




SPECIAL ISSUE ARTICLE

Wastewater treatment by microalgae

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Abstract

The growth of the world's population increases the demand for fresh water, food, energy, and technology, which in turn leads to increasing amount of wastewater, produced both by domestic and industrial sources. These different wastewaters contain a wide variety of organic and inorganic compounds which can cause tremendous environmental problems if released untreated. Traditional treatment systems are usually expensive, energy demanding and are often still incapable of solving all challenges presented by the produced wastewaters. Microalgae are promising candidates for wastewater reclamation as they are capable of reducing the amount of nitrogen and phosphate as well as other toxic compounds including heavy metals or pharmaceuticals. Compared to the traditional systems, photosynthetic microalgae require less energy input since they use sunlight as their energy source, and at the same time lower the carbon footprint of the overall reclamation process. This mini-review focuses on recent advances in wastewater reclamation using microalgae. The most common microalgal strains used for this purpose are described as well as the challenges of using wastewater from different origins. We also describe the impact of climate with a particular focus on a Nordic climate.

1 | INTRODUCTION

Since the industrial revolution, water pollution has increasingly become a concern to the public and societal authorities. With the development of the industrial world and a growing population, the demands for freshwater are drastically increasing. The global water demand for agriculture, industry, and municipalities is expected to rise by 20–30% by 2050 (Boretti & Rosa, 2019). One of the consequences of this increase is the generation of larger quantities and varieties of wastewaters, contaminated with a wide

range and concentrations of chemicals. Besides utilizing several tons of pesticides per year, the agricultural sector also produces considerable amounts of organic waste (Bockstaller et al., 2009), and is one of the most significant sources of water contamination. These pollutants can have dire consequences for the environment and for ecosystems into which they are discharged. Some pollutants, mainly those of organic nature, are generally degradable (either naturally or with the help of microorganisms) and therefore do not cause major problems for the environment. However, some persistent organic pollutants (POPs), typically present in trace amounts, are known to bioaccumulate and exert toxic chronic health effects on animals (Schwarzenbach et al., 2010). Chemical

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contaminants such as polycyclic aromatic hydrocarbons (PAH), dyes, pharmaceuticals, hydrocarbons, hexachlorocyclohexanes, perfluorocarbons, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), bisphenols, or phthalic acid esters (PAEs), are well-known POPs with high impact on the general health of ecosystems and humans (Dong et al., 2018; Klavarioti et al., 2009; Martínez-Huitle & Brillas, 2009; Noszczyńska & Piotrowska-Seget, 2018; Wang et al., 2015; Yunker et al., 2002). Thus, plenty of attention has been focused on the development of chemical removal techniques but unfortunately, they are inefficient when implemented on a large scale. Other pollutants, including heavy metals, can also be problematic as they are nondegradable and can consequentially accumulate in the food chain, thus, posing serious threats to animal and human health (Arceivala & Asolekar, 2006).

With increasing amounts of wastewaters produced and the need to supply freshwater to a growing population, a better understanding of the extent of the challenges ahead has been built. Rout and coworkers (Rout et al., 2020) identified more than 50 new “emerging contaminants” (ECs), all of which with anthropogenic origin that require surveillance and attention. The authors review technologies for the removal of ECs and conclude that both activated sludges and membrane bioreactors are effective for this purpose. As our understanding of the challenges and need for treatment of wastewaters from all sources grows, novel wastewater treatment technologies have been developed with the goals of improving efficiency, reducing costs, and reducing the carbon-footprint of large-scale treatment plants. Some of the most common processes include coagulation/flocculation, precipitation, filtration, oxidation, ion-exchange, solvent extraction, or electrochemical treatment (Crini, 2019), as well as ozonation, adsorption, or membrane bioreactors (Rout et al., 2020). A less conventional and more recently developed technique relies on the use of specific types of biomass to remove pollutants. Microalgae possess the most interesting and most-used type of alternative biomass in current wastewater treatment applications (Acién et al., 2016). They are a diverse group of unicellular photosynthetic organisms, which can grow and even thrive in a wide variety of conditions, including in different types of wastewater. The complex variety of functional groups present in the cell wall of algae allows the binding of pollutants to the cell surface via a phenomenon called biosorption (Michalak et al., 2013; Spain et al., 2021). This rapid and reversible process is independent on the microalgae's metabolism and can thus be performed on living or dead biomass (Michalak et al., 2013). Other compounds are taken up by living cells and are biodegraded. In order to fully comprehend the mechanisms of pollutant removal, the cultivation and growth conditions of microalgae in wastewater must initially be understood (Ubando, 2021).

