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Review

Microalgae systems - environmental agents for wastewater treatment and further potential biomass valorisation

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ABSTRACT

Water is the most valuable resource on the planet. However, massive anthropogenic activities generate threatening levels of biological, organic, and inorganic pollutants that are not efficiently removed in conventional wastewater treatment systems. High levels of conventional pollutants (carbon, nitrogen, and phosphorus), emerging chemical contaminants such as antibiotics, and pathogens (namely antibiotic-resistant ones and related genes) jeopardize ecosystems and human health. Conventional wastewater treatment systems entail several environmental issues: (i) high energy consumption; (ii) high CO_2 emissions; and (iii) the use of chemicals or the generation of harmful by-products. Hence, the use of microalgal systems (entailing one or several microalgae species, and in consortium with bacteria) as environmental agents towards wastewater treatment has been seen as an environmentally friendly solution to remove conventional pollutants, antibiotics, coliforms and antibiotic resistance genes. In recent years, several authors have evaluated the use of microalgal systems for the treatment of different types of wastewater, such as agricultural, municipal, and industrial. Generally, microalgal systems can provide high removal efficiencies of: (i) conventional pollutants, up to 99%, 99%, and 90% of total nitrogen, total phosphorus, and/or organic carbon, respectively, through uptake mechanisms, and (ii) antibiotics frequently found in wastewaters, such as sulfamethoxazole, ciprofloxacin, trimethoprim and azithromycin at 86%, 65%, 42% and 93%, respectively, through the most desirable microalgal mechanism, biodegradation. Although pathogens removal by microalgal species is complex and very strain-specific, it is also possible to attain total coliform and *Escherichia coli* removal of 99.4% and 98.6%, respectively. However, microalgal systems' effectiveness strongly relies on biotic and abiotic conditions, thus the selection of operational conditions is critical. While the combination of selected species (microalgae and bacteria), ratios and inoculum concentration allow the efficient removal of conventional pollutants and generation of high amounts of biomass (that can be further converted into valuable products such as biofuels and biofertilisers), abiotic factors such as pH, hydraulic retention time, light intensity and CO_2/O_2 supply also have a crucial role in conventional pollutants and antibiotics removal, and wastewater disinfection. However, some rationale must be considered according to the purpose. While alkaline pH induces the hydrolysis of some antibiotics and the removal of faecal coliforms, it also decreases phosphates solubility and induces the formation of ammonium from ammonia. Also, while CO_2 supply increases the removal of *E. coli* and *Pseudomonas aeruginosa*, as well as the microalgal growth (and thus the conventional pollutants uptake), it decreases *Enterococcus faecalis* removal. Therefore, this review aims to provide a critical review of recent studies towards the application of microalgal systems for the efficient removal of conventional pollutants, antibiotics, and pathogens; discussing the feasibility, highlighting the advantages and challenges of the implementation of such process, and presenting current case-studies of different applications of microalgal systems.

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1. Introduction

Water is an essential natural resource of Earth, and water scarcity is one of the major problems worldwide. While millions of people have no access to freshwater, some industrial sectors (e.g. agriculture) consume high amounts of available water (Kesari et al., 2021). Concomitantly, massive industrialisation, urbanisation, and agricultural practices generate 359.4×10^9 m^3 of wastewater every year, and only 52% is treated before its discharge into natural bodies (Jones et al., 2021). The continuous disposal of wastewater containing high concentrations of conventional contaminants (organic and inorganic carbon, nitrogen, and phosphorus), contaminants of emerging concern (CECs; e.g. pharmaceuticals, heavy metals, among others), and pathogens can trigger serious water pollution concerns that may lead to the breakdown of water ecosystems, compromising the already low availability of freshwater (Eerkes-Medrano et al., 2019; Gonçalves et al., 2017; Jones et al., 2021; Sousa et al., 2018; Wollmann et al., 2019). Additionally, some Human health hazards are related to low wastewater treatment efficiency. The excessive use of antibiotics, their incorrect disposal, and their continuous excretion into wastewater have a determinant role in increasing antibiotic-resistant microorganisms (Pazda et al., 2019). Studies show that in wastewater treatment plants (WWTPs), sewage sludge is unable to mineralize all the antibiotic residues, and given the high cost associated with the current antibiotic elimination processes, a significant amount of these contaminants are released into the environment (Leng et al., 2020). Hence, WWTPs may act as hotspots for multi-resistant bacteria proliferation and gene exchange, due to the high concentrations of several antibiotic residues (in combination with other stress factors - e.g. the presence of heavy metals) and high bacterial density, which may induce horizontal transfer of antibiotic resistance genes (ARGs) (Guo et al., 2017; Leng et al., 2020; Ruas et al., 2022).

Reuse of treated wastewater can be an eco-friendly alternative to avoid water scarcity in depleted areas and the misuse of untreated wastewater (Kesari et al., 2021). Currently, several physicochemical and biological tertiary treatment methodologies are employed to reduce emerging contaminants and pathogens. Physico-chemical ones include advanced oxidation processes, the addition of activated carbon, the use of membranes, filtration, ultraviolet irradiation, chlorination and ozonation, with the former being considered one of the best disinfection methods (Zahmatkesh et al., 2022). However, besides the costs of implementation, the generation of toxic by-products may occur as well as the synergic hazard effects among them. Furthermore, some of the generated toxic by-products can result in the formation of carcinogenic compounds (Zahmatkesh et al., 2022).

Therefore, biological tertiary treatments are considered a more environmentally friendly alternative to physicochemical ones. Due to their high metabolic flexibility, microalgae have been extensively studied as promising bioremediation agents to be implemented in tertiary wastewater treatment, for conventional pollutants removal (nitrogen and phosphorus), antibiotics removal, and to a lesser extent, for pathogens removal, such as coliforms. Besides the non-generation of toxic by-products, the use of these bioagents has an extra economic advantage particularly promising from an industrial point of view. The biomass obtained at the end of the process can be converted to products with a commercial value such as biofuels or biofertilizers, offsetting any associated implementation costs and corresponding to additional economic input. Namely, microalgal biomass can be converted into biochar, an alternative to coal-based carbon in wastewater treatment processes (Law et al., 2022). Furthermore, the versatility of microalgae cultivation in different types of bioreactors allows to tailor the tertiary treatment setup. Its implementation can be within or near the wastewater generation area (ex. urban WWTP, aquaculture, swine farming, agricultural production site), and built according to the purpose.

However, most of the studies and reviews on this subject only focus on the use of microalgal systems for the removal of one or two types of contaminants. This paper aims to provide an innovative approach through a critical and updated overview of the potential use of microalgal systems as wastewater bioremediation agents, considering the co-removal of several types of pollutants. Critical factors affecting the efficiency of microalgal systems will be discussed, including wastewater composition, microorganism's species selection, and the effects of biotic and abiotic factors on pollutants removal. The focus will be on removing conventional pollutants, antibiotics, and pathogens, describing the microalgal action pathways towards the specific contaminants. In addition, some case studies that reflect the potential of these organisms towards the implementation of a circular economy approach will be presented, as well as future research prospects and challenges in this field.

2. Wastewater composition

Generally, wastewaters contain several types of solid suspensions, microorganisms, inorganic and organic compounds (comprising diverse concentrations of chemical oxygen demand, COD, and– biochemical oxygen demand, BOD), CECs, and persistent organic pollutants (Ahmed et al., 2022; Muylaert et al., 2015). However, wastewater composition greatly depends on its origin and it may vary over time. Wastewaters can be categorized into agricultural, municipal, and industrial. Agricultural wastewaters are discharged from agricultural lands and livestock farms (such as run-off water, swine or poultry) and contain herbicides, pesticides, manure, pathogens, and antibiotics, presenting high levels of nitrogen, phosphorus and organic carbon. Municipal wastewaters originate from household sources and contain CECs (such as pharmaceutical drugs and personal care products) and microplastics, in addition to nitrogen and phosphorus compounds. On the other hand, industrial wastewaters can be discharged from a wide variety of sources, such as food processing, mining, chemical manufacturing, textiles, power plants and energy-related industries, and compounds such as phenols, dyes, heavy metals, among others, (Wollmann et al., 2019). Since wastewater composition is a critical factor in the use of microalgal systems for wastewater treatment, its composition in terms of conventional pollutants, antibiotics (as CECs) and pathogens will be briefly discussed.

