas, biohydrogen, biodiesel, bioplastics and biofertilizers [19], [20] making the products more ethical and the overall process more cost effective and competitive on the market.

Many complex mechanisms are involved in the phycoremediation. Through photosynthesis the algae remove carbon dioxide with the potential to be a carbon reducing system when combined with production of biofuel. Moreover, various metabolisms processes like biosorption, bioconcentration, biotransformation, volatilization provide microalgae the ability of removing environmental toxicants. It is expected that the advanced genomic tools in biotechnology will improve the algal strains for bioremediation by enhancing their photosynthetic efficiency, tolerance to harsh environment conditions, adaptability, and consequently ability to detoxify pollutants in wastewater [21]. In particular, *Chlorella* and *Scenedesmus* species are the microalgal strains that are mainly used in phycoremediation [22].

Among the downsides of phycoremediation implants are the following three [23]:

1. The phycoremediation process is still not cost effective and requires a lot of space and different reactors according to the type of production that is coupled to WWT;
2. Microalgal growth is related to the specific geographical location;
3. The wastewater composition is not constant and stable: these changes interfere with the establishment of a consolidated standard protocol for WWT via phycoremediation.

Moreover, wastewater composition is not optimal for microalgal growth and thus the utilization of trained algal species or modification of wastewater composition to match the microalgal nutrient requirement can be performed [24].

Microalgae for phycoremediation are used in the following four main systems, which can have different shapes and dimensions associated with different advantages and drawbacks [11], [12]:

- Constructed wetlands. Artificial systems are used to filter and detoxify surface, ground and wastewater exploiting the natural activities of plants, soils and microbes. This approach is cost-efficient and has a low environmental impact, beside the fact that it can be used for both solid and liquid waste.
- Algal mat. A thick layer of microalgae and other microorganisms is used as a filter to reduce pollutants concentration in the water that flows through. The slower the water flow is, the higher the detoxification efficiency of the mat is. After a set period of time, algal mats are removed and harvested.
- Open pond. Microalgae are grown in an open pond under mixotrophic condition using photosynthesis and wastewater as nutrient source. These systems are used at commercial scale and can have stirring equipment. The stirring has additional costs but guarantee higher growth rates and nutrient removal efficiency [17].
- Closed systems. The main purpose is to have a deep control of the factors that affect microalgal growth and phycoremediation activity to guarantee high efficiency. Possible decrement of costs for construction, operation and maintenance indicate immense potential for viable large-scale options.

Mutual interactions between bacteria and microalgae cooperate in phycoremediation. Indeed, while microalgae use photosynthesis and nutrients present in wastewater to grow and produce oxygen, bacteria use it for oxidizing organic compounds in the water [8], [16]. This synergic activity suggests a promising role in the phycoremediation of microplastics from the terrestrial and aquatic ecosystems. Microplastics are not only very widespread, non-degrading, and capable of adsorb other pollutants, but can also enter the human food chain. Microalgae can adsorb microplastic materials and thus remove them from the environment [25]. Additionally, together with the microorganism consortia, they can enzymatically degrade some microplastic polymers and use them as carbon source. However, these processes can significantly decrease microalgae growth and reproduction ability [26].

3. Culture Parameters for Microalgal Growth

Culture parameters and conditions must be considered to guarantee optimal microalgal growth in wastewater. Moreover, specific nutrients depletions and stress conditions can guarantee not only efficient production of desired value-added products, but also increase microalgal growth or protection from contamination, and induce the correct balance between saturated and unsaturated fatty acids desired for optimal biodiesel production. For example, microalgae under nitrogen starvation and temperature stress grown conditions produce more lipids reaching up to 90 % and 50 % respectively of their total weight [13], [14], [27], [28]. However, on the downside, wastewater composition is very variable through the year, the geographic zones and human activity making wastewater an unstable and complex culture medium for microalgae.

3.1. Light and Depth of the Bioreactor

Light is crucial in photosynthesis and has to be controlled and regulated in terms of intensity, optical spectra and photoperiods. Too intense light can cause damages to photosystems and decrease efficiency of the process. Ideal light intensity to increase carbohydrates production has been identified between 200–400 µmol photons m^{-3} s^{-1} [14]. When considering optical spectra, a mix of blue (465 nm) and red (660 nm) LED lights seem to guarantee best dry weight increase and nitrate sequestration in *P. tricornutum*, *I. galbana*, *N. salina*, and *N.oceanica* if compared with the sole red or blue light or using fluorescent light [29]. Photoperiods with definite light/dark alternation can increase lipid production in specific microalgal strains.