In this article, the usage of microalgae to treat different types of wastewater is summarized as well as the challenges involved in this kind of reclamation, and the most efficient ways of treating the algae based on the type of reactor systems used.

2 | TYPES OF WASTEWATER

2.1 | Municipal wastewater

Total municipal wastewater production increases proportionally to the growth of the human population. According to a press release from the “Federal Office of Statistics” (Destatis) in Germany, the daily amount of water used per person was around 126 L in 2009. Eighty percent of this was used for personal hygiene and toilet. This results in a need of roughly 3.2 billion m³ of wastewater per year to be cleaned in Germany alone (Schleich & Hillenbrand, 2009), while the amount of water used in the United States is almost twice as much as in European countries. Municipal wastewaters are primarily constituted by water used in households. Thus, their contamination is mostly due to easily degradable organic matter. However, several POPs are also present in micro-concentrations (amounts below the mg/L range). Chemicals within this range of concentrations are often referred to as micropollutants. These micropollutants are, for the most part, pharmaceuticals, hormones, surfactants, plasticizers, flame retardants, and pesticides (Luo et al., 2014). Traditional municipal wastewater treatment is performed in a multi-step system, consisting of a primary (sedimentation/settling for removal of coarse suspended solids), secondary (to reduce biodegradable organic matter dissolved or in colloidal suspension), and tertiary treatment (typically to remove N, P, and potentially harmful microorganisms). Based on the composition of the wastewater, additional steps might be necessary before and after the treatment. Therefore, conventional treatment systems are quite expensive resulting into high costs for water usage per person. Municipal wastewater in general contains high amounts of nitrogen, phosphorus, and organic carbon, but also a large variety of other contaminants such as pharmaceuticals or heavy metals due to mixture with extraneous water and rainfall water (see Table 1). Despite these contaminants, the main focus of cleaning municipal wastewater is the removal of organic matter, nitrogen, and phosphorus as they are one of the main causes of eutrophication.

2.2 | Agricultural wastewater

The growth of the world's population leads not only to larger amounts of municipal wastewater, but also to the need for more food production. Based on the “Proceedings of the UN-Water project in Safe Use of Wastewater in Agriculture” the amount of drinking water required to produce the daily food needed by one person is between 2000 and 5000 L. The agriculture sector is not only the largest consumer of drinking water, but also the largest producer of wastewater (Mateo-Sagasta et al., 2013). Livestock produces “runoff”-water, which is extremely rich in phosphorus and nitrogen (in form of ammonia) (see Table 1). In addition to nutrients, agricultural wastewater can contain a variety of other components such as pesticides or herbicides as well as antibiotics or any other pharmaceutical given to animals. While the manure that comes with the wastewater is often used as fertilizer, the

TABLE 1 Content of total nitrogen (TN) and total phosphorus (TP) found in different wastewaters

| Type of wastewater | Origin | TN (mg/L) | TP (mg/L) | Microalgae used | Reference |
|-------------------------|--------------------------|---------------|--|--|--------------------------|
| Municipal wastewater | Sewage | 27.7 ± 0.11 | 1.59 ± 0.03 | <i>Chlorella vulgaris</i> sp. ZTY4, <i>S. sp.</i> ZTY2, <i>S. sp.</i> ZTY3 | (Zhang et al., 2013) |
| | Secondary treated sewage | 6.3–130 | 0.04–0.88 | <i>Botryococcus braunii</i> | (Sawayama et al., 1992) |
| Agricultural wastewater | Animal waste | 2600 | 120 | <i>Chlorella vulgaris</i> (UTEX 265), <i>Euglena gracilis</i> (SAG 1224) | (Park et al., 2009) |
| | Dairy | 10.66–86.65 | 2.52–9.50 | <i>Scenedesmus quadricauda</i> , <i>Tetraselmis suecica</i> | (Daneshvar et al., 2019) |
| | Dairy | 284.75 ± 7.13 | 77.94 ± 3.05 | <i>Diplosphaera</i> sp. MM1 | (Liu et al., 2016) |
| | Fish processing | 46–50 | 2.7–10.7 | <i>Oocystis</i> sp. | Riaño et al. 2011 |
| | Piggery | 289.89 ± 3.12 | 23.80 ± 2.13 | <i>Chlorella vulgaris</i> (FACHB-8), <i>Chlorella pyrenoidosa</i> (FACHB-5), <i>Haematococcus pluvialis</i> (FACHB-872), <i>Scenedesmus obliquus</i> (FACHB-12), <i>Spirulina platensis</i> (FACHB-440), <i>Porphyridium cruentum</i> (FACHB-1118) | (Wang et al., 2016) |
| | Poultry (diluted) | 76–152 | 6–12 | <i>Chlorella minutissima</i> , <i>Chlorella sorokiniana</i> , <i>Scenedesmus bijuga</i> | (Singh et al., 2011) |
| Swine | 86.4 ± 11 | 20.2 ± 1 | <i>Scenedesmus acutus</i> (P-F-6), <i>Scenedesmus spinosus</i> (P-F-77), <i>Scenedesmus quadricauda</i> (P-F-70) | (Kim et al., 2007) | |
| Industrial wastewater | Paper Mill | 9.93 ± 1.87 | 30.25 ± 3.28 | Mixed culture containing two <i>Scenedesmus</i> sp. | (Usha et al., 2016) |
| | Tannery | 0.93 ± 0.01 | 6.01 ± 0.05 | <i>Chlorella vulgaris</i> | (Das et al., 2017) |
| | Textile ^a | 360 | 4.7 | <i>Chlorella pyrenoidosa</i> | (Pathak et al., n.d.) |
| | Winery | 7.67 ± 0.13 | 21.78 ± 0.25 | <i>Diplosphaera</i> sp. MM1 | (Liu et al., 2016) |