2.1. Conventional pollutants

Carbon, nitrogen and phosphorus compounds are the most common pollutants; however, their forms and concentrations vary according to each type of wastewater. Table SM1 (in supplementary material) presents the composition in terms of conventional pollutants of wastewaters from agricultural, industrial and municipal origin, which can most commonly be treated with microalgal systems. Agricultural wastewaters, such as palm oil mill or piggery, contain higher concentrations of total nitrogen, TN (mainly ammonium, nitrites, nitrates, or urea forms), total phosphorous, TP (as phosphates, esterized phosphorus or pyrophosphates), total carbon (mainly in organic matter form), and some metal ions (Chen et al., 2020a; Ganeshkumara et al., 2018), when compared to municipal (primary and secondary treated effluents) or industrial wastewaters (such as dairy, paper, textile or winery industries) (Table SM1 of Supplementary Material). The typically lower concentrations in conventional pollutants and toxic substances for microalgae (such as heavy metals, and aldehydic- and phenolic compounds) of municipal wastewaters, when compared to industrial or agricultural ones, makes them one of the most studied types of wastewater for microalgal cultures (Li et al., 2019b).

2.2. Antibiotics as contaminants of emerging concern

CECs are defined as natural or synthetic occurring chemicals that enter the environment and can cause harmful effects on the health of an ecosystem. Nowadays, over a thousand substances are considered CECs, belonging to approximately 20 chemical classes. Personal care items, pharmaceuticals, plasticisers, insecticides, and surfactants are some examples of products that contain CECs that are used on a daily basis. Nonetheless, their uncontrolled disposal and inefficient removal in WWTPs are becoming a major concern due to their dangerous impact on human health and ecotoxicological effects (Xabadia et al., 2021).

Particularly, the worldwide overuse of pharmaceuticals and their inefficient removal from wastewater is causing alarm due to toxicity, carcinogenicity, and accumulation effects through the food chain, endangering the integrity of ecosystems (Prosenc et al., 2021; Xabadia et al., 2021). Pharmaceuticals entail an extensive group of chemicals with wide-ranging chemical structures and mechanisms of action. Due to the recalcitrant nature of some pharmaceuticals, in terms of biodegradation, it is usual to detect them in WWTP effluents, ranging from ng L^{-1} to μg L^{-1}. A few examples of the most frequent recalcitrant pharmaceuticals are antibiotics, such as erythromycin found up to 7.2 μg L^{-1} in hospital wastewater, and anti-inflammatory drugs, such as ibuprofen found in concentrations of up to 4.2 μg L^{-1} in municipal wastewater (Kesari et al., 2021; Prosenc et al., 2021). The prevalence of antibiotics in urban wastewater depends mainly on the location and socio-economic development of the countries. The classes of antibiotics most widely used to prevent and treat bacterial infections are penicillin, macrolides, sulphonamides, cephalosporins, and quinolones (Leng et al., 2020). Among the most frequently found in all wastewaters, sulfamethoxazole, ciprofloxacin, trimethoprim, erythromycin, and azithromycin are the most prevalent (Langbehn et al., 2021). Antibiotic contamination levels, sometimes together with disinfectants, and metals (e.g., copper and zinc), have become worrying. Also, the non-complete mineralization of antibiotics may generate by-products with similar or even higher toxicity compared to the parental compound, such as the metabolites N^4-acetylsulfapyridine and N^4-acetylsulfadiazine from sulfadiazine (Kesari et al., 2021; Langbehn et al., 2021). On the other hand, although usually found in the environment at relatively low concentrations, antibiotics can alter the microbial community composition and activities, promoting the development and spread of antibiotic-resistant bacteria (ARB) and ARGs (Xiong et al., 2021). Therefore, this induces a strong selection pressure on human and natural microbial systems, which, according to the first global report from the World Health Organisation in 2014, is a serious clinical and public health issue (World Health, 2014b; Xiong et al., 2021). The exposure of microorganisms/bacteria to antibiotics and other antimicrobials speeds up the ARGs' transfer rate due to the pressure they exert on mobile genetic elements responsible for their dissemination, encouraging resistance to the antibiotic itself or others (Kesari et al., 2021; Nguyen et al., 2021). For example, the antibiotic trimethoprim and the bacteriostatic drug triclosan significantly increased the horizontal gene transfer (HGT) rate of plasmid-encoded multi-drug resistance genes in an activated sludge bacterial community, across genera, at concentrations usually found in wastewater (Li et al., 2019a). This can occur even when antibiotics are 150 times under the minimal inhibitory concentration, as detected in WWTP-activated sludge and effluents (Nguyen et al., 2021). An example of this scenario occurs with tetracycline at a concentration of 10 μg L^{-1}. This antibiotic concentration stimulates the ARG receiver and HGT of ARG, explaining the greater magnitude and diversity of ARG in pharmaceutical wastewater treatment sludge compared to municipal WWTP sludge (Nguyen et al., 2021).

According to the list published by WHO, the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) are the main causative agents of lethal hospital-acquired infections due to the acquisition of multidrug resistance mechanisms against lipopeptides, oxazolidinones, fluoroquinolones, macrolides, tetracyclines, β-lactams, and β-lactam–β-lactamase inhibitor combinations (World Health, 2014a). Moreover, ESKAPE strains may show resistance even to antibiotics of the last line of defence, such as glycopeptides and carbapenems, which is very worrying (Kesari et al., 2021; Langbehn et al., 2021).

2.3. Pathogens

The pathogens found in sewage vary according to socioeconomic conditions and community habits (Ruas et al., 2022). Given the difficulties in correctly identifying all the pathogens occurring in a certain habitat, the monitoring of faecal indicators, *Escherichia coli* and enterococci, is commonly used instead (Rodríguez-Chueca et al., 2013). *E. coli* exists in large amounts in the intestinal tract of warm-blooded animals and humans. Depending on the virulence of the strain, it can cause several intestinal diseases, severe illness, or death. Enterococci are used as indicator bacteria due to their incapacity to grow in other environments, such as water and soil, indicating that their occurrence is only due to faecal contamination (Ruas et al., 2022). Other aerobic bacteria are also relevant as sanitary indicators, such as *Pseudomonas*, *Acinetobacter*, *Klebsiella* and *Aeromonas* spp. Namely, *P. aeruginosa* (an ubiquitous bacteria usually found in wastewater, water and soil) is liable for ear and eye infections. Its main transmission is related to the contact of susceptible tissues with polluted water (Malato et al., 2009).

As mentioned before, raw urban wastewater provides a constant input of ARB and ARGs and extremely diverse pathogenic and commensal bacteria from human and animal microbiomes assembled in the WWTPs. Particularly, genera such as *Pseudomonas* and *Acinetobacter* are often detected in wastewater as active ARGs vectors and carriers, with plasmids mediating the ARG exchange (Nguyen et al., 2021). Li et al. (2018) reported that inside the activated sludge microbial community, there were different plasmid transfer frequencies across diverse forms of ARG-carrying plasmids and plasmid-donor bacteria such as *Pseudomonas putida* and *E. coli*. The plasmid recipient community was composed mainly of members of the genus *Acinetobacter* and families *Enterobacteriaceae* and *Pseudomonadaceae*. Hence, bacteria such as *P. aeruginosa*, enterococci, and enterobacteria have been considered ARB surrogates due to their omnipresence in the wastewater ecosystem. They are also identified as active ARG vectors and carriers (Nguyen et al., 2021). Reducing the abundance of pathogens and ARB in the WWTP effluents is vital to prevent increased human morbidity and mortality caused by these water vectors (Nguyen et al., 2021).

3. Wastewater treatment with microalgal systems

Microalgae are able to undergo photoautotrophic, organoheterotrophic or mixotrophic metabolisms according to external conditions, which enables them to remove several kinds of pollutants, from conventional ones to CECs. Moreover, microalgae have a role in hazard bacteria removal, making microalgae-based systems an environmentally friendly and effective tertiary wastewater treatment (Ji, 2022; Jiang et al., 2018; Wollmann et al., 2019; Yong et al., 2021).

3.1. Microalgal systems composition

Microalgal systems can be considered a community of microalgal cells composed of: (i) a single microalgal species (monoculture); (ii) several species of microalgae (polyculture); or (iii) microalgae and bacteria (microalgae-bacteria consortia). Several different compositions of microalgal systems have been studied for wastewater treatment considering different scenarios, using either real or synthetic wastewater, at lab or pilot-scale, as will be presented in the following sections. Fundamental studies regarding wastewater treatment have been carried out mostly at a lab scale and using synthetic or sterilised wastewater. These studies usually rely on microalgae monocultures or well-defined microalgae-bacteria consortia to understand the mechanisms and the effects of isolated biotic and abiotic factors. However, in a real scenario, it is very unlikely to maintain a microalga monoculture in wastewater treatment due to the presence of other microalgal species, bacteria and other microorganisms in the wastewater. Hence, microalgal polycultures, either forming or not a consortium with bacteria, are among the most common microalgal systems studied for further application on a larger scale. The use of microalgal polycultures for wastewater treatment has two main advantages over the use of monocultures (Mandal et al., 2018; Ray et al., 2022; Singh and Singh, 2022; Stockenreiter and Litchman, 2019; Tran et al., 2020b; Vargas-Estrada et al., 2021): (i) higher efficiency in terms nutrient uptake and consequently higher biomass production; and (ii) higher insurance of robustness, scalability, self-reliance, and viability of the bioremediation process due to the different nutritional requirements and abiotic adaptation capabilities of the different species. However, it should not be neglected that the interaction among the different microalgal species may result in a competition for nutrients and the excretion of allelochemicals in unfavourable (stress) conditions that may block their growth – such as nutrient starvation, low light intensity and temperature, and high pH values. Thus, the selection of the microalgal species is a critical point (Cembella, 2003; Gonçalves et al., 2017; Subashchandrabose et al., 2011).