Depth of the bioreactor must be shallow enough to prevent self-shading effects but also to protect microalgae from evaporation. Ideal depth for open pond systems ranges between 15 and 30 cm (or few millimetres to 2 cm in thin layer ponds). Moreover, deeper bioreactors guarantee a higher residence of CO_2 and other gasses supplied to the medium [27].

3.2. Mixing

Mixing must prevent sedimentation of the biomass as well as guarantee homogeneity in the culture medium in terms of nutrients, oxygen and CO_2. Mixing is also crucial in permitting light to penetrate to lower parts of the reactor. However, mixing intensity should be tailored to not cause shear stress on microalgal cells and to limit costs due to the use of propellers [30]–[32].

3.3. pH and CO_2

As CO_2 concentration affects pH value, both parameters must be considered to guarantee maximal yield. High concentration of CO_2 can lead not only to a decreased pH but also to inhibit the activity of Photosystem II, which is essential for photosynthetic activity [14], [27]. Optimal pH for microalgal growth ranges between 6 and 9: at higher pH the culture collapses as cell metabolisms is impaired. However, some species of *Chlorococcum* and *Spirulina* are resistant to very acid and very basic pH making these microalgal strains very useful in stress conditions to prevent contamination from other organisms [28], [33].

3.4. Temperature

Usually, microalgae grow best between 25 to 35 °C but marine microalgae are more sensitive to temperature and at temperatures over 28 °C productivity decreases [28], [34].

Generally, while lower temperature affects enzymatic activity, especially RuBisCo's (Ribulose 1,5-Biphosphate Carboxylase Oxygenase) which is the key enzyme in photosynthesis, increased temperatures decrease CO_2 solubility causing increased photorespiration [35]. However, some strain of *Chlorella* can grow at temperatures of 35–40 °C. The high temperature resistance is an interesting feature that can help to prevent contamination in open pond bioreactors. Moreover, temperature can modulate the fatty acids production since at higher temperatures microalgae produce more saturated than unsaturated fats [28].

3.5. Salinity

Salinity can be an important factor involved in prevention of contamination in open pond reactors. Moreover, salt concentration in the medium can shift the metabolism of the microalgae. For example, higher salinity induces the production of smaller compounds to prevent osmotic effects on the cells [14], [27].

3.6. Macro- and Micronutrients

Nutrients can be divided into two main categories: macronutrients (C, N, P, S, K) that are necessary in high concentrations and micronutrients (including heavy metals such as Fe, Co, Mn, Cu, and Zn) that must be present in small quantities to sustain microalgal cultures [22]. N and P are two of the essential micronutrients that are used to produce protein, nucleic acid, lipids and many other cellular structures [24]. All the nutrients must be present in correct concentration to guarantee proper microalgal growth. However, some specific nutrient depletion condition can be used to enhance production of specific wished metabolites.

Concerning nitrogen, microalgae can uptake ammonia, nitrites, and nitrates. Ammonia is the more indicated nitrogen source since it is assimilated firstly due to its easy uptake, which does not require any redox process unlike nitrites and nitrates uptake. Wastewaters are rich in ammonia especially streams coming from agriculture sources as nitrogen is one of the main components of many fertilizers. Nitrogen must be present in sufficient amount as nutrient to guarantee proper microalgal growth. For example, in *Chlorella* it was found that 17 mg/L of ammonia concentration guaranteed a 0.92/d growth rate while 39–143 mg/L of ammonia caused a 0.33/d growth in microalgae. Also, a 39 mg/L concentration of ammonia guaranteed a lipid productivity of 23.3 mg/L/d of mostly unsaturated C16 and C18 [33].

Phosphorous is an essential macronutrient because it is crucial for cell metabolism, energy, lipid, and protein production. It can be assimilated in the form of salts of $H_2PO_4^-$ and HPO_4^- . This element can also be stored in Ca-P rich-bodies, i.e. membrane-less compartments located inside the vacuolar lumen in which polyphosphates are accumulated as a reservoir [36]. P is very common in manure wastewaters and is also commonly used in agriculture, but it has the problem of requiring processing to be recovered and reused in agriculture. Microalgae can be grown in wastewater, uptake phosphorus and then be used as biofertilizers [7], [33].

