The Environmental Biorefinery: Using Microalgae to Remediate Wastewater, a Win-Win Paradigm

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Date Submitted: 2018-11-27

Keywords: water and nutrient recycling, biofuel, microalgae, bioremediation, wastewater treatment

Abstract:

Microalgae have been shown to be a source of multiple bio-based products ranging from high value molecules to commodities. Along with their potential to produce a large variety of products, microalgae can also be used for the depollution of wastewaters of different origins (urban, industrial, and agricultural). This paper is focused on the importance of harnessing the bioremediation capacity of microalgae to treat wastewaters in order to develop the microalgae industry (especially the microalgae biofuel industry) and to find other alternatives to the classic wastewater treatment processes. The current research on the potential of microalgae to treat a specific wastewater or a targeted pollutant is reviewed and discussed. Then, both strategies of selecting the best microalgae strain to treat a specific wastewater or pollutant and using a natural or an artificial consortium to perform the treatment will be detailed. The process options for treating wastewaters using microalgae will be discussed up to the final valorization of the biomass. The last part is dedicated to the challenges which research need to address in order to develop the potential of microalgae to treat wastewaters.

Citation (overall record, always the latest version): LAPSE:2018.0865
Citation (this specific file, latest version): LAPSE:2018.0865-1
Citation (this specific file, this version): LAPSE:2018.0865-1v1

DOI of Published Version: https://doi.org/10.3390/en9030132 energies

MDPI

Review

The Environmental Biorefinery: Using Microalgae to Remediate Wastewater, a Win-Win Paradigm

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Academic Editor: Paul L. Chen
Received: 20 December 2015; Accepted: 18 February 2016; Published: 25 February 2016

Abstract: Microalgae have been shown to be a source of multiple bio-based products ranging from high value molecules to commodities. Along with their potential to produce a large variety of products, microalgae can also be used for the depollution of wastewaters of different origins (urban, industrial, and agricultural). This paper is focused on the importance of harnessing the bioremediation capacity of microalgae to treat wastewaters in order to develop the microalgae industry (especially the microalgae biofuel industry) and to find other alternatives to the classic wastewater treatment processes. The current research on the potential of microalgae to treat a specific wastewater or a targeted pollutant is reviewed and discussed. Then, both strategies of selecting the best microalgae strain to treat a specific wastewater or pollutant and using a natural or an artificial consortium to perform the treatment will be detailed. The process options for treating wastewaters using microalgae will be discussed up to the final valorization of the biomass. The last part is dedicated to the challenges which research need to address in order to develop the potential of microalgae to treat wastewaters.

Keywords: wastewater treatment; bioremediation; microalgae; biofuel; water and nutrient recycling

1. Complementarity between the Wastewater Treatment and the Microalgae Industries

Microalgae have multiple potential applications, of which the most promising future objective on a large-scale is their use as a biofuel feedstock [1]. A number of microalgae-based products are already well established in other high-value markets, for example as a human dietary supplement (nutraceuticals) and as a component in animal feed [2]. Nevertheless, considerable advances in the field of biology and substantial processing improvements are required to achieve economic, environmental, and energetic sustainability in the production of microalgae biofuels [3]. Wastewater constitutes a great opportunity for microalgae as it can be considered as a medium for growing them at a low-cost and as a new potential market. Through their various modes of nutrition (phototrophy, heterotrophy, mixotrophy), microalgae can effectively remove a broad range of chemicals from aqueous matrices. Amongst the various strategies possible for economical large-scale production of microalgal biomass, a coupling of wastewater treatment with algal farming is possibly the most sensible due to the similar scale and production facilities that both industries rely on (as it will be illustrated and discussed in this article). The additional benefit from such coupling is the promotion of on-site local industries and more importantly, the elimination of a large negative environmental footprint that would otherwise arise from the pollution associated with nutrient manufacturing, transportation and change in land use.

1.1. Microalgae Industry: A Need for Wastewater

Several studies have shown that the use of wastewater is a necessity for the development of the microalgae biofuel production industry [4–7]. Microalgae production is done at a very high cost nowadays. Actual costs are in the order of magnitude of 100 €/kg of biomass. For example, The production cost of a real microalgae production plant of 30 m^3 of tubular photobioreactors was estimated to be 69 €/kg of dry weight using data collected during two years of continuous operation [4]. In France, spirulina, a well-known easy-to-cultivate cyanobacteria, is usually sold at a price of 150–200 €/kg of dry weight. The two main contributing factors to the high price are the unoptimised processes employed as well as the small-scale of operations (economies of scale). Future costs of large-scale production are estimated through techno-economic extrapolations. The results vary greatly between studies depending on the hypotheses and the value chosen for the key parameters (such as lipid productivity). On average, the estimated production cost of microalgae biodiesel is around 2.5 €/L [8]. Still, these costs are too high to address the current energy market (0.6 €/L for petroleum diesel) and not competitive enough to convince the petrochemical industry that microalgae could become a valuable feedstock even in the long term. The major challenge of microalgae biofuel production is to reduce the production cost. Limiting the use of industrial nutrients could contribute since they have a non-negligible impact on the production cost (between 1% and 10% depending on the process [3]).

In addition, nutrients and water have to be used rationally due to three major facts: (1) life cycle analyses have shown that nutrients have a high impact on the environmental efficiencies of the microalgae production [9]; (2) water scarcity is a well-known global problem [10]; and (3) phosphorus is a non-renewable resource [11]. These economic and environmental drawbacks can be partly overcome by using wastewater (industrial, agricultural or urban) as growth substrate for microalgal biomass production. The demand for freshwater and industrial nutrients can be significantly reduced, thus bringing down the production cost and environmental impact of the whole process. Additionally, the cost of wastewater treatment using conventional processes can be as high as 0.682 $/$m^3$ if membrane bioreactors are used [12]. Part or of this cost can be recuperated in the form of credits for the positive environmental impact created by wastewater remediation by microalgae.