Until recently, the bacterial presence in microalgal cultures was perceived as a source of contamination, but now the synergic relationship between microalgae and bacteria is deemed promising for various applications, putting microalgae-bacteria consortia in the spotlight in terms of wastewater treatment, as discussed in the following sections (Deng et al., 2022; Yong et al., 2021). While microalgae can transform solar energy into chemical energy, producing O_2 and organic matter, bacteria use the O_2 for respiration, decompose the organic compounds, and supply CO_2 to microalgae, as well as growth-promoting factors and vitamins (B12, B1 and B7) that boost microalgal growth (Higgins et al., 2018; Wang et al., 2022; Yong et al., 2021). On the other hand, the microalgal surface can be a habitat for bacteria, protecting them against unfavourable environmental conditions and providing extracellular metabolites that improve bacterial growth (Lee and Lei, 2019). Other cooperative relationships can also occur due to the bacterial excretion of chemical signal substances, such as N-acyl-homoserine lactones and indole-3-acetic acid, which are responsible for mediating several actions such as ecological niche formation (due to the induction of granules formation together with microalgae), and induce microalgal growth, respectively (Ramanan et al., 2016; Wang et al., 2022). Hence, the use of microalgal-bacterial consortia for wastewater treatment gathers several advantages such as (Akizuki et al., 2019; Huo et al., 2020; Leong et al., 2019a, 2019b; Wang et al., 2022): (i) the enhancement of conventional pollutants uptake, including the reduction of stress caused by high concentrations of a certain pollutant, e.g. bacterial consumption of NH_3, a toxic nitrogen form for microalgae; (ii) the increase of microalgal biomass production; (iii) easy microalgal harvesting by flocculation due to the release of extracellular polymeric substances (EPS) by bacteria; and (iv) greater robustness and resistance of the consortia to fluctuations in the environmental conditions.

However, microalgal-bacterial consortia also may have some issues that should be considered, such as (Gonzalez-Camejo et al., 2020; Lee and Lei, 2019; Sahoo et al., 2019; Wang et al., 2022) (i) the nutrient competition between the two microorganisms; (ii) the shading effect of bacteria on microalgae, negatively affecting the latter as they need light for photosynthesis; (iii) the release of algicidal substances by bacteria; (iv) the production of antibacterial metabolites by microalgae; and (v) bacterial growth inhibition due to an increase in pH and O_2 concentration, as a result of microalgal photosynthesis.

Hence, it is crucial to establish and optimise the proper consortium according to the purpose and existing conditions. Furthermore, it is essential to understand how each type of wastewater's pollutants concentration and other characteristics (biotic and abiotic) can influence microalgal removal mechanisms and efficiency to optimise wastewater treatment. These are important considerations to set a microalgal system for conventional pollutants, antibiotics, and pathogens removal, which are discussed in the following sections.

3.2. Microalgal systems in conventional pollutants removal

3.2.1. Microalgal pathways for conventional pollutants removal

Microalgae have specific mechanisms for each conventional pollutant removal, which may entail pathways to store nutrients in the biomass or to assimilate them into essential molecules for microalgal growth. Microalgae are mainly photoautotrophs, using light as the energy source and CO_2 from pure, simulated or real gaseous streams, or bicarbonates (HCO_3^-) in the culture medium as a carbon source, converting it into energy-storing molecules such as carbohydrates through photosynthesis and CO_2 fixation (Singh and Dhar, 2019; Su, 2021; Umdu and Univ, 2020). Most microalgal species have carbon-concentrating mechanisms that involve the reversible intracellular or extracellular conversion of HCO_3^- to CO_2. These mechanisms allow efficient CO_2 fixation by obtaining inorganic carbon, even when the atmospheric CO_2 concentration is low (Singh and Dhar, 2019; Umetani et al., 2021). Microalgal growth mainly occurs at pH between 7.0 and 8.4, pH values at which HCO_3^- typically represents most of the available inorganic carbon in the culture medium (Onyeaka et al., 2021). Inorganic carbon can enter the microalgal cell through three different processes (Prasad et al., 2021; Singh et al., 2016): (i) direct passive diffusion of CO_2; (ii) transformation of HCO_3^- to CO_2, catalysed by an extracellular carbonic anhydrase, followed by passive diffusion of CO_2; and (iii) direct active transport of HCO_3^-, aided by inorganic carbon uptake transporters. However, hydroxyl radicals are produced due to the conversion of HCO_3^- to CO_2, which can contribute to an increase in the culture's pH (Whitton et al., 2015). Inside the cell, CO_2 is fixed in a reaction that initiates the Calvin-Benson cycle, catalysed by ribulose biphosphate carboxylase/oxygenase (Rubisco), ultimately yielding carbohydrates (Yang et al., 2017). Even though CO_2 is the preferred carbon source, in the presence of certain organic molecules (e.g., sugars, acetate, and alcohols) and the absence of light, some microalgae, such as *Chlorella* spp., can adopt a heterotrophic metabolism, using these molecules as a carbon source (Siqueira et al., 2018; Su, 2021). Furthermore, mixotrophic metabolism is common in some of these microorganisms, in which CO_2 fixation co-occurs with photosynthesis and the organic compounds are facultatively used as an energy source for respiration (Prasad et al., 2021; Siqueira et al., 2018). Nitrogen is a crucial nutrient for microalgae; it is found in peptides, and proteins, including enzymes, chlorophylls, and nucleic acids, thus being essential for algal growth and metabolism regulation. Microalgae can assimilate this nutrient from wastewaters in the form of inorganic nitrogen, such as NH_4^+, NO_2^-, NO_3^-, and organic nitrogen, such as urea, purines, amino acids and pyrimidines (Cai et al., 2013; Chen and Wang, 2020; Kumar and Bera, 2020). The inorganic nitrogen species are actively transported into the microalgal cell, and NH_4^+ can be directly incorporated into 2-oxoglutarate, leading to glutamate production and, consequently, amino acid synthesis through the glutamine synthetase/glutamate synthase cycle (Vega, 2020). However, NO_3^- and NO_2^- have to be reduced to NH_4^+ before assimilation: NO_3^- is primarily converted to NO_2^- in the cytosol by the action of nitrate reductase, and NO_2^- is then reduced to NH_4^+ in the chloroplast, in a reaction catalysed by nitrite reductase (Chai et al., 2021; Sanz-Luque et al., 2015). Consequently, NH_4^+ is the preferred and most readily taken-up form of nitrogen by most microalgae as it does not involve redox reactions, thus having a lower energy demand than other nitrogen sources (Wang et al., 2017). Furthermore, some cyanobacteria, formerly described as blue-green algae, can also fix molecular nitrogen, converting it to NH_3 in a reaction catalysed by a nitrogenase enzyme complex, which can be transformed into NH_4^+ for assimilation (Kumar and Bera, 2020). Besides the production of hydroxyl radicals, the positive uptake of H^+ via the co-transportation of nutrients such as nitrogen and phosphorus through the microalgal cell membrane can lead to an increase in the medium's pH (Whitton et al., 2015). As a result of this pH increase or due to high temperatures, an indirect microalgal NH_4^+ removal from wastewaters may occur through its conversion into gaseous NH_3 and consequent volatilisation from water (Wang et al., 2017; Whitton et al., 2015). However, it must be noted that this is not a desirable event since NH_3 is a precursor of N_2O, a greenhouse gas.

In wastewater, phosphorus can exist in the form of either organic or inorganic compounds. However, the primary source of phosphorus for microalgae is inorganic phosphate in the forms of $H_2PO_4^-$, HPO_4^{2-} or PO_4^{3-} (Su, 2021; Whitton et al., 2015). These compounds are transferred through the cell membrane by active transport using inorganic phosphorus transporters. Once inside the cell, they can be used directly to synthesise nucleic acids, phospholipids, and adenosine triphosphate (ATP) through phosphorylation (Su, 2021; Whitton et al., 2015). Moreover, in the presence of light, phosphates can be converted into acid-soluble polyphosphates in a reaction catalysed by polyphosphate kinase and used for deoxyribonucleic acid (DNA) and protein synthesis (Su, 2021; Whitton et al., 2015). When phosphorus is in excess, some microalgae can also convert phosphates into acid-insoluble polyphosphates and store them as granules inside the vacuole. This process can happen when microalgae are starved of phosphorus and then re-fed (starvation uptake) or, when exposed to excess phosphorus (luxury uptake), consuming more than what is required for their growth (Singh et al., 2018; Zafar et al., 2021). On the contrary, in conditions with low inorganic phosphorus availability, some microalgae have phosphatases to consume external organic phosphorus found in compounds, such as phosphate esters, to support the essential cellular processes (Cai et al., 2013; Mühlroth et al., 2017). Besides microalgal assimilation and storage, phosphorus can also be indirectly removed from the wastewater through precipitation by complexation with Ca, Mg, and Fe, due to the high pH (>8) as a consequence of microalgal growth (Wang et al., 2014; Whitton et al., 2015).