^aAlso contains heavy metals.

water itself enters conventional wastewater treatment. Nutrients from the manure that are not taken up by crops will enter the water body, resulting in a high risk of eutrophication. Therefore, proper treatment of agriculture waste and wastewater is mandatory.

2.3 | Industrial wastewater

Industrial wastewaters come in a wide variety. Water consumption and contamination are particularly high within the paper, cloth, and plywood industries. The C&A foundation reported that their cloth supply chain requires between 5.7 and 9.7 billion m³ of water per year (Franke & Mathews, 2013). The lack of reliable data about water consumption by the paper industry, makes an accurate estimation of the total used water almost impossible, however, the total water footprint of one A4 sheet of paper was estimated to be between 13–20 L (Hoekstra, 2015). These wastewaters usually contain much smaller amounts of nitrogen and phosphorus, but higher concentrations of different carbon sources. Though less than the two previous examples, the plywood industry is also one of the more significant water pollution sources. Wastewaters from this industry are characterized

by a high concentration of solids, organic matter, and nitrogen, as well as toxic chemicals, such as phenol or heavy metals like copper, cadmium, or lead (Heng et al., 2004; Mukherjee et al., 2007; Muñoz et al., 2006; Prasad et al., 2019). The impact of this industry in the total amount of wastewaters produced is also on the rise, particularly in rich countries, where household and office furniture are regularly replaced. These are examples of the primary industrial consumers due to the sheer amount of water they use.

Globally, industrial wastewaters are the primary source for POPs as well as heavy metal contamination of the aquifers. The real impact that these activities have on ecosystems is very hard to determine. Before wastewater treatment, the load and nature of pollution generated by the industrial sector can be categorized by the type of activity: the same type of industry will roughly generate the same amount of wastewater with the same profile of contaminants. However, often inefficient treatments (or even no treatments) are applied, due to the lack of regulatory frameworks and their respective legal enforcements. Thus, examples can be found where industrial wastewaters are almost 100% recycled and reused, as well as being discharged into natural aquifers without any treatment whatsoever.

3 | WASTEWATER RECLAMATION BY MICROALGAE

The use of algae for wastewater reclamation has been studied since the 1960s and is currently trending even more for circular blue bioeconomy approaches. Microalgae have been applied to the treatment of wastewater due to their ability to utilize organic and inorganic carbon, nitrogen, and phosphorus, while simultaneously accumulating biomass and reducing N, P, and chemical oxygen demand (COD) in the wastewater (Ferro, Gentili, et al., 2018; Sawayama et al., 1992; Zhang et al., 2013). Several microalgae are even capable of removing heavy metals from industrial wastewater via biosorption. Combined removal of nitrogen, phosphorus, and heavy metals was shown to be possible using industrial wastewater as microalgal growth medium (Chinnasamy et al., 2010). Microalgae are not yet used on a large scale in the treatment of wastewater, however, notable examples of commercial systems exist. The US-based company *Algae Systems LLC* have a floating photo-bioreactor (PBR) designed to operate with environmental light and CO₂ to remove nutrients downstream of their source (Novoveská et al., 2016). *Algal Enterprises* (Australia) have a solution destined for a wide range of wastewaters which consists of a closed PBR system coupled to an anaerobic digester to produce biogas (Montingelli et al., 2015). *RNEW* technology of *Microbio Engineering* (US) uses open raceway ponds enriched with CO₂ for removal of N and P from municipal wastewater and produce biomass for biofuels (Craggs et al., 2011). These are some examples of commercial systems based on suspended microalgae cultures. Immobilized systems are also commercially available, however for the treatment of lower volumes. Companies like *HydroMentia*, *OneWater*, and *Gross-Wen Technologies* all commercialize wastewater treatment solutions based on immobilized microalgae (or a combination microalgae/bacteria) with various configurations (Wollmann et al., 2019). However, a huge number of different factors are affecting microalgal growth and the removal of nutrients from wastewater (Al Ketife et al., 2019).