1.2. Microalgae, an Opportunity for the Wastewater Treatment Industry

In recent years, concern has grown over the presence of Pharmaceutical and Personal Care Products (PPCPs, Endocrine Disrupting Compounds (EDCs) and heavy metals in water. In Europe, the Water Framework Directive (Directive 2000/60/EC) does not require wastewater treatment plants to treat these micropollutants but it has listed a number of substances that need to be monitored by member states. Additionally, the REACH regulation (2006) has strengthened the EU regulation by requiring the evaluation of the risks of 30,000 chemical substances. The European Union IPPC (Directive 2008/1/CE) and then the Industrial Emission Related Directive (Directive 2010/75/UE) have replaced Water Framework Directive. These directives request the application of the best available techniques (BAT) taking into account technically and economically preventive measures, pollution control technologies and efficient resource consumption. Considering the bioremediation capabilities of microalgae, there is therefore a niche opportunity to develop and implement new microalgae based wastewater bioremediation technologies. In the US, the Environmental Protection Agency (EPA) has included 12 PPCPs/EDCs substances in a list in order to evaluate their related occurrence and safety risks. However, no requirement exists on concentration levels of these pollutants in water (drinking or treated water).

Microalgae are known to be pollutant scavengers for a broad category of chemicals issued from the domestic, industrial and agricultural sectors. Besides the usual organic and inorganic compounds present in the wastewater, *i.e.*, nitrates, phosphates, ammonium, microalgal cells can also assimilate and break down more persisting molecules such as hydrocarbons, antibiotics, PPCPs, EDCs and heavy metals. While bioremediation of excess nutrients in the water by microalgae has been extensively documented over many years, in comparison, little is known about their degradation capacity and efficiency for micropollutants (pollutants with toxic effect at very low concentration [13].

Concerns over micropollutants are rising and regulation is expected to become more stringent and rigorous over time. Conventional activated sludge processes or chemical processes can be ineffective for treating some organic pollutants. In the French AMPERES project, 97 substances were followed and 24 of them showed conventional activated sludge treatment efficiency below 30% [14]. Therefore, water companies are developing dedicated treatment processes in order to reduce the level of micropollutants in the effluent water of wastewater treatment plants (WWTPs). Using microalgae can offer an alternative for treating these molecules. In an extensive review on the ability of microalgae to degrade organic pollutants, the authors have listed the organic pollutants that have been successfully treated using microalgae [15]. The major advantage of using microalgae is that decreasing concentration of pollutant will not limit their growth as opposed to strictly heterotrophic microorganisms. Moreover, microalgae can contribute to the sequestration of carbon (CO_2), coming from the atmosphere or industrial flue gases, thereby contributing to the mitigation of greenhouse gases (GHG) emissions. In addition, the aquatic ecology in wastewater represents a suitable farming ground for microalgae. The relation between microalgae and bacteria is indeed very synergetic [16]. Microalgae provide oxygen for bacteria while bacteria provide carbon dioxide for microalgae, this leads to a significant decrease in the oxygen needs of the wastewater treatment process. Oxygen aeration represents more than 50% of the energetic needs of a WWTP [17]. Bringing oxygen through microalgae photosynthesis without any energy consumption can therefore lead to important savings in terms of energy demand, GHG emission and electricity cost for the WWTP.

2. Current Knowledge on Microalgae to Treat Wastewaters

The potential of microalgae to treat wastewaters has been evaluated through three different approaches: (1) the efficiency of microalgae-based high-rate algal ponds (HRAPs) treating urban wastewater; (2) the ability of microalgae to treat specific wastewaters (agricultural or industrial) and; (3) the ability of microalgae to treat a specific pollutant (generally a micropollutant or an industrial pollutant). These three approaches will be reviewed and discussed in this section. Table 1 gathers typical examples of studies where microalgae have been used to remove a specific pollutant.

2.1. Urban Wastewater Treatment

The three main pollutants found in urban wastewaters are carbon (C), nitrogen (N) and phosphorus (P). The ability of microalgae to treat mineral pollution such as mineralized forms of nitrogen (ammonium, NH_4^+ or nitrate, NO_3^-) and phosphorus (phosphate, PO_4^{3-}) is well known and documented. Since the 1950's, extensive studies have been done by Prof. Oswald and his team of the University of California. For example, they observed very good removal rates for ammonium (NH_4^+-N, 85%–90%) and phosphorus (PO_4^{3-}-P, 95%–99%) in two 1000 m^2 pilot-scale HRAPs [18]. Since then, a lot of other studies have demonstrated the ability of microalgae to treat urban wastewater and focused mainly on process intensification. For example, urban wastewater treatment has been managed on the long-term and a mean biomass productivity of 16.7 $g/m^2/day$ (maximum of 24.7 $g/m^2/day$) being obtained in a pilot-scale HRAP working at four days of hydraulic retention time (HRT) [19].

Besides mineral pollutants, microalgae can also reduce the organic loading rate (C). Several studies have shown this aspect. For example, 70% chemical oxygen demand (COD) reduction (3000 to 400 mgO_2/L) in 13 days was obtained on a centrate from urban WWTP by a PBR inoculated with *Chlorella* sp. [20]. A mix of *Chlorella* sp. and *Scenedesmus* sp. in a pilot-scale 16 m^2 open pond could remove 90% of the COD of an urban wastewater (from 180 to less than 20 mgO_2/L, [21]). More recently, microalgae have been shown to grow on different carbon substrates in wastewater open ponds, from simple molecules (glucose, lactose) up to quite complex ones (α-cyclodextrin, Tween 40 and 80) [22].

Table 1. Typical examples of studies on the use of microalgae to degrade specific pollutant.