Some heavy metals, which are very dangerous for aquatic environment and human health, are essential for microalgal growth and other heavy metals can even enhance microalgal growth if present at specific concentrations [37]. Two main pathways are used to detoxify waters from heavy metals. The first pathway is passive adsorption (or biosorption). The mechanism is based on electrostatic interaction between metal ions that in water are mostly positively charged and cell wall which presents heavy metals-binding functional groups that can interact with both anionic and cationic heavy metals. The second pathway is intracellular positive diffusion and accumulation. This mechanism is slower and implies firstly the transport of heavy metals into the cell by diffusion and then their binding to specific intracellular ligands (proteins, metal transporters and glutathione) [11].

3.7. Pharmaceuticals, Personal Care Products and Degradation Mechanisms

A serious ecological risk in water is represented by chemical reagents for pharmaceuticals for human and animals' therapy, and care products for personal hygiene and beauty purposes. These chemicals are currently ubiquitous in every environmental compartment and can cause antibiotic resistance and endocrine disruption. Microalgae can eliminate these chemicals through various degradation mechanisms:

- Bioadsorption: a rapid passive mechanism based on the interaction between pharmaceuticals and personal care products (PPCPs) and the cell wall or extracellular polymeric substances produced by microalgae. The nature of these interactions is mainly electrostatic. Among the compounds eliminated through this process are tetracycline, triclosan, 7-amino-cephalosporanic, carbamazepine, ibuprofen, diclofenac, paracetamol. However, it has been found that the efficiency of the removal process and the nature of the consequent bioadsorbed compounds vary concerning the species of microalgae, the pH of the medium and the hydrophobicity of the compounds [9].
- Bioaccumulation: a slow active mechanism that occurs after bioadsorption and characterized by the travelling of the pollutants from the extracellular environment into the cellular cytoplasm [9].
- Biodegradation: an active process of metabolic degradation of PPCPs mediated by enzymes. Xenobiotic compounds in microalgae are degraded into three steps:
  1. Detoxification performed by cytochrome P450 transforming lipophilic compounds into hydrophilic compounds;
  2. Bonding molecules with electrophilic groups to glutathione units; and
  3. Degradation of the xenobiotic compounds in several less complex molecules [38], [39].
- Photo-degradation: solar light degrades PPCPs through indirect or direct photolysis [40]. Indirect photolysis consists in the liberation of carboxylic acid because of high Reactive Oxygen Species (ROS) concentrations in the microalgal cell. Then, carboxylic acids induce the formation of other ROS that degrade the pollutants. Direct photolysis consists in the bond breaking as a consequence of direct UV irradiation [41].
- Volatilization: it is a compound specific mechanism very relevant in open ponds in which volatile compounds are removed according to Henry's Law which describes the relation between the amount of gas dissolved in a solution and its partial pressure of the gas outside the solution [9].

4. CULTURE CONDITIONS

Generally, microalgae are photoautotrophic microorganisms that fix CO_2 through photosynthesis and convert solar energy into biochemical energy (photoautotrophic cultivation). However, microalgae can also be cultivated providing all the nutrients they need to grow (heterotrophic cultivation) or providing some nutrients so that photosynthesis is still performed (mixotrophic cultivation). Finally, microalgae can be cultivated with another microorganism (co-cultivation). In the following sections the different cultivation modalities are discussed.

4.1. Photoautotrophic Cultivation

Photoautotrophic cultivation is the economically more convenient method to grow microalgae because no nutrients are supplied to the culture as microalgae produce nutrients through photosynthesis. Four main cultivation systems are used.

1. Open cultivation systems (raceway pond): open rectangular tanks with specific length, width and depth are used to guarantee optimal microalgal growth. These systems are convenient as they only require energy for the mixing and they are easy to maintain. However, they have numerous disadvantages: lower light utilization, scarce mixing, evaporation, contamination, and request of large spaces [42]. To reduce the contamination problems, microalgae can be grown under stressful conditions, like high salinity, high nutrient concentration, low and high pH levels [10], [12];
2. Closed cultivation systems: Closed tanks of various shapes (tubular, flat plates, or column photobioreactors) are more expensive and require artificial light supply. However, they guarantee correct mixing, sterility, and higher production rates [22], [24];
3. Membrane photobioreactors: The bioreactor has a core in which wastewater flows continuously and a peripheral area in which microalgae are grown. The two sectors are separated by a membrane that allows nutrient permeation and biomass retention [20];
4. Hybrid photoautotrophic systems: Microalgae are firstly grown in a closed photobioreactor to guarantee high growth rates and sterility. Once desired conditions are reached, microalgae are moved to an open pond with nutrients addition [20].