3.2.2. *Effects of biotic and abiotic factors on conventional pollutants removal*

Several abiotic and biotic factors influence the effectiveness of microalgal systems on conventional pollutants removal. Among abiotic factors, pH, temperature, light intensity, hydraulic retention time (HRT), wastewater composition, and water turbidity are the most critical. Concerning biotic factors, the microalgal-bacterial ratio and microalgal species are the most studied (Huo et al., 2020; Lee and Lei, 2019; Nguyen et al., 2020b).

Regarding the abiotic factors, the pH can significantly affect nitrogen and phosphorus removal, mostly due to phosphorus precipitation and NH_3 stripping in alkaline conditions. There is an increase in the availability of free CO_2 at higher pH values, promoting its absorption. However, extremely high values can inhibit microalgal metabolism and reduce nutrient assimilation (Prasad et al., 2021; Wang et al., 2017).

Overall, conventional pollutants removal and biomass productivity are enhanced with the increase of light intensity and temperature. The ideal temperature for microalgal growth is typically 15–30 °C. Lower or higher temperatures can reduce the activity of certain enzymes such as Rubisco, inhibiting carbon sequestration, and temperatures above 35 °C can be lethal for several species. This directly impacts conventional nutrient removal. As observed by Zhang et al. (2021), fermented high-strength mariculture wastewater treatment with *Chlorella vulgaris* was optimal at 25 °C, removing 99% of COD and 68.8% of ammonium, while at a temperature of 30 °C, the pollutants removal efficiency decreased to 94.4% and 44.6%, respectively, due to a reduction of the activity of Rubisco. Furthermore, if the light intensity is too high, exceeding the species-specific saturation point, oxidative damage can occur due to photoinhibition of microalgae, leading to a reduction in nutrient removal (Dasgupta et al., 2019; Mohsenpour et al., 2021; Prasad et al., 2021; Whitton et al., 2015). High light intensity can also affect the nitrite-oxidizing bacteria, particularly from microalga-bacteria consortium in granules. It was reported that light intensities greater than 180 μmol m^{-2} s^{-1} induced the inhibition of the nitrite-oxidizing bacteria, leading to a decrease in the bioremediation efficiency and a nitrite-nitrogen (NO_2–N) accumulation in the reactor (Meng et al., 2019).

Furthermore, low HTR values can lead to a wash-out of the microbial population composition in the photobioreactor, lowering the number of microalgae cells and modifying the composition of the bacterial community, namely in terms of dominance. This event can lead to a reduction in the efficiency of microalgal systems in specific conventional pollutants removal, as observed in piggery wastewater (Garcia et al., 2019).

The mechanisms described for nitrogen, phosphorus, and carbon removal by microalgae appear to be linked; thereby, the external nutrient concentration is also a key factor, with N/P mass ratio values in the range of 5–30 being considered optimal (Choi and Lee, 2015). For instance, low nitrogen concentrations can limit phosphorus uptake, as nitrogen is necessary to synthesise proteins to allow phosphorus assimilation. Moreover, phosphorus limitation can decrease the synthesis of ATP, which is essential for microalgal metabolism, such as the carbon concentrating mechanisms (Su, 2021; Whitton et al., 2015). In some cases, a wastewater pre-treatment is required to ensure efficient bioremediation by microalgae due to high suspended solids concentration (and thus turbidity) and NH_3 content, which negatively affect microalgal growth (Li et al., 2019b). For example, a sedimentation pre-treatment can promote a better bioremediation performance in agricultural wastewaters such as piggery or winery (Ganeshkumara et al., 2018). However, depending on the microalgal species, sedimentation may not be enough, and filtration or dilution pre-treatments are necessary at times due to the ammonium concentration tolerance of several microalgal species, which ideally is aimed to be in the range of 25–1000 μmol NH_4–N L^{-1} (Chen and Wang, 2020). On the other hand, industrial wastewaters have very different compositions depending on the type of industry, whether it is food processing (dairy, palm oil mill, winery), textile, pharmaceutical, or paper industry, as shown in Table SM1 (in Supplementary Material). Thus, to ensure efficient remediation, diluting or mixing different types of wastewater may also be helpful to achieve optimal conditions for microalgal growth according to the microalgal elemental stoichiometry, particularly concerning the conventional pollutants ratio (Mohsenpour et al., 2021).

The effects of biotic factors are related to the presence of other microorganisms besides microalgae and their interactions, namely with bacteria due to their cooperation or inhibition effects. Several authors have reported that the interactions between microalgae and bacteria might change depending on the species used, the ratio between these two types of organisms, and the cultivation phase (Cheng et al., 2020; Fan et al., 2020; Huo et al., 2020; Nguyen et al., 2020a). Particularly in a microalgae-bacteria consortium, competition for nutrients and space between these two types of microorganisms can be the cause of growth suppression of one type of microorganism. This was observed in a system composed of *Chlorella* sp. and *Bacillus firmus*, at which, in a later cultivation phase, the microalgal growth was inhibited, even though there were still nutrients in the culture medium, probably due to the increasing concentration of bacteria in the culture (Huo et al., 2020). However, this dynamic between microalga and bacteria concentration is very species-specific. When the pair *Chlorella* sp. and *Beijerinckia fluminensis* was used, the opposite situation occurred, as the number of microalgae increased at a later cultivation phase, whereas the number of bacteria decreased significantly. This occurrence was probably caused by an increase in metabolites released by microalgae, which can have a bactericidal effect (Huo et al., 2020).

3.2.3. *Wastewater treatment efficiency*

Organic matter, nitrogen, and phosphorus removal are commonly performed by activated sludge systems. However, these processes are inefficient and cannot always effectively remove nitrogen and phosphorus, increasing the probability of water eutrophication and the associated harmful consequences (Fan et al., 2020). Currently, microalgal-bacterial systems have become a green and low-cost alternative treatment to remove conventional pollutants from secondary treated wastewaters. In recent years, several authors have evaluated the wastewater treatment by growing various microalgal species in municipal (Tran et al., 2020a; Zhong et al., 2021), agricultural (Chen et al., 2020b; Ganeshkumara et al., 2018), food processing and industrial wastewaters (Behl et al., 2020; Silva et al., 2021). Overall, microalgal systems were able to grow successfully in wastewaters with satisfying biomass productivities, providing high TN, TP, and/or organic carbon removal efficiencies (up to 99%, 99%, and 90%, respectively).

Several research studies report successful results using microalgal polycultures, native and artificial consortia, for wastewater treatment (Table SM2 of Supplementary Material). Namely, a proper selection of microalgal species allows the combination of high bioremediation rates with high biomass productivity and increased content in high-value products. For instance, a microalgal polyculture composed of *Chlorella zofingiensis* and the auto-flocculating yellow-green strain *Tribonema* sp. (in a 1:1 ratio) was used to treat an effluent composed of swine wastewater diluted with fishery wastewater (Cheng et al., 2020). Besides resulting in TN removal above 80%, and high microalgal biomass productivity with increased lipid accumulation, it also had the extra advantage of promoting an efficient recovery of biomass due to cell aggregation as a result of the EPS produced by *Tribonema* sp. (Cheng et al., 2020).

The application of microalgal-bacterial consortia in wastewater treatment is being tested mainly in two forms (Zhu et al., 2019a): (i) microalgae and selected bacteria; and (ii) microalgae and activated sludge. In both situations, microalgae are primarily responsible for nitrogen and phosphorus assimilation, whereas bacteria contribute to COD removal, which demonstrates the strong cooperation between microalgae and bacteria and the advantages of their application in wastewater treatment (Huo et al., 2020; Nguyen et al., 2020a; Zhu et al., 2019b). Table SM3 of Supplementary Material provides an overall view of several microalgal-bacterial consortia that were successfully used to remove conventional pollutants (organic matter, nitrogen, and phosphorus) from various wastewater sources.