Reference	Microalgae Species	Pollutant	Temperature	Culture Volume	Agitation	Light Intensity	Light Mode	Carbon Source	Removal Rate
[23]	*Chlorella vulgaris* and *Coenochloris pyrenoidosa*	p-chlorophenol	25 °C	150 mL in 250 mL flasks	100 RPM	52.5 $\mu mol \cdot s^{-1} \cdot m^{-2}$	16 h light/8 h dark	CO_2	10 mg/L/day
[24]	*Scenedesmus obliquus*	2,3-dichlorophenol	30 °C	50 mL bottle	-	50–60 $\mu mol \cdot s^{-1} \cdot m^{-2}$	24 h light	glucose	9 µmol/day
[24]	*Scenedesmus obliquus*	2,4-dichlorophenol	30 °C	50 mL bottle	-	51–60 $\mu mol \cdot s^{-1} \cdot m^{-2}$	24 h light	glucose	10 µmol/day
[24]	*Scenedesmus obliquus*	2,5-dichlorophenol	30 °C	50 mL bottle	-	52–60 $\mu mol \cdot s^{-1} \cdot m^{-2}$	24 h light	glucose	9 µmol/day
[24]	*Scenedesmus obliquus*	2,6-dichlorophenol	30 °C	50 mL bottle	-	53–60 $\mu mol \cdot s^{-1} \cdot m^{-2}$	24 h light	glucose	13 µmol/day
[24]	*Scenedesmus obliquus*	3,4-dichlorophenol	30 °C	50 mL bottle	-	54–60 $\mu mol \cdot s^{-1} \cdot m^{-2}$	24 h light	glucose	6 µmol/day
[24]	*Scenedesmus obliquus*	3,5-dichlorophenol	30 °C	50 mL bottle	-	55–60 $\mu mol \cdot s^{-1} \cdot m^{-2}$	24 h light	glucose	0 µmol/day
[25]	*Scenedesmus obliquus*	progesterone	25 °C	150 mL in 250 mL flasks	150 RPM	3000 lux	12 h light/12 h dark	carbonate and CO_2	0.3 µmol/day
[25]	*Chlorella pyrenoidosa*	progesterone	25 °C	150 mL in 250 mL flasks	150 RPM	3000 lux	12 h light/12 h dark	carbonate and CO_2	0.3 µmol/day
[25]	*Scenedesmus obliquus*	norgestrel	25 °C	150 mL in 250 mL flasks	150 RPM	3000 lux	12 h light/12 h dark	carbonate and CO_2	0.3 µmol/day
[25]	*Chlorella pyrenoidosa*	norgestrel	25 °C	150 mL in 250 mL flasks	150 RPM	3000 lux	12 h light/12 h dark	carbonate and CO_2	0.2 µmol/day
[26]	*Chlorella pyrenoidosa*	triclosan	22 °C	100 mL in 250 mL flasks	120 RPM	4000 lux	16 h light/8 h dark	acetate	104 mg/L/h
[27]	Consortium with *Chlorella vulgaris*	tetracycline	10.0–17.5 °C	14 L fed-batch HRAP	-	10 W PAR$\cdot m^{-2}$	24h light	atmospheric CO_2	Not calculated but continuous degradation
[28]	*Chlorococcum* sp.	α-endosulfan	22 °C	5 mL glass test tubes	-	2000 lux	24 h light	atmospheric CO_2	0.135 mg/L/day
[28]	*Scenedesmus* sp.	α-endosulfan	22 °C	5 mL glass test tubes	-	2000 lux	24 h light	atmospheric CO_2	0.140 mg/L/day
[29]	Review of 63 bioremediation cases out of 265 microalgae-heavy metal couples (other cases are adsorption experiments on dead cells with or without pretreatment)—pH from 4 to 9								From 0.02 to 1378 mg/g
[30]	*Selenastrum capricornutum*	PAHs + heavy metals	22 °C	100mL in 250 mL flasks	160 RPM	40 $\mu mol \cdot s^{-1} \cdot m^{-2}$	16 h light/8 h dark	atmospheric CO_2	Positive effect of heavy metals on PAHs removal

This study showed that out of the five strains of green algae (3 *Scenedesmus* sp. and 2 *Chlorella* sp.) exposed to 31 types of carbon substrates, two of the algae could effectively grow on two thirds of the substrates, thus highlighting the wide adaptation capacity of microalgae to various organic compounds. Tertiary wastewater can also be efficiently treated using microalgae. Three strains (*Scenedesmus obliquus*, *Chlorella vulgaris*, *Chlorella kessleri*) and a natural bloom were successfully grown on a treated wastewater submitted to pretreatment, primary settling, activated sludge and secondary settling [31]. The treated water could then meet the most restrictive currently imposed water regulation (European Directive 98/15/CE).

Anaerobic digestion is the most frequent process for the treatment of activated sludge produced during urban wastewater treatment. It produces a biogas and also high loads of nitrogen (in the form NH_4^+) and phosphorus (in the form PO_4^{3-}) in their liquid effluents. These nitrogen and phosphorus are a result of protein and organic matter transformation [32]. Therefore, wastewater treatment plants with anaerobic digestion processes have to include specific processes in order to treat this excess of nitrogen and phosphorus in their effluents or, otherwise, recirculate these effluents into the process finally accounting for about 20% of the total pollutant loading rate [33]. These higher nitrogen and phosphorus loading rates imply additional energy consumption for aeration for nitrification, and an extension of the plant size or the addition of chemicals for phosphorus removal [34]. Therefore, the ability of microalgae to treat these anaerobic digestion effluents has been studied. The color can be problematic for light penetration but after dilution, batch experiments have shown great nitrogen and phosphorus removal rates (8.5 mgN-NH_4^+/L/d for [35] and 99% reduction of nitrogen and phosphorus concentrations after 21 days for [36]). These results obtained in batch cultures need to be confirmed on continuous pilot-scale experiments where dilution can be more easily managed by adjusting the HRT.

2.2. Industrial or Agricultural Wastewater Treatment

Another research approach for the use of microalgae in wastewater treatment is to evaluate the ability of some microalgae strains to remove pollution from specific wastewaters (industrial or agricultural) which are poorly treated using the conventional activated sludge process.

Industrial wastewaters from molasses-based distilleries are generated in large volumes (15 L of effluent per liter of alcohol produced) with high Biological Oxygen Demand (BOD) and COD concentrations (average ranges of 40–50 gO_2/L and 80–100 gO_2/L, respectively) [37]. The COD of a pH-adjusted alcohol distillery wastewater (pH = 6.0–7.0) could be decreased from 20 to 1.5 gO_2/L in 3 days in a 50 L PBR using *Chlorella sorokiniana* (with a 95% decrease in nitrate, 77% in phosphate and 35% in sulfate) [38].