3.1 | Nutrient concentration, pH value, color of wastewater

Concentration and availability of nitrogen or phosphorus impact the microalgal metabolism in specific ways. Many microalgae will continue to produce biomass under N-starvation, but instead of producing proteins, they will increase the amount of lipids and/or carbohydrates (Gojkovic et al., 2020). A dependency between the uptake of N and P has been observed. Nitrogen uptake is enhanced by the presence of phosphorus, and a surplus of phosphorus can be taken up by the algae (luxury uptake) in the presence of N (Bougaran et al., 2010). The pH value is important for the availability of nutrients (Posadas et al., 2015). It affects, for example, the solubility of ammonium or phosphate as well as the formation of precipitates. High pH values of 9 or higher can induce the formation of calcium phosphate, which is unavailable for microalgae (Laliberté

et al., 1997). The pH values also determine the charge of functional groups at the microalgal surface and therefore the ability to bind, for example, heavy metal ions.

The color of wastewater plays an important role in wastewater cleaning as it affects microalgal growth and nutrient removal (Marcilhac et al., 2014). Dark brownish-grayish and opaque wastewater (e.g. livestock wastewater, pulp, and paper) show high absorbances of light, limiting photosynthetic light absorption of microalgae and therefore their growth and nutrient uptake (Lee & Lee, 2001; Lee & Shoda, 2008).

3.2 | Commonly used microalgal strains

Although many microalgal strains have the potential to remove pollutants from wastewater, a selective few seem to be more frequently used than others, most likely due to their rapid growth rate, low production cost and high tolerance to extreme, and potentially stressful environmental conditions (i.e. low or high temperature, pH, light intensity).

The genus *Chlorella* is composed of unicellular, nonmotile spherical green microalgae of 2–10 µm in diameter. *Chlorella* is currently the best-studied and most cultivated microalgae worldwide, mostly due to its high photosynthetic efficiency and high nutritional value (Masojidek & Torzillo, 2008). On many occasions, *Chlorella* species have shown their high biosorption capacities and efficiency to remove pollutants from various aqueous solutions. *Chlorella vulgaris* displayed a removal efficiency of total phosphorous (TP) content of around 85% and a removal efficiency of total nitrogen (TN) content of around 89% (Wang et al., 2015). *Chlorella minutissima* and *Chlorella sorokiniana* were shown to remove up to 41 and 34% of the TN and up to 70% of the TP (Singh et al., 2011). Immobilized *Chlorella* sp. cells were able to achieve a removal of 90% of not only phosphate, but also ammonium and nitrate from synthetic wastewater (Shi et al., 2007). Other strains of *Chlorella* have shown to be efficient biosorbents of not only nitrogen and phosphorous, but also of metal ions such as Al³⁺, Ca²⁺, Fe²⁺, Mg²⁺, Mn²⁺, and Zn²⁺. *Chlorella* sp. was able to remove up to 100% of Fe²⁺ and Mn²⁺, 70–87% of Al³⁺, and 80–98% of Mg²⁺ from four different types of wastewater (Wang et al., 2010). Overall, *Chlorella* is one the most commonly used genus of microalgae for wastewater treatment as it can adapt well to many types of wastewaters and is extremely efficient at removing a variety of pollutants (Wu et al., 2019).

The genus *Scenedesmus* contains colonial green microalgae, which can commonly be found in groups of four or eight cells, arranged side by side or within the same mother wall. *Scenedesmus* is primarily found in freshwater lakes and rivers, where it usually dominates over other species of microalgae (Kim et al., 2007). As well as being commonly used in the food and pharmaceutical industries, *Scenedesmus* species are well studied for their biosorption capacities for applications in wastewater treatment. *Scenedesmus* sp. can remove 87% of TN, 83% of TP, and 92% of suspended solids from swine urine (Kim et al., 2007; Prandini et al., 2016).