However, it should be noted that the selection of the microalgal-bacterial consortia should be done according to the type of wastewater. While to treat agricultural wastewater, it is more likely to keep a selected microalgal-bacterial consortia, the latter is more dubious to be applied in municipal wastewater treatment. For example, agricultural wastewater from vinegar production was successfully treated with a defined microalgal bacteria consortia composed of *Chlorella* sp. (1.0 x 10^5 cells mL^{-1}), and 1% (v/v) or 10% (v/v) of *B. firmus* and *B. fluminensis* cultures (1.0×10^7 CFU mL^{-1}). In both microalgal-bacterial consortia, COD, TN, and TP removal rates were increased by 22%, 20% and 8%, respectively, compared to single microalgal cultures (Huo et al., 2020). However, a suppression of microalgal growth was observed at a later phase, demonstrating that bacterial load had a crucial role in this system. On the other hand, in a study using synthetic wastewater, the *C. vulgaris-Bacillus licheniformis* consortium with a higher concentration of bacteria, in a 1:3 ratio (initial microalgae and bacteria concentration of 1 x 10^5 cells mL^{-1} and 1×10^5 CFU mL^{-1}, respectively) revealed a much faster COD, TN and TP removal, attaining removals of 86.5%, 88.9% and 80.3%, respectively (Ji et al., 2018). However, when using the *Microcystis aeruginosa-B. licheniformis* consortium, in the same ratio, lower removal efficiencies were observed (Ji et al., 2018).

For municipal wastewater treatment, the use of activated sludge bacteria seems to be more suitable, as well as the use of microalga native species. Besides, by using consortia of microalgae and activated sludge, it is possible to combine the secondary and tertiary wastewater treatment into a single-stage process, lowering the oxygenation costs of activated sludge tanks, once microalgae can provide the oxygen needed to bacteria (Fan et al., 2020; Nguyen et al., 2020b). The first microalgal-bacterial consortia systems to treat wastewater were developed in the '50s and consisted of (HRAPs) for domestic wastewater remediation (Oswald and Gotaas, 1957). These systems are still nowadays widely used to treat various effluents such as agricultural, municipal and industrial wastewaters (García et al., 2019; Molinuevo-Salces et al., 2019; Zhu et al., 2019a). In this scenario, the microalgae-activated sludge ratio is critical as well. The initial sludge concentration affects the microalgal biomass yield due to a possible shading effect that decreases the photosynthetic efficiency (Nguyen et al., 2020a). Using synthetic wastewater, Nguyen et al. (2020a) found that *Chlorella* sp. specific growth rate proportionally decreased with the reduction of the microalgae-activated sludge ratio, but with a microalgae-activated sludge ratio of 3:1 was possible to combine optimal biomass productivities (total biomass concentration of 400 mg L^{-1}) and nutrient removal efficiencies (TN, TP, and COD removal efficiencies of 86%, 79%, and 99%, respectively).

Furthermore, the presence of microalgae aids the activated sludge bioremediation performance, particularly in wastewaters containing a low C/N ratio that limits bacterial growth as registered when using *C. vulgaris* and activated sludge (COD, ammoniacal-nitrogen - NH_3–N - and TP removal efficiencies of 83%, 76% and 100%, respectively) (Zhu et al., 2019a, 2019b). Besides, the combination of activated sludge with microalgae allows a wastewater system with reduced GHG emissions and high contents of microalgal biomass, due to limited bacterial proliferation (Zhu et al., 2019a, 2019b).

3.3. Microalgal systems for antibiotics removal

As stated before, microalgae-based systems have been seen as an emergent tool for wastewater treatment, particularly for removing pharmaceuticals such as antibiotics (Bai and Acharya, 2019; Villar-Navarro et al., 2018; Xiong et al., 2017a). However, the microalgal antibiotic removal rate depends on several factors, such as the algal species, growth conditions, and antibiotic degradation mechanisms, in which several experiments were performed and described in Table SM4 of Supplementary Material.

3.3.1. Microalgal mechanisms for antibiotics removal

The microalgal mechanisms for antibiotic removal mostly include bioadsorption, bioaccumulation, and biodegradation, as depicted in Table SM4. These mechanisms may occur as follows (Xiong et al., 2019, 2021): (i) fast passive adsorption due to the physicochemical properties of the cell surface and pollutants, followed by (ii) fairly slow mass transfer through the cell membrane, and (iii) culminates either with bioaccumulation, biodegradation, or both. Adsorption is an extracellular process, and its performance varies according to the structure, hydrophilicity, and functional groups of diverse antibiotics (Xiong et al., 2019). The interaction between antibiotics and microalgal cell walls can be classified as a non-metabolic and passive mechanism, mostly due to adsorption reactions, surface complexation and ion-exchange reactions, micro-precipitation, and chelation. Hydrophobic antibiotics can be absorbed into the microalgal cell walls or organic molecules discharged by microalgae into their adjacent environments, such as EPS (Sutherland and Ralph, 2019). EPS comprise a combination of high molecular weight polymers, including polysaccharides, lipids, proteins, nucleic acids, and humic substances, that protect cells from severe environments (Xiong et al., 2021). Usually, in response to antibiotics toxicity and as an adaptive mechanism, microorganisms excrete more EPS (Xiong et al., 2021). Microalgal EPS can be closely bound, weakly attached to the cell surface or soluble when excreted by microalgae. Additionally, microalgal cell walls and EPS generally have a negative charge due to hydroxyl carboxyl and phosphoryl functional groups. Hence, positively charged antibiotics can be adsorbed due to electrostatic forces (Xiong et al., 2019). The composition and quantity of EPS affect the non-polar antibiotics sorption. When a high ratio of proteins/polysaccharides is present in EPS, stronger hydrophobicity is created; thus, there is a higher number of adsorption spots for this type of antibiotic (Xiong et al., 2021). Also, non-living microalgal biomass can be used as a bioabsorption promoter, encompassing several benefits over the use of living microalgae, including (i) absence of toxicity concerns; (ii) increased algal sustainability and ecologically acceptability (if spent biomass can be used); and (iii) lower operating costs. For instance, de-fatted biomass (DB) of *Chlorella* sp. was able to remove cefalexin (initial concentration of 50 mg L^{-1}) in a percentage of 71.2 with a calculated theoretical adsorption capacity of 63 mg g_{DB}^{-1}, a removal efficiency very close to living *Chlorella* sp. biomass (82.7%) (Angulo et al., 2018). Similarly, lipid-extracted (LE) *Scenedesmus quadricauda* biomass showed a very high adsorption capacity in the removal of tetracycline (295 mg g_{LE}^{-1}) (Daneshvar et al., 2018).

In living microalgal cells, accumulation is a metabolic intracellular active pathway for antibiotics uptake mostly due to the binding of antibiotics to intracellular proteins (Xiong et al., 2019). Algae accumulation has been described as having a central role in removing antibiotics like sulfamethoxazole, trimethoprim, and doxycycline (Bai and Acharya, 2017). However, antibiotics accumulation can (Xiong et al., 2021): (i) induce the production of reactive oxygen species (ROS) to restore cells' baseline balance that otherwise could cause cellular damage or eventually death; or (ii) increase depletion of antibiotics, indicating that accumulation is a pre-step for biodegradation. In algal cells, the combined effect of accumulation and biodegradation significantly contributes to the full assimilation of some antibiotics, as observed for sulfamethazine and levofloxacin with *Chlorella pyrenoidosa* (Sun et al., 2017) and *C. vulgaris*, respectively (Xiong et al., 2017a). Otherwise, antibiotic accumulation in the organisms alone and possible amplification over the food chain can eventually induce antibiotic resistance.

In biodegradation, antibiotics are broken down within or outside the algal cells, and the originated derivatives are further consumed by algae (Leng et al., 2020). In intracellular degradation, the antibiotic is first adsorbed on algae, then gradually transmitted through the algal cell walls, and lastly fragmented by algal enzymes inside the cell (Yu et al., 2017). In extracellular degradation processes, the antibiotic is fragmented due to the action of extracellular enzymes, and the intermediates/end-products are metabolised inside algal cells (Leng et al., 2020). Moreover, the antibiotic degradation metabolism for algae can be categorized as (i) co-metabolic degradation patterns and (ii) metabolic degradation. In metabolic antibiotic degradation, which requires the action of specialised enzymes, antibiotics are the sole carbon and energy source, while in co-metabolic degradation another carbon and energy input is required. On the other hand, in co-metabolic degradation, non-specific enzymes are used to digest antibiotics, thus requiring an additional carbon source and energy input. Xiong et al. (2017a) showed that *C. vulgaris* could carry out both of these patterns in levofloxacin biodegradation. In terms of microalgal antibiotics degradation pathways, it was found that while *Scenedesmus obliquus* processed sulfamethazine by hydroxylation, methylation, and sulfamethoxazole degradation entailed deamination, nitrosation, hydroxylation and methylation (Xiong et al., 2017c, 2019). On the other hand, *Chlorella* sp. L38 metabolic degradation pathway of sulfadimethoxine occurred mostly by deamination reaction resulting in its transformation into hydroxyl- and amino-derivative compounds (Sun et al., 2018).