Numerous other industrial wastewaters can be treated using microalgae. For example, microalgae can also be efficient in treating wastewaters from the pulp and paper industry. A consortium from a stabilization pond was able to remove up to 58% of COD, 84% of color and 80% of adsorbable organic halogens (AOX) from a diluted pulp and paper industry wastewater [39]. The treatment of dairy wastewaters by microalgae has also been studied. The level of nitrate could be reduced by 90%, ammonia by 90%, phosphorus by 70% and COD by 60% in a dairy wastewater using *Chlamydomonas polypyrenoideum* in 10 days in 250 mL flasks [40]. Using an outdoor 40L PBR, *Chlorella* sp. was able to reach removal rates of 41.31, 6.58, and 2.74 mg/L/day for COD, total nitrogen (TN) and total phosphorus (TP) respectively when grown on a dairy wastewater [41]. Microalgae can also process oil refinery wastewaters: 97% reduction of ammonium, 69% reduction of TN and 90% reduction in TP have been obtained after three days of batch treatment [42]. Carpet wastewaters have been successfully processed by a consortium of 15 native microalgae isolated from these carpet wastewaters [43]. The process could efficiently reduce the pollution in 10 days in four 950 L raceway ponds, namely the COD (from 1 412 mgO_2/L to 106–183 mgO_2/L), the BOD (from 331–487 mgO_2/L to 2–21 mgO_2/L), the Total Kjeldahl Nitrogen (TKN, from 32.6–45.9 mg/L to 3.97–5.53 mg/L) and PO_4^{3-} (from 20.31–35.10 mg/L to 17.59–21.95 mg/L).

Acid mine drainage (AMD) is another type of wastewater that causes major environmental pollution in countries having historic or current mining industries. Pilot-scale experiments in 1 m^3 biological treatment test cells have been performed to treat AMD. A cyanobacterial-microbial consortium trapped in a substrate (containing powdered goat manure, wood chips, and soil) was used, forming a microbial mat [44]. Promising removal rates were observed for metals: 95% for Fe, 79%–97% for Cu, 84%–86% for Zn, 88% for Pb, 59%–83% for Co, 22%–62% for Ni, and 28%–45% for Mn.

Microalgae are also effective for treating agricultural wastewaters. For example, 97 strains were screened for treating 20-fold diluted swine manure wastewater [45]. Two of them were selected (growth rate of 0.536 and 0.433 d^{-1}) and validated in a two-step culture (first mixotrophic and then photoautotrophic). Olive mill wastewaters can also been treated using microalgae using *Scenedesmus* sp. for example, although phenolic compounds inhibited the depollution [46].

2.3. Specific Pollutant Degradation: Types and Mechanisms

Many studies have also investigated the potential of microalgae to degrade specific pollutants (PPCPS, EDCs, heavy metals, . . .). Typical examples will be reviewed in this section, and also gathered in Table 1. For example, a review) has listed a high number of micropollutants (over 25) for which degradation by microalgae has been studied [15]. For example, *p*-chlorophenol can be degraded at a rate of 10 mg/L/day by a consortium of two species (*Chlorella vulgaris* and *Coenochloris pyrenoidosa*) isolated from a water polluted with several aromatic pollutants [23]. It has been shown that the degradation of phenolic compounds is directly related to photosynthesis for *Scenedesmus obliquus* [24]. This green alga is capable of degrading phenol at a concentration of 1.5 mM (141 mg/L) and in some cases dichlorophenols when a carbon source and light are provided.

Hormones can also be transformed by microalgae [25]. In a 5 days experiment, *Scenedesmus obliquus* and *Chlorella pyrenoidosa* degraded 1.6 μM (0.5 mg/L) of progesterone (>95% reduction) or 1.6 μM (0.5 mg/L) of norgestrel (100% for *S. obliquus* and 60% for *C. pyrenoidosa*). Hormones were transformed by the microalgae via hydroxylation, hydrogenation and dehydrogenation [25]. *Chlorella pyrenoidosa* was also very efficient in degrading triclosan, a commonly used biocide [26]. The authors noted that *C. pyrenoidosa* could remove 50% of triclosan at 800 mg/L in one hour. Also, 77.2% of triclosan at 800 mg/L could be degraded within 4 days. Antibiotics can be processed using microalgae as well. For example, tetracycline, a veterinary antibiotic, could also be removed in a HRAP by photodegradation [27]. HRAP as compared to the conventional activated sludge process offers the additional advantage that the water is retained in much shallower ponds, thereby allowing better light penetration through the water column. Not only does the shallow depth promote better photon capture by the algal photosynthetic apparatus, it also enhances the photodegradation of photosensitive molecules.

Endocrine disruptors are another major class of micropollutants. *Chlorococcum* sp. and *Scenedesmus* sp. have been shown to degrade two endocrine disrupting chemicals, α-endosulfan (a cyclodiene insecticide) and to a lesser extent its oxidation product, endosulfan sulfate, through biosorption and then biotransformation [28]. However, at high concentrations, endocrine disruptors can be toxic to microalgae by impacting their photosynthetic activity. Indeed, the photosystem II energy fluxes of two green microalgae and two cyanobacteria were affected by 4-octylphenol, 4-nonylphenol and β-estradiol [47].

Heavy-metals are pollutants frequently encountered in industrial wastewater. The mechanism of heavy metal detoxification is mediated by class III metallothioneins (MtIII) in microalgae as detailed in a review focusing on the biological mechanisms of heavy metal accumulation and detoxification by microalgae [48]. They have also listed different examples of successful heavy metal bioremediation by microalgae, and the genus *Scenedesmus* (U^{6+}, Cu^{2+}, Cd^{2+}, Zn^{2+}) appears to be one of the most efficient species for bioremediation purposes. A more recent review proposed a well-documented list of heavy metal bioremediation by microalgae (Cd^{2+}, Co, Cr^{3+}, $Cr_2O_7{}^{2-}$, Cu^{2+}, Fe^{3+}, Hg^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}) through detoxification and also biosorption (heavy metal bindings on dead microalgae cells) [29].

Although there is an extended bibliography on the capabilities of algae to treat pollutants, there are still a lot of possible algae-pollutant(s) combinations that have to be explored. The great microalgae diversity needs indeed to be explored but the synergistic or antagonistic effects between pollutants needs also to be investigated. For example, a positive effect of heavy metals on the biodegradation of polycyclic aromatic hydrocarbons by *Selenastrum capricornutum* was revealed [30].