4.2. Heterotrophic Cultivation

Microalgae can be cultivated supplying all the nutrients they need for growing, including organic carbon sources. This kind of cultivation guarantees higher growth rates, larger cells, high cell density and thus lower harvesting and extraction costs. However, heterotrophic cultivation is more expensive as it needs nutrients to guarantee microalgal growth and it is more energy intensive [42], [43].

4.3. Mixotrophic Cultivation

The mixotrophic culture system is a combination of the previous two cultivation modalities: microalgae are both supplied with nutrients to increase growth rate and density (heterotrophic cultivation) and they are exposed to light to induce carbon fixation through photosynthesis (photoautotrophic cultivation). A constant biomass production is guaranteed because it overcomes the decreased growth during dark phase common in photoautotrophic cultivation [44].

4.4. Co-Cultivation Approach

Co-cultivation is an approach that consists in cultivation microalgae with another microorganism. Fungi cultures are commonly coupled to microalgae in bioreactor as the interaction with mycelia simplifies harvesting [37]. In proper culture conditions, algae and fungi create spherical structures that are more stable, increase mass transfer and thus the ability to increase homogeneity in the medium and can be easily separated from the broth making the harvesting more economically feasible [37], [45]. Moreover, while microalgae produce oxygen usable in fungi respiration, fungi produce extracellular enzymes that make nutrient assimilation more efficient for microalgae [46]. If compared with mono-cultivation, co-cultivation can significantly reduce total nitrogen, phosphorous and chemical oxygen demand in wastewater. The case of *Filamentous* fungi in co-cultivation with microalgae [20], [47] is discussed in Table 1 for the advantageous and disadvantageous aspects.

TABLE 1. ADVANTAGES AND DISADVANTAGES OF CO-CULTIVATION OF MICROALGAE WITH *FILAMENTOUS* FUNGI [20], [47].

Advantages	Disadvantages
- Better utilization of available nutrients by microalgae and better fungi respiration;	- Competitive interactions between microalgae and fungi can be detrimental for biomass growth;
- Easier mass separation and harvesting process;	- Difficulty in mass transfer in large pellets;
- High mechanical stability and increased biomass quality for biobased products;	- Biomass quality depends on many more variables and is thus difficult to control and stabilize;
- Increased mass transfer rate;	- Higher maintenance costs of co-cultures;
- Better removal of polluting compounds;	- Increased risk of contamination;
- Lower costs in WWT.	- The process is still at a laboratory level

5. HARVESTING OF MICROALGAE BIOMASS

Harvesting of microalgae biomass is a very expensive process and in biodiesel production it can account for up to 30 % of the final product cost [48], [49]. Different methods are available, but none has guaranteed economic feasibility. Four methods are described in the following.

- Chemical and biological harvesting. Polymers and electrolytes (ferric chloride, aluminum sulfate and chitosan sheds) are used to mask the charge on the cell wall and to allow aggregation and thus sedimentation of microalgae. Synthetic polymers and metal salts have negative impacts on the safety of the final product so it is preferred to identify biopolymers that can induce bio-flocculation and auto-flocculation [20], [50]. This strategy is associated with high pH that induces liberation of positively charged ions that interact with negatively charged microalgae inducing aggregation and precipitation [51].
- Mechanical harvesting. Centrifugation is the main technology, which guarantees the highest yield. However, it is very energy demanding, requires expensive machinery and can cause shear stress to the microalgae cells [20], [37]. Sedimentation is an alternative, which is not expensive but very slow. Flotation is another possible approach for large scale purposes. It consists in the utilization of air bubbles beneath the sedimentation tank to induce gravity separation of algal biomass and to create a surface film of microalgae. By coupling the utilization of coagulants, the efficiency of the mechanical harvesting process is increased. Even though this approach is less energy intensive it has high running costs. Another option for microalgae recovery is the utilization of membranes, which do not require a lot of energy, but high operation costs in case of cleaning, membrane fouling, and eventually substitution.
- Electric or magnetic harvesting. Through the application of an electric field, microalgae are forced to move since they present negatively charged proteins on their cell wall [52]. This technology is attractive as it does not require the addition of any substance to the medium. However, it is very energy intensive making it a non-economically sustainable option [53]. The magnetic separation is a possible alternative, where external magnetic particles are added to the culture and bound to the microalgae cell wall throughout electrostatic interactions. Finally, microalgae are

separated from the culture broth using a magnetic field with low energy and land use, simple operation and no clogging or fouling problems [54]. In this approach pH and magnetic particle concentration are crucial to guarantee proper harvesting [37].