Scenedesmus quadricauda was used to treat wastewater from the dairy industry, which can contain a large range of different compounds such as lactose, fats, washing detergents, nutrients, sanitizing agents, and abnormally high quantities of nitrogen and phosphorous (Daneshvar et al., 2019). *Scenedesmus quadricauda* was able to remove approximately 92% of the TN and 71% of PO_4^{3-} , far more than *Tetraselmis suecica*, whose removal efficiency of PO_4^{3-} was 41% lower. *Scenedesmus* species were further used to remove heavy metals (chromium [VI], cadmium [II], and copper [II]) either as single metal species or as a mixture of two or three metals from artificial wastewater. Although the algae could remove the metals when present as single species (with a removal efficiency of 24% for cadmium alone), the removal was far more efficient when the metals were present in mixtures (Cd + Cr: 65%; Cu + Cd: 59%) (Pena-Castro et al., 2004). This information is very important to take into account when treating wastewater that contains a variety of heavy metals that have different interactions with one another and, by consequence, with the algal biomass.

Although it is not that common, the slow-growing algae *Botryococcus braunii* is a promising strain for wastewater treatment and biomass valorization. This green freshwater microalgae lives mainly in colonies that float in large masses on the top of the water (Borowitzka, 2018). This algae is well known for its capacity to produce large quantities of long-chain hydrocarbons and lipids, which can be used to produce biofuel. As seen in Table 1, *B. braunii* is also used for the removal of nitrogen and phosphorous from wastewater. This alga thrives in wastewater that has high TN and TP values, and can not only remove nitrogen and phosphorous from the wastewater, but in turn use it to produce more hydrocarbons (An et al., 2003).

Other microalgal strains are equally interesting for wastewater treatment due to their ability to remove heavy metals and pharmaceuticals. *Desmodesmus* sp. and *Heterochlorella* sp. were used to remove copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) from their growing environment. *Desmodesmus* sp. was especially efficient at removing iron (up to 86% after 16 days). *Heterochlorella* sp. was more efficient at removing Mn, with an adsorption percentage of 84% at 10 mg L^{-1} . The removal of the ion happened both by adsorption and by uptake within the cell (up to 99% was accumulated inside the cell) (Abinandan et al., 2019).

The microalgae *Nannochloris* sp. is able to remove triclosan, an antimicrobial, from the growth medium during light/dark cycles and even in total darkness. 100% of the triclosan was removed when the algae was grown for 7 days in the light/dark cycles and 97% was removed when the algae was grown in dark conditions for 14 days (Bai & Acharya, 2016). The green microalgae *Selenastrum capricornutum* was shown to be effective at removing hormones such as 17β -estradiol and 17α -ethinylestradiol from wastewater. Hormones in wastewater have become a major concern as they are endocrine-disrupting compounds, which can have serious negative effects on human and animal health (Roudbari & Rezakazemi, 2018). The algae could remove 17β -estradiol and 17α -ethinylestradiol with a removal efficiency of 88–100% and 60–95%, respectively (Hom-Diaz et al., 2015).

4 | CHALLENGES FOR ALGAL-BASED WASTEWATER RECLAMATION

4.1 | Climate

Biotic (e.g. algal pathogens such as bacteria, fungi, and viruses; indigenous photosynthetic microbial community) as well as abiotic (e.g. light, temperature, pH, salinity) factors have the strongest impact on algal-based wastewater treatment (Christenson & Sims, 2011; Hwang et al., 2016; Park et al., 2011; Posadas et al., 2017). Environmental parameters such as light intensity, light period, and temperature play a key role in influencing the performance and cost effectiveness of algal treatment systems. Importantly, the impact of these factors is also interlinked (Hwang et al., 2016; Mohsenpour et al., 2021). Therefore, the location of the algal-based wastewater reclamation system plays a key-role for cost-efficiency of the process. Seasonal changes in day length and light intensities are location-specific and have an important role on algal communities and their macromolecular structures (Ferro et al., 2020; Posadas et al., 2017). The employment of native strains is advantageous in exploiting the inherent suitability to specific environmental conditions, an important consideration in achieving maximal effectiveness with minimal energy input (low light and low temperature). Screening of culture collections (e.g. HAMBI [University of Helsinki Culture Collection, <http://www.helsinki.fi/hambi/>] (Lynch et al., 2015) and/or the MicroBioRefine collection of local Nordic strains (Ferro, Gentili, & Funk, 2018) resulted in the identification of native isolates with high potential for integrated wastewater treatment and biofuel production. Recently, the potential of indigenous Nordic algal strains has been documented on water remediation, as feedstock for biofuel and high-value compounds in either lab or pilot scale under Nordic conditions (Cheregi et al., 2019). Algal strains from Sweden (Ferro, Gentili, et al., 2018; Ferro, Gorzsás, et al., 2018; Lindberg et al., 2021), from Finland (Jämsä et al., 2017; Lynch et al., 2015), and from Quebec, Canada (Abdelaziz et al., 2014) have been employed for their ability to treat municipal wastewater in cold climate. Even Arctic and Antarctic strains have been screened for their potential use in outdoor wastewater treatment systems at cold temperatures. A promising Polar cyanobacterial strain has been identified with satisfactory growth rates and an advantage for harvesting due to the formation of floc-aggregates. However, at warmer temperatures, green algae outcompeted the polar strains (Tang et al., 1997). A sequential combination using cold and moderate strains could potentially extend the period for outdoor wastewater treatment applications.