The literature on the toxicological effects of pollutants on microalgae is also very abundant since they are often used in ecotoxicological studies. The toxicity of various pollutant classes towards microalgae has been investigated: fossil fuels [49], PFCAs [50], organophosphorus compounds [51], organotin compounds [52], Polycyclic aromatic hydrocarbon (PAHs) [53], organochlorinated compounds [54], . . . These studies are crucial to gather information on the biological mechanisms that are impaired by the pollutant as well as to discover resistant microalgae strains.

3. Seeding Approaches for Efficient Bioconversion of Nutrients and Pollutants in Wastewater

Two strategies have been adopted for the inoculation of the process with microalgae: either select a well-suited microalgae strain through a screening method or allow a natural, indigenous consortium to evolve and become established in the water.

Screening methods are means to scan the biodiversity in order to determine the best microalgae strain for a specific application. It has been used only quite recently for treating a wastewater or removing a particular pollutant. The growth of 14 strains (from *Chlorella*, *Haematococcus*, *Scenedesmus*, *Chlamydomonas*, and *Chloroccum*) was tested on centrate (*i.e.*, the process water coming from the dewatering processes in a WWTP) [55]. All were able to grow and *Chlorella kessleri* showed the highest final biomass concentration (2.01 g/L). 100 local strains from Quebec (Canada) have been screened on 12-well plates using artificial medium (Bold's Basal Medium) and a real secondary effluent from a WWTP at 10 and 22 °C [56]. The authors used criteria such as biomass productivity, lipid content and nutrient removal. These techniques, combined with latest molecular biology advances can be very efficient for characterizing microalgae strains and selecting the ones with the highest potential. However, the results of these screenings cannot be directly applied on the large-scale. The robustness of the selected strain has to be tested first. Wastewaters are contaminated with various microorganisms that can be detrimental to the microalgae growth. More importantly, the environmental conditions are constantly varying (mainly climate and wastewater characteristics) and the microalgae have to withstand and adapt to sustain these changes.

The use of consortia to enhance the wastewater treatment is well documented. A review on the use of wastewater to bring the microalgae cultivation to economic viability cited numerous studies stating benefits of consortia, either bacterial-microalgal consortia or consortia between multiple microalgae strains [57]. The microbial interactions were well described in another review [16]. The concomitant release of carbon dioxide through bacterial heterotrophy and of oxygen through algal photosynthesis ensures a gaseous equilibrium in the water which benefits both the algal and bacterial flora. The synergistic effects between microalgae and bacteria in consortia on pollutant removal rates have been demonstrated [58]. Indeed, the best removal rates of aromatic pollutants (>85%) were recorded when both microalgae and bacteria were incubated under continuous lighting. These consortia are less subject to fluctuations in the environmental conditions and more resistant to contaminations. Moreover, microalgal-microbial flocs settled more easily than microalgal flocs, thereby creating a natural bioflocculation phenomenon which is very important for efficient harvesting of the biomass [59]. The treatment of aquaculture wastewater was tested using axenic and non-axenic culture of *Chlorella* sp., *Scendesmus* sp. and an indigenous consortium [60]. The authors found that microalgae were good at removing nitrogen but that bacteria were needed for removing organic pollutants.

Furthermore, microalgae growth can be promoted by bacteria [61]. Better chlorophyll *a* content was also obtained in the co-cultures of *Chlorella vulgaris* and *Bacillus licheniformis* than in the cultures of *Chlorella vulgaris* alone. Additionally, the best removal rates for NH_4^+ and TP were obtained for the co-cultures in comparison to single cultures. When treating urban polluted river with *Neochloris* *oleoabundans*, a natural bacterial consortium would develop itself with other native green microalgae and diatoms [62]. This consortium could more efficiently remove nitrogen and phosphate.

Consortia of multiple microalgae strains are also of great interest. The potential for wastewater treatment of these consortia have been investigated. For example, a microalgal consortium of more than 20 strains was shown to remove total organic carbon (TOC, 86%), TN (90%), Ammonia (89%), TP (70%) and Orthophosphates (76%) from sewage wastewater [63]. In addition, a natural microalgal consortium could efficiently reduce the nitrogen and phosphorus pollution of a centrate obtained from dewatering anaerobically digested municipal sludge [64]. These studies are showing that natural and indigenous consortia can be a good start for treating a specific wastewater.

4. Process Options

4.1. Cultivation

Microalgae can be cultivated either in open systems or closed systems (called PBRs). A focus will also be made on attached cultivation system (which are often implemented in open systems but can also be implemented closed systems) due to their potential for wastewater treatment.

4.1.1. Open Ponds and High-Rate Algal Ponds (HRAP)

Stabilization ponds have been used for the treatment of urban wastewater for a long time [65] but they need a lot of land to be efficient. To optimize the wastewater treatment process, high rate algal ponds (HRAPs) have been developed. HRAPs are shallow raceway-type open ponds of single or multiple loops where a water velocity of 0.15–0.3 m/s is obtained by the use of a paddlewheel [5]. Their depth is generally between 0.2–0.4 m (sometimes up to 1 m). CO_2 can be added in a sump of about 1.5 m depth. Compared to stabilization ponds, HRAPs reduce the surface needed by a factor of 5 [66] and the biomass productivity while achieving a three-fold improvement in yield from 10 ton/year/ha [67]. Despite needing 50 times more space land than activated sludge systems (the most common wastewater treatment technology), HRAPs' costs are significantly reduced compared to activated sludge systems: by a factor of two for the capital costs and by a factor of five for the operational costs [67].

4.1.2. Photobioreactors

PBRs are closed systems where microalgae can be cultivated in axenic and controlled conditions (good resistance to contaminations). Also, the volumetric productivities are significantly higher than in open systems. For example, better biomass and lipid productivities (+144% and +271% respectively), as well as N and P removal rates (+38% and +15% respectively) were found in a PBR than in flasks for the culture of *Chlamydomonas reinhardtii* on wastewater [68]. However, the cost is significantly higher for these closed systems than for open systems (more than 10 times higher for the same production capacity [69]).