– Fungal assisted harvesting. Fungi are co-cultivated with microalgae after injection of fungal spores into microalgal growth medium, so that pelletization and harvesting occur at the same time [47]. Alternatively, pellets generated from filamentous fungi are added to microalgae. The consequent formation of fungal-microalgal pellets helps harvesting microalgae.

6. VALUE-ADDED PRODUCTS FROM MICROALGAE

The process of wastewater treatment can be efficiently coupled to the production of biomaterials to reduce overall costs and make the bioproducts more competitive on the market. Bioproducts for human consumption (like foods, pigments and cosmetics), animal feed and biofuels (like biodiesel, biogas and bioethanol) are typical microalgae applications.

– Food application. Not all microalgal species are suitable for human consumption. Three main species can be consumed by humans: *Spirulina, Dunaliella* and *Chlorella* and they already cover a large portion of the microalgae market. Microalgae are considered particularly valuable aliments especially for their high protein content (40–60 % wt% of dry matter) [55]. Additionally, microalgae contain amino acids that are essential for human diet with a correct profile, thus they seem a valuable option in vegetarian and vegan diets. Microalgae contain also non-essential amino acids that can have significant health benefits and have a role in immunity, cell signaling, gene expression and antioxidant responses [56]. In the past, microalgae have been used mainly as supplements or health food products, but they can also be used as additional ingredients for common foods to increase their nutritional value.
– Pigments. Chlorophylls, carotenoids and phycobilins are the main responsible for microalgal color. These pigments can be used as colorants in food products, in pharmaceuticals and in nutraceutical products as they have beneficial effects on human health and represent a more environmentally sustainable option if compared with synthetic dyes [57]–[59].
– Supplements. Microalgae are rich in Poly Unsaturated Fatty Acis (PUFAs) such as omega 3 and omega 6 which are considered essential for proper brain development and many vital functions. Moreover, microalgae are alternative sources of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are commercially valuable PUFAs with much less undesired strong smell if compared with fish derived PUFAs and are less contaminated by toxic compounds such as heavy metals and residual pesticides that often leech into water and accumulate into fish [60]. Microalgae represent a promising alternative source of DHA and EPA compared to fungi, that require external carbon sources to accumulate these compounds and have a slower growth [61], and to higher plants that would need genetic engineering to produce long chain PUFAs [62]. Other essential fatty acids are alpha linoleic acid, gamma linoleic acid, linoleic acid and arachidonic acid, which are present at high concentrations. In addition, microalgae are rich in vitamins that are essential cofactors for cellular metabolism. The high content of vitamins such as B12, which is essential and lacks in vegan diets and is higher in microalgae than in other plant or animal food source [63], can make microalgae a suitable source for nutritional supplements [55].
– Biofertilizers. Some microalgae are used as fertilizer providing promising results.

For example, *Acutodesmus dimorphus* is used in cultures of Roma tomatoes to enhance the germination, growth and fruit production. It has been observed that the use of microalgae mixed with vermiculite provides an increased weight of 7–33 % [64].

- Supplements in animal feed both for animals and fish industry. The use of microalgae as supplements in animal feed increases their body weight, immune response, and resistance to diseases of bacterial and viral nature. Today 50 % of *Arthrospira sp.* production is used in animal feed application [55]. The use of microalgae in feeds, however, is determined by their type, composition, nutrition values and the animal adaptation to the ingredient [65].
- Pharmaceuticals and cosmetics. 1,3-β-glucan is a compound extracted from *Chlorella* that is used for multiple therapeutic purposes: immunostimulation, quenching of free radicals, reduction of blood lipids but also prevention of gastric ulcers and atherosclerosis. Moreover, many secondary metabolites and pigments are used in the development of cosmetics [66].

Biofuels are alternative options to fossil derived fuels and coupling phycoremediation to biofuels production has two main scopes: reduction of the overall cost of the process and guarantee of proper microalgal growth. From microalgae metabolites three main classes of biofuels can be generated, as listed in the following.