3.3.2. Effects of biotic and abiotic factors on antibiotic removal

Various abiotic and biotic factors can impact the microalgal removal of antibiotics from wastewater. Overall, the processing conditions that promote algal growth are the same that induce antibiotics removal. Among the abiotic factors, pH, salinity, light, and temperature have a major role in antibiotics removal efficiency (Norvill et al., 2017). The pH of the culture could intermediate the hydrolysis of some ionic antibiotics. While alkaline pH can induce the hydrolysis of tetracycline, improving its removal efficiency, other antibiotics like sulfamethoxazole and trimethoprim are stable when the pH is basic (Bai and Acharya, 2019; Norvill et al., 2017). pH variation does not affect the removal rate of antibiotics such as 7-amino cephalosporanic acid (7-ACA) due to its stability in the pH range of 6.3–8.0 (Guo et al., 2016).

The addition of saline compounds that stimulate algal growth, such as NaCl and sodium acetate, can also increase some antibiotic removal performance, such as levofloxacin. For example, *S. obliquus* and *C. vulgaris* increased levofloxacin bioaccumulation, and consequent intracellular biodegradation, in 88% and 3-fold respectively, when salinity stress was induced with 171 mM NaCl, in comparison to the experiment without salt (Xiong et al., 2017b, 2017c). Also, ciprofloxacin removal showed to be increased by *C. mexicana* in 3-fold with the addition of 4 g L^{-1} of sodium acetate (Xiong et al., 2017b).

Studies have also revealed that light can reduce antibiotic concentration in wastewater due to photodegradation, which can occur directly or indirectly (Norvill et al., 2017; Villar-Navarro et al., 2018). Direct photodegradation happens when the target pollutant absorbs light and is degraded. In contrast, indirect photodegradation can occur due to light absorption by dissolved organic compounds generating ROS, which in turn degrades the antibiotics (Norvill et al., 2017). It was observed that applying high light intensities to microalgal cultures, besides increasing dissolved oxygen (DO) and pH, also induced the production of ROS due to indirect photodegradation, which in turn promoted the removal of tetracycline (Norvill et al., 2017). Irradiance of 20 MJ m^{-2} d^{-1} stimulated the removal of norfloxacin, ciprofloxacin, and ofloxacin antibiotics by a polyculture composed mainly of *Coelastrum* sp. (90%) in a six-month operation HRAP (Villar-Navarro et al., 2018).

Regarding the biotic factors, there are two main aspects to consider when selecting the proper algal species to remove antibiotics from wastewater: (i) microalgal antibiotic inhibition; and (ii) microalgal antibiotic removal rate. Antibiotics can suppress the production of molecules related to algal growth, such as pigments (e.g. chlorophyll-a), and/or the activity of enzymes (e.g. catalase and superoxide dismutase) (Bashir and Cho, 2016; Leng et al., 2020). Hence, it is crucial to select microalgal species that are not affected by the antibiotic content in the wastewater. For example, it was observed that tetracycline and kanamycin decreased the photosynthetic activity and growth of *Dictyosphaerium pulchellum* in a concentration of 5 mg L^{-1} and *Micractinium pusillum* at 30 mg L^{-1}, due to their effect on protein synthesis (Bashir and Cho, 2016). Furthermore, micromolar concentrations of streptomycin compromised the photosynthetic activity of *C. vulgaris* (Perales-Vela et al., 2016). Antibiotics' inhibitory effect on microalgae is generally measured using the half-maximum effective concentration (EC_{50}), which consists in the antibiotic concentration that can inhibit 50% of the algal growth. However, in general, the EC_{50} values of most antibiotics are some orders of magnitude greater than the quantities in wastewater; hence, microalgae can resist their presence under these conditions. For instance, in municipal wastewater, while the concentrations of ciprofloxacin, tetracycline, and sulfamethoxazole were about 1 μg L^{-1}, the EC_{50} of *Pseudokirchneriella subcapitata* is 3.31 mg L^{-1}, of *Chlamydomonas mexicana* was 65 mg L^{-1}, and of *P. subcapitata* was 0.146 mg L^{-1}, respectively (Leng et al., 2020; Välitalo et al., 2017; Wang et al., 2017). Nevertheless, it should not be neglected that the inhibitory effect of a combination of antibiotics can be harsher than the one caused by a solo antibiotic and thus reduce the order of magnitude of EC_{50}. For example, it was observed that the *C. vulgaris* EC_{50} values after 96 h of exposition to erythromycin (85.7 μg L^{-1}), and enrofloxacin (124.5 μg L^{-1}), decreased to 39.9 μg L^{-1} when they were combined, demonstrating a synergistic effect among the two antibiotics (Zhu et al., 2019a).

The antibiotic removal rate greatly depends on algal species. Many studies indicate that *Chlorella* is quite effective in removing antibiotics. *C. pyrenoidosa* is often used to eliminate 7-ACA, cephalexin, amoxicillin, ceftazidime, cefixime and cefradine, among other antibiotics (Table SM4). Xiong et al. (2017a) observed a greater removal rate of levofloxacin and enrofloxacin using *C. vulgaris* compared to other species. However, other algae species may be less successful at removing antibiotics. For example, *C. mexicana* could only remove 13% of the ciprofloxacin (initial concentration of 2 mg L^{-1}) (Xiong et al., 2017b). Trimethoprim (0–11%, initial concentration of 1.6 ng L^{-1}) and sulfamethoxazole (11–32%, initial concentration of 18 ng L^{-1}) were only partially removed by *Nannochloris* sp. (Bai and Acharya, 2017). Nevertheless, besides *Chlorella,* other microalgae can remove other antibiotics. For example, *S. obliquus* could remove cefradine (initial concentration of

100 mg L^{-1}) by more than 60%, whereas *C. pyrenoidosa* could only remove it by less than 30% (Yang et al., 2017).

Nevertheless, the inoculum concentration and the HRT of the photobioreactor must be adjusted according to the algal species and antibiotic concentration, since algae need to extend their lag phase to adapt to an adverse environment (Villar-Navarro et al., 2018). This was observed in polycultures isolated from a wastewater bioreactor, mainly composed of *Chlorella* spp., when the tetracycline concentration increased from 1 μg L^{-1} to 20 mg L^{-1}, or due to the content of inhibitory substances like N-heterocyclic compounds (Leng et al., 2020).

3.4. Microalgal systems for pathogens removal

Microalgal systems have also been considered as an alternative for wastewater disinfection. It was observed that when growing microalgae in wastewater, hostile conditions for many pathogens were generated, mostly related to operational characteristics (Muñoz and Guieysse, 2006; Ruas et al., 2018). Additionally, some physiological characteristics of microalgal systems may play a key role in pathogens removal such as (Liu et al., 2018): (i) the content in humic substances; (ii) the production of antimicrobial metabolites and toxins; and (iii) the capacity of attachment to bacteria and sedimentation. Hence, microalgal systems are seen as a potential tool to develop new strategies and methods for wastewater disinfection by decreasing pathogens, such as faecal coliforms (Bouki et al., 2013; Ruas et al., 2022).

However, although abiotic factors have been pointed out as having a major role in pathogens removal, their elimination is complex and very strain-specific. Usually, it involves interactions among several abiotic factors, such as pH, DO, CO_2 concentration, light exposure, photoperiod, and HRT, that may directly or indirectly create adverse conditions for some pathogens (Liu et al., 2018; Muñoz and Guieysse, 2006; Ruas et al., 2018, Ruas et al., 2022). In earlier studies, light exposure (115–1973 μmol m^{-2} s^{-1}), DO (1.2–8.18 mg L^{-1}) and pH (above 8.5) induced *E. coli* removal, while *Enterococcus faecalis* removal was driven mostly by DO and light intensity, demonstrating that pathogens removal with high pH and DO values alone are not necessarily effective unless combined with light exposure (Posadas et al., 2017). Furthermore, the supplementation of CO_2 in microalgal systems, particularly operated in HRAPs, has been evaluated for the removal of both aerobic and facultative potentially pathogenic bacteria, such as *P. aeruginosa*, enterococci, *E. coli*, and total coliforms (Ruas et al., 2018). However, gas supplementation effects seem to be very specific. While a CO_2 supply of 30% (v/v) in the microalgal-bacterial consortium *C. vulgaris*-activated sludge improved the total coliform removal efficiency from 88.7% (1.1 log) to 99.4% (2.8 log), the same effect was not observed for either *E. coli* or enterococci removal (Ruas et al., 2018). The absence of CO_2 effects upon *E. coli* removal does not seem to be related to the wastewater and microalgal system composition. Either in anaerobically digested sewage treated with *Scenedesmus* sp., or in raw domestic wastewater treated with the microalga-bacteria consortia *C. vulgaris*-activated sludge, *E. coli* removal efficiency was high (>99% and 98.6%, respectively), either at high or neutral pH values (>9, or 7.7), regardless of the CO_2 supplementation (20% or 30% (v/v) CO_2) (Posadas et al., 2017; Ruas et al., 2018). On the other hand, the light intensity seems to have a synergetic effect with CO_2 supplementation on pathogens removal, as studied by Ruas et al. (2022). Using the same said *C. vulgaris* - activated sludge consortia, but with a light intensity of 439 ± 100 μmol m^{-2} s^{-1} and a photoperiod of 16:8 light:dark cycle (LDC), the CO_2 supplementation of 30% (v/v) increased *E. coli* removal by 11%. *Ent. Faecalis* removal seems to be negatively affected by CO_2 supplementation despite the use of high light intensities, with a 24% decrease in its removal compared to no gas supply. However, a photoperiod of 24:0 LDC (439 ± 100 μmol m^{-2} s^{-1}) seemed to be more effective in removing *Ent. Faecalis* when compared to a 16:8 LDC photoperiod. Nevertheless, positive results were not observed in *E. coli* and *P. aeruginosa* elimination. Additionally, other photoperiods with extended light cycles showed to be effective in pathogens removal (Ruas et al., 2022). Urban wastewater was effectively treated in terms of total coliform bacteria using a microalgal system composed of *Pseudochlorella pringsheimii*, in an indoor pond, with an 18:6 LDC (at 300 μmol m^{-2} s^{-1}) (Kumar et al., 2021). After 5 d, total coliform bacteria (initial load of 6 × 10^3 CFU mL^{-1}) dropped to non-detectable counts, and after 14 d, the total bacterial load was reduced in 8 log, including heavy metal resistant and multiple ARB.