PBRs have been designed in order to increase the volumetric productivity of microalgae cultures. Their most useful advantage for the microalgae industry is that they keep cultures axenic, allowing the growth of fragile strains that produces high-value molecules. Since a lot of microorganisms are naturally present in wastewaters, this precious advantage is lost when cultivating microalgae using wastewater. The gain in volumetric productivity does not counterbalance the high cost of PBRs in the case of urban or agricultural wastewater treatment. However, it could be of interest in cases where a high value molecule produced during the process can counterbalance the high cost of PBRs, or when the cost of wastewater treatment is not a problem (a wastewater containing highly hazardous pollutants for example).

4.1.3. Attached Microalgae Cultivation

In attached cultivation, microalgae are immobilized and fixed onto supporting materials. The supporting materials are immerged in the nutrients (wastewater in our particular case). An exhaustive review has been performed recently on the use of attached microalgae cultivation systems to treat wastewater [70]. They noticed that the few available comparisons of wastewater treatment performance gave comparable results for suspended and attached algal systems. Their main conclusion is that there is a need for more research studies on this attached cultivation systems on factors that affect algal growth, nutrient mass transport, species selection, algal–bacterial interactions, and upscaling of laboratory research. However, attached microalgal cultivation systems have been applied to a few wastewaters with promising results. For example, the use of benthic microalgae in an attached cultivation system treating dairy manure could reduce by 26% the land area required for an equivalent nitrogen uptake rate compared to the conventional corn/rye rotation process (23% for phosphorus) [71].

Biofilm rotating disks reactors are very promising and efficient attached cultivation systems for wastewater treatment using microalgae with good biomass productivity (Figure 1). Biomass productivities between 20–31 $g/m^2/day$ and nutrient reduction rates as high as 14.1 $g/m^2/day$ for nitrogen and 2.1 $g/m^2/day$ for phosphorus have been reported [72]. Similarly, an average biomass productivity of 20.1 ± 0.7 $g/m^2/day$ was obtained in a rotating biological contactor based PBR and the authors maintained the culture over a period of 21 weeks without re-inoculation [73]. These rotating biofilm disks reactors provide a better surface area to volume ratio as compared to HRAPs. The rotation through the partially filled culture vessel allows for an enhanced gas-to-biofilm mass transfer due to the higher time of exposure to the gaseous phase.

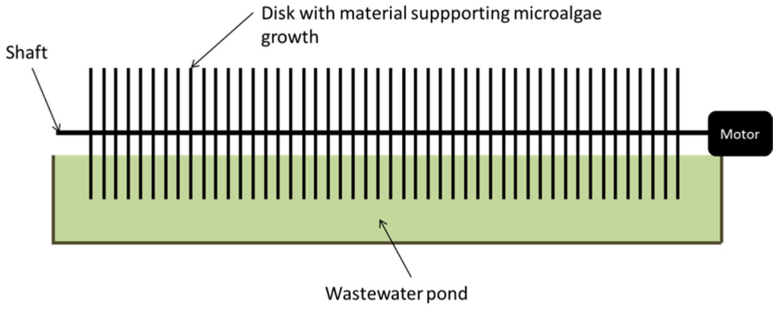

Figure 1. Schematic view of a microalgae biofilm rotating disks reactor used for wastewater treatment.

4.2. Harvesting the Biomass: Concentration and Dewatering

Microalgal cultures are typically extremely diluted, with biomass concentrations ranging from 0.3 to 5 g/L at best [74]. The recovery of essentially 99% of the water from the culture remains a major challenge for solids separation technology. Similarly to the wastewater treatment industry, two steps will be needed for harvesting the biomass. However contrary to activated sludge, microalgae do not form flocs naturally or settle as easily as activated sludge (their density is close to 1 [75]), therefore in most cases, the use of a coagulant or a flocculant will be needed for this first step. A second step called dewatering is then required to remove the extracellular water. The choice between centrifugation and filtration will be discussed.

4.2.1. First Step: Harvesting of the Biomass by Coagulation-Flocculation

Coagulation is the physico-chemical process which neutralizes the surface charge of particles in order to allow them to aggregate in flocs. To neutralize the surface charge, two methods can be applied: the use of chemicals (a coagulant) or the modification of the environment (for example a change in pH or application of an electric current). Flocculation is the process which aggregates small flocs into larger ones by the use of flocculants (generally polymeric substances). These polymers agglomerate the small flocs making bonds between them leading to larger flocs. The whole coagulation-flocculation process is often simplified by the term "flocculation". Flocculation is an efficient, low-cost harvesting technique with low energy requirements which is particularly adapted to microalgae culture [76]. Moreover, this is a method that may be easily scaled up by reproducing and adapting processes that are currently used in the treatment of wastewater. Various techniques are available: bioflocculation (use of bacteria to enhance the flocculation of microalgae, [59]), autoflocculation (induced by a pH increase, [77] or a pH decrease, [78]) and electroflocculation (use of an electric field, [79]). Although only a few studies exist on the use of flocculation to harvest microalgal biomass grown on wastewater [80,81], the technique should efficiently work for harvesting microalgae since it is frequently used in both the wastewater and microalgae industries. Decantation is commonly used after flocculation. However, flotation can also be used as an effective alternative depending on the density of the microalgae [82].

4.2.2. Second Step: Dewatering by Centrifugation or Filtration

Centrifugation is the most practical and common technique to harvest and concentrate a microalgal biomass [83]. It can be used directly on the microalgal biomass or after a flocculation step. However, it is quite expensive and more importantly it consumes a large amount of energy [3]. Therefore, its application to the dewatering of microalgal biomass grown on wastewater where large volumes need to be centrifuged should be dedicated to very specific applications: when the biomass (or part of it) can be valorized at a high value or when no other dewatering technique is available.

In this context, the use of filtration and especially dedicated, low-cost and low-energy consuming belt filter press could be a good option for dewatering the microalgal biomass grown on wastewater [3]. Indeed, belt filter press has been shown to use around six times less energy than centrifugation for the same results [84]. Investigation and research on this kind of dewatering techniques are still at a very early stage but should be encouraged considering these promising first results.