Nordic isolates have been shown not only to remove N and P from municipal wastewater, but also pharmaceuticals. Swedish microalgae strains showed promising removal of several pharmaceuticals with satisfactory growth performances in lab-scale photobioreactors (PBRs) (Gojkovic et al., 2019; Lindberg et al., 2021). A pilot-scale algal cultivation achieved partial or total removal of pharmaceuticals from urban wastewater with a mixed population of wild freshwater green algae under natural light and with the addition of flue gases (Gentili & Fick, 2017; Lindberg et al., 2021).

Although cold-adapted algal strains demonstrated biomass accumulation and wastewater remediation in cold climate, there is still concern about outdoor performance in the winter season. At cold temperatures algal metabolism slows down, additionally, the low light availability in winter places a serious threat (Ferro, Gorzšás, et al., 2018). Hence, the integration of algal cultivation into greenhouse infrastructures presents an opportunity in Nordic countries to operate year-around. Several pilot-scale algal-based wastewater treatment systems are covered by greenhouses to ensure essential growth conditions in cold regions (Ferro et al., 2020; Gentili & Fick, 2017; Salazar et al., 2020). Integrating algae cultivation into power plants is another opportunity to be considered to cope with the harsh winter conditions. In Iceland, for example, a local microalga was cultivated in a geothermal brine, where heating was provided by geothermal electricity, enriched with low percentages of Walne growth medium to supply additional nutrients (Cheregi et al., 2019).

Using algal-based wastewater reclamation in hot climates has its own challenges (AlMamani & Örmeci, 2020; Posadas et al., 2017). A temperature increase above the optimal growth conditions drastically decreased growth and productivity (AlMamani & Örmeci, 2020; Park et al., 2011). Moreover, prolonged exposures to excess light intensities and direct sunlight can cause photodamage and photoinhibition resulting in the reduction of algal culture growth and eventually induce cell death (Christenson & Sims, 2011; Posadas et al., 2017). It is important to consider that in warm climate evaporation raises, which leads to increased salinity causing osmotic and cellular ionic stress (Hwang et al., 2016).

4.2 | Harvesting

Promising downstream process applications (e.g. harvesting) must ensure a cost-efficient wastewater treatment for large-scale operation, posing still one of the major bottlenecks for an efficient, sustainable, and low-cost operation (Christenson & Sims, 2011; De Godos et al., 2017; Hwang et al., 2016; Lavrinovičs & Juhna, 2017; Mohsenpour et al., 2021). The natural buoyancy of many microalgae restricts their sedimentation just by gravity. According to life cycle analysis, just the recovery of microalgae from the liquid stream accounts for about 20–30% of the total costs (Jacob-Lopes et al., 2015; (De Godos et al., 2017; Lavrinovičs & Juhna, 2017; Sukačová et al., 2020). Although several harvesting techniques have already been developed (Christenson & Sims, 2011; De Godos et al., 2017; Lavrinovičs & Juhna, 2017), only low-cost methods will make algal systems economically feasible. The harvesting technique has impact on system design and operation for both upstream and downstream processes. To separate the microalgal biomass from its aqueous media, usually more than one-step harvesting is required due to the small size of microalgal cells and their negative surface charge.

Most widely used harvesting operations are based on chemical, biological, electrical, and mechanical methods (Kadir et al., 2018). Some common methods can be listed as filtration, gravity

sedimentation, centrifugation, flotation (Chen et al., 2011; Kadir et al., 2018). Implementation and operational costs of these techniques, however, are often not effective in large-scale wastewater treatment. Chemical methods, for example, chemical flocculation, provide economically more feasible solutions to harvest microalgae biomass (De Godos et al., 2017; Lam & Lee, 2012). Nevertheless, these methods require the addition of a relatively large amount of chemicals to the water and therefore are not environmentally friendly.