- Biodiesel is produced through the catalyzed transesterification of triacylglycerols that are accumulated in high quantities in microalgae. It has been shown that using *Chlamydomonas* in dairy wastewater treatment has a lipid productivity of 87 ± 2 mg/L/d and provides an optimal mix of fatty acids for biodiesel production including stearic, linoleic, and linolenic acid [33], [64].
- Biogas. Methane, hydrogen, and a mixture of the two, i.e. bio-hythane, can be produced in anaerobic digestion facilities. Microalgal biomass can be digested with sludge (25/75 %) to produce biogas in continuous reactors. Another way to produce biogas is through biophotolysis or dark fermentation of microalgae [33], [64].
- Bioethanol. This biofuel is produced through fermentation of fermentable sugars present in microalgae after their extraction. Even though microalgae have low concentration of sugar, their cell wall does not contain lignine, that makes sugar extraction in higher plants more difficult because of the strong chemical stability of the lignin polymer.

7. The Use of Three Microalgae to Detoxify Wastewater

Several microalgae can be used to detoxify wastewater. *C. minutissima*, *Scenedesmus sp.* and *N. muscorum* were used as single strain and also in consortium to evaluate their effects on primary treated wastewater. pH, total dissolved solids (TDS), dissolved oxygen (DO), nitrate-nitrogen (NO_3-N), ammonium-nitrogen (NH_4-N), phosphorous, potassium, biological oxygen demand (BOD) and chemical oxygen demand (COD) where measured before and after microalgal cultivation after 25 days at open ambient temperature and light. Microalgae were grown in plastic trays of 45 cm of length and 30 cm of breadth and inoculation was performed with a microalgal concentration of 0.7 g/l [19].

Performances of the 3 strains [67] were very similar, while the consortium performed best only for P removal (15 %) and COD removal (19 %). Overall *C. minutissima* and *Scenedesmus* were the strains with better performances. Considering TDS and NO_3-N removal, values of 96 % and 87 % were obtained with *C. minutissima*, respectively. *Scenedesmus sp.*, on the other hand, removed more NH_4-N. It can be concluded that if both

NH_4 and NO_3 are available, microalgae prefer to assimilate ammonia as it requires a passive form of assimilation. The microalgal cultivations increased the pH level of the solution because of removed CO_2 due to photosynthetic activity.

In consideration of microalgal growth, *C. minutissima* and *Scenedesmus* strains had accumulated better fresh and dry weight with *Scenedesmus* reaching the best growth rate of 0.38 ±0.01 g/L on day 10^{th}.

In terms of biomass production, lipid content, lipid productivity and fatty acids contents are generally evaluated. Experiments suggest that *Scenedesmus* and *C. minutissima* were suitable for WWT producing a significant amount of biomass in 25 days [67], [68].

Other experiments were conducted with *Chlorella pyrenoidosa* in wastewaters coming from different sources: POME (Palm Oil Mill Effluent), mixed kitchen, piggery and domestic wastes [67]. Lipid content was monitored over 4 days and the best result was of 182 mg/l obtained by cultivation on POME. After 20 days total nitrogen, cell dry weight and optical density were measured. In these experiments, POME has performed best for all these parameters guaranteeing OD600 of 0.7, a decrease in total N from 590 mg/L to 210 mg/L and yield of dry weight of almost 600 mg/L after 20 days.

Baldev's group has performed experiments using *Scenedesmus sp*. Strain NTMDB07 to treat three different samples of domestic wastewater [68]. Results in P, NO_3, NO_2, NH_3, SO_4 sequestration were analyzed, and it was found that unsaturated fatty acids were the most common component of the total FAME production. In particular linoleic acid was 34.23 %.

8. Conclusions

The utilization of microalgae in wastewater treatment is a chance to guarantee a wiser utilization of water resources for growing population, and reduces the amount of pollutants including nitrogen, phosphorus, heavy metals and PPCPs that reach the ocean and that have negative effects on the environment and on human health. The utilization of microalgae provides a sustainable alternative to the production of biofuels and other valuable products for human consumption such as supplements, pigments, pharmaceuticals and cosmetics.

Overall, the utilization of microalgae in WWT is very promising but the main limitation is the economic feasibility of the process. Further study is necessary to identify ideal culture conditions and bioreactors that could enhance the production of valuable compounds associated with microalgae in order to make the process more economically sustainable. It is necessary to find standard processes to cope with the inconstant nature of the wastewater flow especially when considering municipal wastes. Microalgae are really promising because they can fixate carbon at higher rates than land plants and they can absorb and/or degrade many contaminants present in wastewaters.

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