4.2.3. Biomass Valorization

Once the biomass has been harvested and the extracellular water removed, the dry weight concentration is generally around 15% to 25% [85]. The harvested biomass can be used in the agricultural sector, either as an animal feed or as a fertilizer. However, for these applications, microalgae biomass should not contain high concentration of persisting pollutants such as heavy metals or persisting organic pollutants that could be transferred into the animals or the soil. For those uses, drying would be required. Two techniques are particularly adapted since they do not denature the biomass: spray drying or solar drying. Spray-drying is very effective [86] but is very energy-intensive due to the use of a hot gas (nitrogen or air) to dry the biomass. Solar drying is very efficient and has a very low energy demand but requires a large surface area [87]. After drying the biomass can be used as animal feed [88] or as a fertilizer [89]. The wet biomass can also be used as a feedstock for composting. Composting has been successfully performed at the pilot-scale for macroalgae [90] and is envisaged to be as equally successful with microalgal biomass. Indeed green seaweed compost could effectively increase the growth and water resistance of tomato plants [91].

After drying, the biomass can also be used as feedstock for high-value molecules depending on the dominant microalgae strains in the wastewater grown biomass. As an example, cyanobacteria are a good source for pigment such as phycocyanin [92]; this water-soluble pigment is easily extracted from the biomass. Other high-value molecules such as omega 3 [93] or carotenoids [94] are very interesting on an economical point of view. However, the productivity of these molecules in wastewater grown microalgal biomass is likely to be very low since it needs specific conditions to be optimized (axenic cultures, optimum temperature and medium, *etc*...). Furthermore, strict regulations imposed by the food, pharmaceutical and cosmetic industries would probably impede the entry of wastewater-grown microalgal extracts on those markets.

Therefore, the most promising use of this biomass would be the energy market. For energy applications, drying should be avoided [3]. Wet processes have to be used to convert the biomass into energy. Lipids can be extracted through wet extraction techniques [95] and then converted through transesterification [74]. Promising results are coming from recent screenings, for example, strains were found to grow on wastewater and accumulate lipids at the same time (up to 23.7 mg/L/day) [56]. However, it is still difficult to adjust the microalgae metabolism to lipid accumulation in a microalgae culture growing on wastewater. High lipid productivities (at least over 200 mg/L/day) are needed for economic and energetic viability of microalgae to biofuel processes [3].

Direct wet conversion processes of the whole biomass such as anaerobic digestion or hydrothermal liquefaction (HTL) are therefore more adapted. Anaerobic digestion is the conversion of a biomass through dark fermentation into a biogas. It is efficient on microalgae with theoretical yields between 260 and 414 mL of CH_4/g of volatile solids [96]. Unfortunately, the economic value of biogas is too low at present (at most 1.33 €/Nm^3 of CH_4 using the highest electricity buy-back rate of Electricité de France). Nowadays, anaerobic digestion is not an economically profitable solution for microalgae biomass valorization. HTL is a thermochemical process which converts wet biomass into a biocrude (heavy oil, yields between 20 and 87% [97]), gas (>95% of CO_2 that can be recycled to the cultivation step [98]), some residual solids and an aqueous phase that contains large amount of nutrients. The potential to recycle the aqueous phase has been studied in order to reduce the cultivation costs and increase the overall sustainability of the process [98–100]. Growth can be inhibited at first but after an adaptation period, higher biomass productivities have been observed probably due to mixotrophic growth [98,99]. The biocrude can be directly burned in a boiler or upgraded through hydrotreating into a biofuel (a mix of naphta, gasoline and jetfuel [68]). HTL converts the whole biomass, therefore, there is no need for a monoclonal, monospecies, high l ipid-producing microalgae in comparison to the lipid extraction and conversion pathway. The HTL biomass conversion process is agnostic to the type of feed and hence, broadens the range of biomass and mixtures of organic material (including the activated sludge (bacteria) and algal biomass, as well as zooplankton generated from wastewater treatment) that can be used. The concept is represented on Figure 2. Additionally, HTL pathways are getting closer to economic viability with estimated biofuel production cost around 2.5 €/L of biofuel [101].

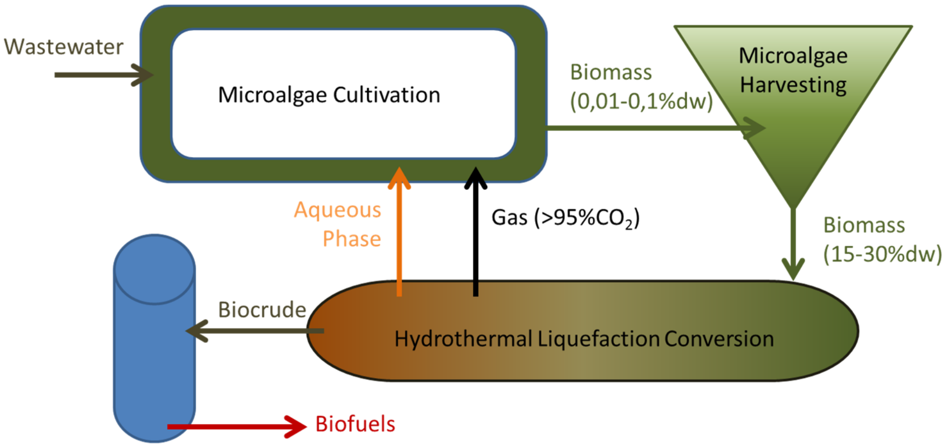

Figure 2. Typical pathway for biofuel generation using wastewater, microalgae and hydrothermal liquefaction.

Microalgal biomass grown on wastewater may not conform to chemical and biological safety regulations to be reused in the raw state, for example, in instances when the biochemical composition does not meet the desired criteria, or when there is too much fouling by pathogenic organisms or by toxic pollutants. In such cases, conversion of the low-grade biomass into biochar through pyrolysis becomes an interesting value-adding alternative option. Depending on the composition of the biochar, it can then be used as soil amendment [102] with reduced risk of leaching of toxic material such as heavy metals, since the pyrolysis process aids in capturing the metals in the solid matrix [103].

5. Future Research and Development Needs

Further research in the use of microalgae for performing wastewater treatment is still needed. Table 2 lists the actual and validated knowledge against the challenges that the microalgae wastewater treatment industry will face. These challenges are discussed in this section.