Although commonly algal wastewater treatment systems are suspension cultures, applications of immobilized algal systems in the form of biofilms and beads are promising approaches (Eroglu et al., 2015; Mallick, 2002; Wollmann et al., 2019). The advantage of the immobilized microalgal systems is the facilitation of easy harvesting and handling of biomass through separation of the algae from the treated water before discharge. The system also ensures high effectiveness in the removal of nutrients, heavy metals, or different contaminants (Christenson & Sims, 2011; Kesaano & Sims, 2014; Mallick, 2002, 2006). For more details on biofilm-based systems see Section 5.2.

5 | MICROALGAE REACTORS FOR WASTEWATER RECLAMATION

To maximize the growth of microalgae in contact with wastewater, various reactor configurations have been reported to date. Their goal is to ensure optimal micro-algae productivity as well as to achieve high pollutant removal yields while accommodating large volumes of wastewater.

An important parameter affecting the performance of the microalgae-based wastewater treatment is the exposure of the biomass to the media. Reactors can be classified as suspended and non-suspended systems and further sub-categorized as either open to the environment or enclosed. In Table 2, characteristic features of open and closed systems are compared. The main consideration of the bio-reactor design is its cost. Below PBR, high-rate algal ponds (HRAP), matrix-immobilized micro-algae, and attached micro-algal biofilms systems are described (Borowitzka & Moheimani, 2013).

5.1 | Suspended-biomass reactors

In suspended-biomass reactors, microalgae freely move within the fluid media. Because of their relatively low implementation costs and ease of operation, these systems are very common. Open systems can either be stirred or nonstirred ponds. While nonstirred ponds are cheaper and simpler to manage, stirred ponds provide proper aeration, light, and nutrient distribution improving the growth of microalgae.

HRAP are large shallow-water basins (20–60 cm) that are common in temperate and tropical climates because they often employ solar light as irradiation source. In these usually elliptical ponds, water is impelled through paddle wheels and recirculates in a loop channel. The wheels operate continuously and contribute to homogenize

TABLE 2 Main design parameters of open and closed systems (adapted from [Carvalho et al., 2006])

| Feature | Open system | Closed system |
|-------------------------------------|--------------------|--------------------------------|
| Area to volume ratio | Large | Small |
| Algal species | Restricted | Flexible |
| Main criteria for species selection | Growth competition | Shear resistance |
| Population density | Low | High |
| Harvesting efficiency | Low | High |
| Cultivation period | Limited | Extended |
| Contamination | Likely | Unlikely |
| Water loss (evaporation) | Possible | Reduced |
| Light use efficiency | Poor/fair | Fair/excellent ^a |
| Gas transfer | Poor | Fair/high |
| Control of temperature | None | Excellent |
| Most costly variables | Mixing | Oxygen and temperature control |
| Capital investment | Small | High |

^aDependent on transparency of the construction material.

temperature along the channel, avoid undesired sedimentation, ensure distribution of nutrients, carbon dioxide, and minerals, remove the oxygen produced by the microalgae and improve the light utilization. These open systems are not axenic and support a rich symbiotic community of microalgae and bacteria that together remove pollutants.

PBR are closed configurations, where the microalgae float continuously through a set of transparent tubes that are irradiated externally. To ensure optimized photosynthesis the culture undergoes bubbling with CO₂ and degassing of produced O₂. Either horizontal/vertical tubular reactors or flat panel reactors have been designed. Compared to HRAP these reactors allow fine-tuning of key parameters such as the light exposure, CO₂ concentration, dissolved O₂, and pH. Optimized growth parameters result in higher concentrations of biomass. However, higher installation and operational costs restrict their use on the production of products with commercial interest, where wastewater provides low-cost culture medium (Cantrell et al., 2008).

5.2 | Biofilm-based systems

The immobilization of microalgae include natural or induced biofilm formation (passive immobilization) and entrapment of the cells in hydrogel polymer matrices (active immobilization). Immobilization of the algal cells takes advantage of the microalgal tendency to form biofilms. Inorganic and organic compounds adhere to the surface of a bedding material, creating a favorable environment for microbial growth (Qureshi et al., 2005; Stephens et al., 2015). Once microalgae and bacteria have colonized the surface, they begin secreting extracellular substances composed of nucleic acids, proteins, polysaccharides, and phospholipids, which serve to improve adherence to the bedding