In summary, it is challenging to combine maximum pollutants (conventional and antibiotics) uptake with high rates of pathogens removal. While pollutants uptake is dependent on the optimum microalgal growth, which requires optimal biotic and abiotic conditions (pH around 7–8, light intensity according to microalgal species requirements, and CO_2 availability), pathogens removal efficiency is closely related to alkaline pH (9–11), high light intensities, and high CO_2 or DO concentrations. Facing all the reports described above, for agricultural and municipal wastewater, two-stage cultivation systems should be considered in future studies, using microalgal polycultures and consortia with bacteria, namely with microalgae and bacteria belonging to *Chlorella*, and *Bacillus* genera, respectively, in a ratio of 3:1 (Huo et al., 2020; Ji et al., 2018). On the other hand, systems with autochthonous microalgal species and sludge bacteria seem to be a more promising solution for municipal wastewater treatment, with an extra possibility to combine secondary and tertiary treatments. In the first stage, inoculum concentration should be adjusted according to the pollutants concentration, namely antibiotics, and pH would be controlled with CO_2 sparging (that besides lowering the pH, would also increase the photosynthetic rate and removal of some pathogens such as *P. aeruginosa)*, to keep it in the range of 7–8.5, the optimal values for microalgal growth and pollutants availability and uptake (Onyeaka et al., 2021; Ruas et al., 2018; Villar-Navarro et al., 2018). At this stage, light intensity should be around 100–180 μmol m^{-2} s^{-1}. This range would prevent light limitation or light excess, avoiding microalgal photoinhibition or bacterial inhibition due to the creation of oxidative stress conditions (Meng et al., 2019). Then, in the second stage, the lack of pH control leads to alkaline conditions (above 8.5), creating hostile conditions to pathogens (namely faecal coliforms) and promoting additional hydrolysis of some antibiotics, such as tetracycline (Awuah et al., 2002; Bouki et al., 2013; Ruas et al., 2022). Additionally, light intensities should be increased, not only to avoid light restrictions to microalgae but also to increase DO and pH values, inducing direct and indirect photodegradation, due to the production of ROS, which would promote the removal of antibiotics (Norvill et al., 2017). Nonetheless, HTR conditions should be regulated according to pollutants concentrations in wastewater, namely carbon, nitrogen and phosphorus, and microalgal systems cell density, to avoid cell wash-out (Choi and Lee, 2015; Villar-Navarro et al., 2018). Furthermore, when using agricultural wastewater, a pre-sedimentation step should always be set to avoid a high concentration of suspended solids that would decrease microalgal systems' efficiency (Ganeshkumara et al., 2018).

4. Integrated wastewater treatment by microalgae: case studies

Along this review, the potential of microalgae to remove conventional pollutants, CECs, such as antibiotics, and potentially hazardous bacteria, has been discussed individually. However, the full potential of microalgae should be evaluated from an integrative perspective, in which microalgal systems can simultaneously remove biological and inorganic hazards. Due to global water scarcity and the expensive operation and maintenance cost of wastewater treatment, several research works and government projects have been proposed and developed using microalgal systems for wastewater treatment with further biomass valorisation. Microalgal systems have a crucial role in changing the paradigm of considering wastewater as disposable waste, and start to be considered a valuable resource, from which new added-value products can be obtained and proficiently used. Additionally, their use gathers high environmental and economic potential in the same process, strongly supporting a circular economy.

As presented in the introduction section, the agricultural sector is among the highest water-demanding sectors and is also the one with the highest wastewater generation. The European project 'Innovative Eco-Technologies for Resource Recovery from Wastewater' (INCOVER) developed an experimental microalgae treatment system for agricultural runoff and urban wastewater reuse, co-generation of added-value products, and reuse of treated wastewater (Uggetti et al., 2018). This setup used a microalgal system composed of a microalgal polyculture (including several eukaryotic and prokaryotic species of microalgae) in consortia with bacteria (Fig. 1A). The effluent inflow was adjusted to maintain a low concentration of heterotrophic bacteria (by keeping a low carbon concentration) and to particularly promote the growth of cyanobacteria for the accumulation of polyhydroxybutyrates in biomass, to be further used for bioplastics production. Concomitantly, the remaining biomass from polyhydroxybutyrates production was used