Table 2. Validated knowledge and future challenges for wastewater treatment using microalgae.

Validated Knowledge	Future Challenges
Ability to treat different wastewaters or specific pollutants	Technical feasibility at large-scale (HRT and area needed) Economic feasibility
Screening several microalgae strains for their degradation of a pollutant or their treatment of a wastewater	High-throughput screening methodology
Natural consortium for the treatment of a specific wastewater or pollutant	Artificial specifically designed consortium
HRAPs for the treatment of urban wastewater	Optimized systems with reduced footprint and HRT
Long-term operation with variation in treatment efficiency	Contaminations control and consortium protection
Research studies are dependent on the availability of real wastewater	Design a representative and easy-to-make synthetic wastewater

5.1. High-Throughput Screening Methodology

The biodiversity of microalgae is immense and estimated to be between 200,000 and several millions compared with about 250,000 for higher plants [86]. Only a few of them has been described (less than 10,000 [104,105]). There is thus a huge potential within this broad biodiversity for industrial applications. High-throughput screening methodologies are needed in order to investigate the microalgal biodiversity. Screenings of a dozen of microalgae strains are quite common now (13 for [106]; 14 for [107]) and their results are very promising for wastewater treatment using microalgae. But high-throughput methodology using cultivation on well plates and adapted analytical equipment are needed for rapid selection of promising strains. A screening methodology based on 12-well plates was applied on more than 100 strains for wastewater treatment and biodiesel production [55]. They found strains with relatively good biomass productivity (around twice less than in an optimized synthetic medium) and excellent lipid productivity (only 20% less than in the optimized synthetic medium). The laboratory equipment has also to be adapted for allowing high-throughput screening. For example, a microplate-based photobioreactor was recently developed for screening and medium optimization purposes [108]. This approach needs to be further developed since the applications are numerous (specific wastewater treatment, specific pollutant reduction, wastewater treatment in specific climatic conditions . . .).

5.2. Artificial Specifically Designed Consortium

Results of large high-throughput screening could help in designing efficient microalgae consortium for specific wastewater characteristics. These artificial consortia need to be tested and their efficiency evaluated. Synergistic or antagonistic effects could be observed. Depending on the variation of the environmental parameters (principally the wastewater characteristics and the meteorological conditions), the consortium will naturally evolve and adapt to these changes. For example, different compositions of the microalgae community were found depending on the season [44]. The challenge is to develop a robust consortium with a broad pollutant affinity that is tolerant to subtle and drastic changes in the wastewater composition. However, some pure axenic cultivation of each strain used in the consortium should also be kept ready in case of a major loss of biomass.

5.3. Process Intensification

In wastewater treatment using microalgae, the most important operating parameter is the HRT which is directly related to the growth rate and pollutants removal rates. It drives the cost and energy consumption per cubic meter of treated water as well as the footprint of the installation. Reducing the HRT is crucial for the development of microalgae based WWTPs. This could be done by process intensification and use of innovative systems, such as attached microalgae cultivation systems, combined with the selection of fast-growing pollution microalgal scavengers.

5.4. Contaminations Control and Consortium Protection

Contaminations are a great threat for microalgae cultivation, especially when microalgae are grown in raceways or HRAPs [5]. Grazing by herbivorous protozoa and zooplankton can reduce algal concentrations drastically in just a few days. Zooplankton (rotifers and cladocerans) at high concentrations can destroy 90% of the algal biomass in just two days [109]. Daphnia [110], viruses [111], bacteria [112] and even other microalgae can be a big constraint for mass cultivation of microalgae [113].

Physical (filtration), chemical (use of herbicides) methods or change of the operating conditions (light, temperature, pH) have been investigated as means to control contamination but with few industrial applications. Further research studies are needed to control these contaminations [114]. Possible synergistic effect in consortium should be investigated such as protection by a specific species, symbiosis.

5.5. Synthetic Wastewater

Real wastewaters are not always accessible and convenient for scientific studies. Handling them is not an easy task because it has to be rapidly used at the risk of having its composition vary. Therefore, synthetic wastewaters are often used in this kind of studies but they are often not representative of real wastewaters. Several research teams use classic nutrients to simulate pollutants or even known medium (like BG11 for [115]). But the bioavailability of these commercial nutrients is far less superior to pollutants found in wastewaters. There is a need for an easy-to-make (from chemicals that are accessible from common chemicals suppliers for laboratories) and representative synthetic wastewater. The difficulty lies in the chemical complexity of wastewaters where organic and mineral, soluble and particulate coexist for nearly every pollutant (carbon, nitrogen, phosphorus for the principal ones).

6. Conclusions

Growing microalgae on wastewater offers new insights for the microalgae industry as well as the wastewater treatment industry. The use of wastewaters for cultivating microalgae is necessary in order to reduce the cost of microalgae production. This is a prerequisite for microalgae to enter the energy market through biofuels. The wastewater treatment industry is facing challenges (such as micropollutants fate) that induce the development of other alternatives. Microalgae-related processes can be an interesting alternative to the conventional activated sludge process. Despite these two opportunities, many research and development challenges have still to be overcome in order to benefit from the full potential of the combination of microalgae production and wastewater treatment, namely in the development of robust, productive wastewater-adapted microalgal species, and in the improvement and innovation of cultivation and downstream processing systems which will allow for better growth, harvesting and conversion of the algal biomass.

Acknowledgments: The authors are very grateful to the editor for offering the opportunity to publish a paper in the special issue "Algae Fuel 2015". Part of this research was funded by the New Energy Technologies program of CEA.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

AOX	Adsorbable Organic Halogen
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
EDC	Endocrine Disrupting Compound
HRAP	High-rate algal pond
HRT	Hydraulic Retention Time
HTL	Hydrothermal Liquefaction
PBR	Photobioreactor
PAH	Polycyclic Aromatic Hydrocarbon
PFCA	Perfluorinated Carboxylic Acid
PPCP	Pharmaceutical and Personal Care Product
TKN	Total Kjeldahl Nitrogen
TN	Total Nitrogen
TOC	Total Organic Carbon
TP	Total Phosphorus
WWTP	Wastewater Treatment Plant

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