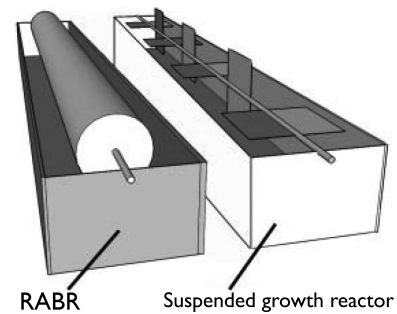


FIGURE 1 Scheme of a bench scale RABR and a suspended growth reactor (Christenson & Sims, 2012), reproduced with permission from John Wiley and Sons

material, but also to entrap and concentrate nutrients necessary for cell growth (Mohsenpour et al., 2021; Qureshi et al., 2005). Microalgal biofilms can grow on many different surfaces with optimal moisture and irradiation, and commonly the attached algae are harvested by scrapping. Materials include plastics such as polyvinylchloride, polyethylene, polyurethane polymethyl methacrylate, polystyrene, polycarbonate, polyamide; different natural materials: old reed stems, green reeds, bamboo pipe, granite, andesite; and many other materials such as borosilicate glass, cardboard, loofah sponge, fibrous scrubber and ceramic tiles. During active immobilization, the algal cells are entrapped into polymer nontoxic natural (e.g. alginate, chitosan, carrageenan, nanocellulose) or synthetic (e.g. acrylamide) matrices (Kosourov et al., 2018) (Kosourov et al., 2018).

Reactors for biofilm-based systems commonly are: (1) Parallel Plate Microalgae Biofilm Reactor (PPMB), (2) Vertical Submerged Biofilm Reactor (VSB), (3) Enclosed Biofilm Tubular Reactor (EBT), (4) Moving bed biofilm Reactor (MBB), or (5) photo-rotating biological contactor (PRBC) (Katarzyna et al., 2015). An example for the configuration of a rotating algal biofilm reactor (RABR) can be observed in Figure 1 (Christenson & Sims, 2012). In these type of reactors, microalgal growth takes place in a biofilm on the surface of a cylinder that is partially submerged in wastewater and rotates to alternate exposure of the biomass to the wastewater and to the air.

Applications of immobilized microalgae in wastewater treatment provide an array of advantages compared to the regular suspended approach. Among the most relevant advantages, special attention should be drawn towards the following: (1) larger flexibility in the photobioreactor design; (2) increased reaction and uptake rates, arising from higher cell density; (3) enhanced operational stability; (4) avoidance of cell washouts; (5) easier cultivation, harvesting, and handling of the produced bio-mass; (6) easy replacement of the algae; (7) provision of shelter and protection of cell integrity from harsh environmental conditions such as salinity, metal toxicity, variations in pH, and any product inhibition; and (8) continuous utilization of algae in a nondestructive way (Eroglu et al., 2015; Escudero-Oñate & Ferrando-Climent, 2019). However, some drawbacks still limit large-scale and commercial uses of immobilized microalgal systems: the polymeric matrices used for cell immobilization are prone to degradation over

time resulting in leaching of the cells (Mohsenpour et al., 2021), and the production of these systems (particularly for high volumes of wastewater) faces cost and technical know-how constraints (Gonçalves et al., 2017). In addition, immobilized microalgal biomass requires a high surface area to ensure an efficient performance. For example, it was estimated (Acién et al., 2016) that the land area necessary to accommodate these systems would be at least twice as much as the area taken by conventional treatment systems. Nevertheless, the authors conclude that the profits can outweigh the increased costs in municipal treatment plants for small cities. The immobilization matrix should provide mechanical and chemical stability, while exhibiting good mass transfer characteristics to favor an effective transport between phases. Although synthetic polymers, such as polyacrylamide, polyurethane, and polyvinyl might be used as matrix, commonly immobilization methods are based on natural biopolymers such as agar, alginate, carrageenan, and nanocellulose (Jämsä et al., 2018; Kosourov et al., 2018; Mohammed et al., 2018). Their environmental friendliness, low toxicity, and high transparency justify this selection.

6 | CONCLUSIONS

Wastewater can contain significant levels of pollutants, which can be dangerous to animals, humans, and the environment. As industrial activities expand to fulfill the needs of our growing population, more and more wastewater is being produced as a “by-product.” Solutions to effectively treat the large quantities of produced wastewater have to be found rapidly. Microalgae can be seen as potential candidates for wastewater treatment as they can assimilate nitrogen, carbon, and phosphorous and can also adsorb or uptake other problematic elements such as heavy metals. Furthermore, their high adaptability to new environments allows them to grow in a wide variety of conditions, including municipal, industrial, and agricultural wastewater. However, microalgal-based wastewater reclamation presents some challenges including the choice of growing conditions (mainly the light intensity, light period, and temperature) and the harvesting process. Harvesting is still one of the main bottlenecks in any biotechnological application that involves microalgae and needs to be studied further. Current harvesting techniques in wastewater treatment are either expensive, time consuming, or costly and could be improved based on the choice of bioreactor or culture system used for the wastewater treatment process. While microalgae can contribute to a circular bioeconomy, further research and development are needed to overcome the current challenges.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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