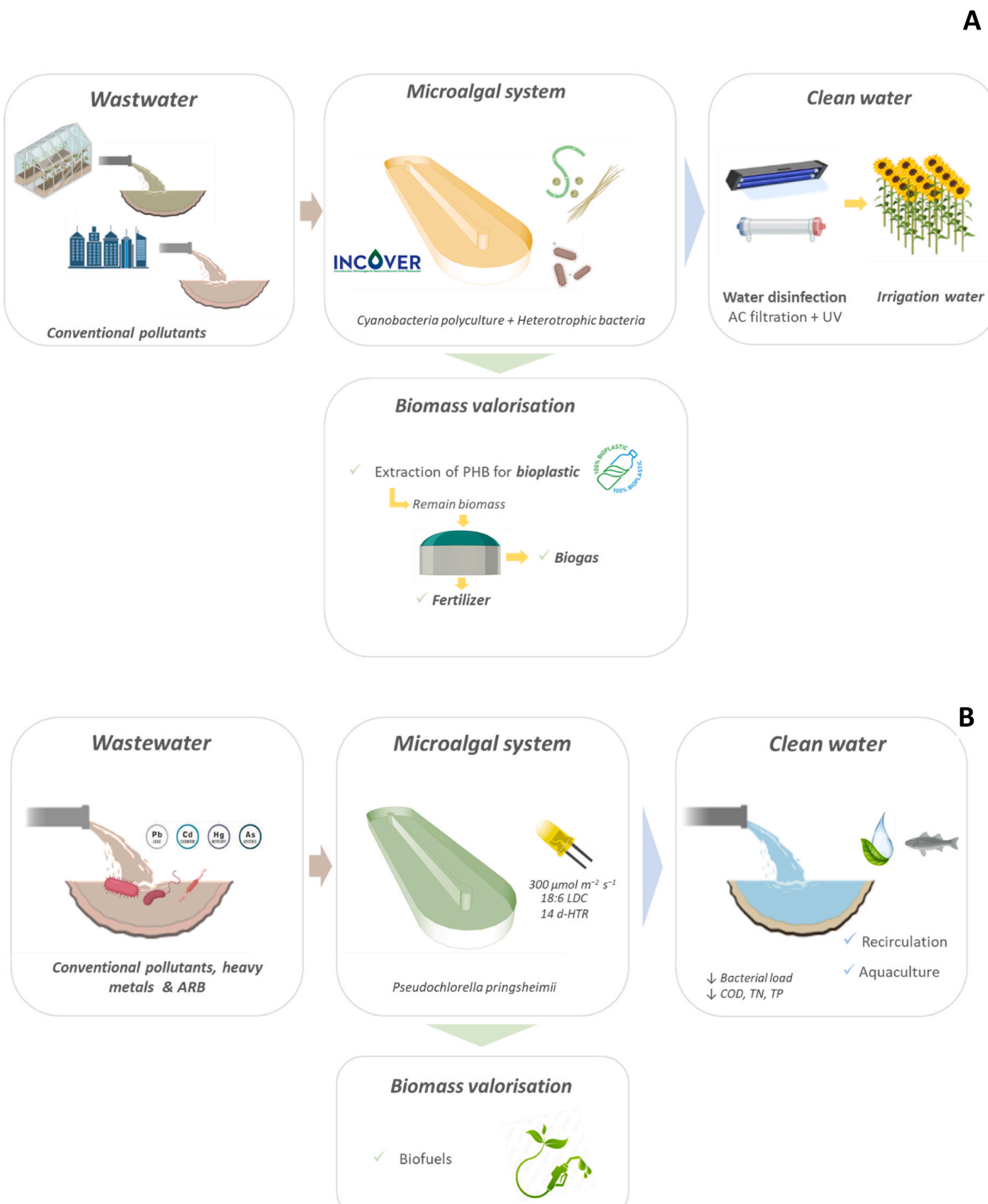

Fig. 1. Schematic representation of case-studies: (**A**) European project INCOVER, for the removal of conventional pollutants from agricultural and municipal wastewater, using a microalgal polyculture in consortia with bacteria. Microalgal biomass was used to co-produce PHB for bioplastics formulation, biogas, and fertilizers, and treated water for agricultural application; (**B**) Research work developed by Kumar et al. (2021) for conventional pollutants, heavy metals, and antibiotic-resistant bacteria (ARB) mitigation, using the microalga *Pseudochlorella pringsheimii*. Reuse of treated wastewater in aquaculture and microalgal biomass as a source of biofuels was envisaged.

to produce biogas by anaerobic co-digestion, along with secondary sludge, and the digestate was further processed to produce biofertilizers. The treated wastewater was then submitted to ultrafiltration and disinfection, through a solar-driven process that combines activated carbon filtration and UV disinfection, and then reused in a smart irrigation system to grow energy crops. The INCOVER setup proved to be possible to treat 6.9 m^3 of urban wastewater and agricultural runoff daily, producing around 2 kg d^{-1} of polyhydroxybutyrate-rich biomass and 150 L d^{-1} of biogas, and while using the treated water for irrigation of a 125 m^2 sunflowers plantation.

As discussed throughout the present review, the high content of CECs in urban wastewater has a strong contribution to the development of ARB. In this context, Kumar et al. (2021) developed a 105-L pilot-scale pond reactor for phyco-mitigation of conventional pollutants, heavy metals and ARB from urban wastewater using the microalga *P. pringsheimii* (Fig. 1B). This microalgal system provided an 83.2% reduction in COD, and TN and TP removals of 95.1% and 97.2%, respectively. Also, after 5 d of cultivation, total coliforms dropped to non-detectable counts (the initial count was 6×10^3 CFU mL^{-1}), and a reduction of 8 logs in the total bacterial load after 14 d of treatment was observed. As discussed before, (i) the depletion of nitrogen, phosphorus and carbon; (ii) the use of extended LDC (18:6) and high light intensity (300 μmol m^{-2} s^{-1}); (iii) the increase of DO concentrations and pH values; and, (iv) the adsorption of bacteria to microalgal cell surface, have a role in the total coliforms reduction. However, even after 14 d, three out of ten initially identified antibiotic-resistant strains remain on the treated water, being *Pseudomonas peli* the dominant bacterial species, proving the difficulty of reducing such opportunistic bacterial genus. After this process, due to a higher lipid and carbohydrate content, 21% and 30% respectively, when compared to the control (grown with Bold's Basal Media), the obtained microalgal biomass in this system showed potential to be used for biofuel production (Kumar and Bera, 2020). Furthermore, treated wastewater was successfully used to cultivate *Catostomus* fishes (average survival rate of 84%) when compared the raw wastewater (survival rate of 0%), proving that the treated wastewater is a valuable water resource.

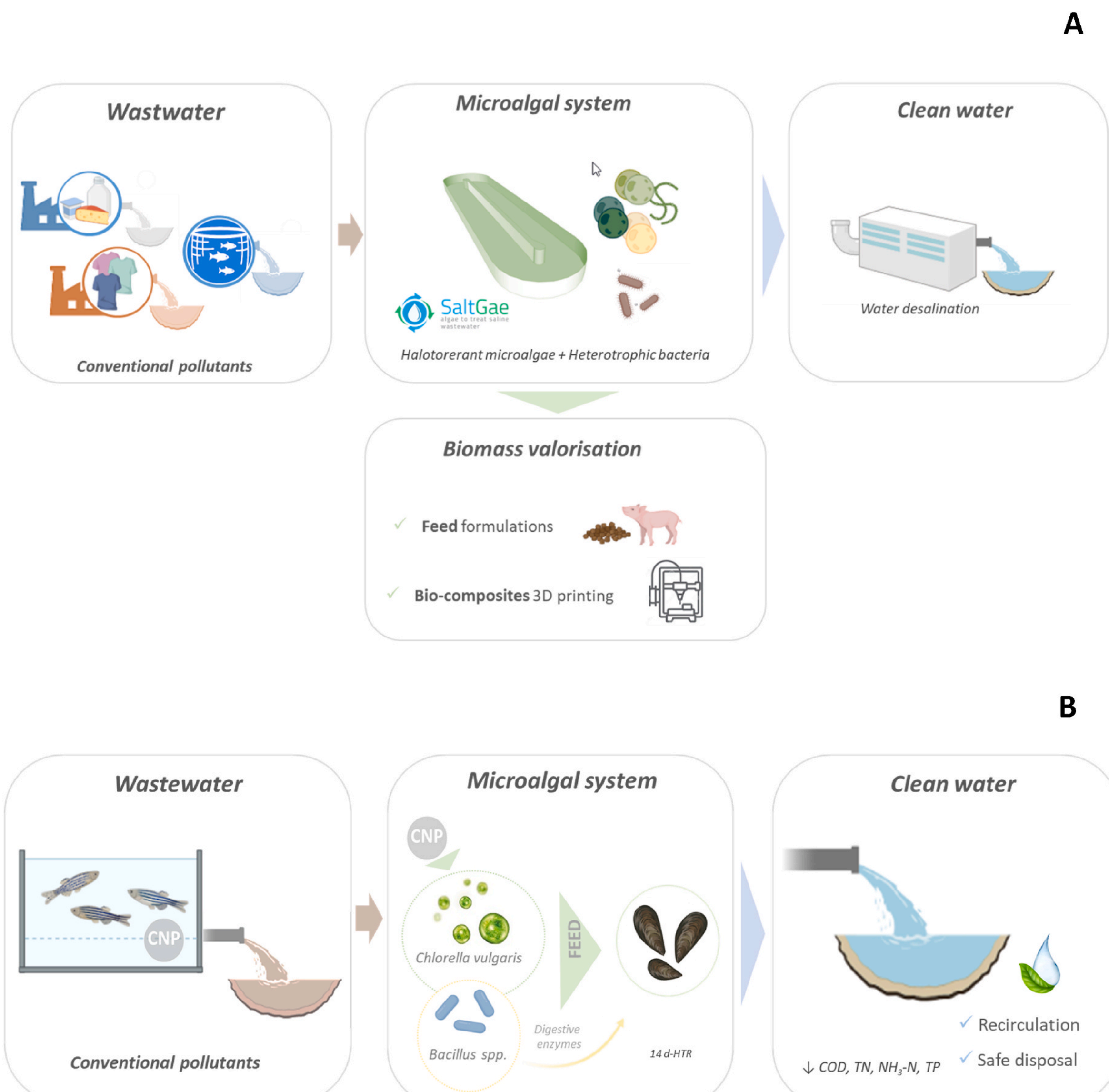

Fig. 2. Schematic representation of case-studies: (**A**) European project SaltGae, for the removal of conventional pollutants from industrial salted wastewater, using halotolerant microalgal species in consortia with bacteria, with a further desalination process of treated wastewater. Microalgae biomass was further used to feed formulations and obtain biocomposites for 3D printing; (**B**) Research work developed by Geng et al. (2022) for the removal of conventional pollutants from intensive aquaculture wastewater, using a consortium composed by the microalga *Chlorella vulgaris* and the bacteria *Bacillus subtilis* and *Bacillus licheniformis*. Treated wastewater was safely disposed and microalgal biomass was fed to mussels.

Industrial wastewater treatment is challenging due to its diverse composition. Effluents with high concentrations of salts and organic matter (e.g., from meat processing, canned fish, pickled vegetables, aquaculture or tanneries) are very difficult and expensive to treat, namely for small businesses. Aiming to reduce the economic and environmental impact of saline wastewater, SALTGAE's, a H2020 project funded by the European Union, proposed the use of halotolerant algae/bacteria consortiums systems, in HRAPs, for the elimination of organic matter, conventional pollutants, and co-production of high added value by-products. In this microalgal system, species such as *Dunaliella salina*, *Spirulina*, *Tetraselmis suecica* and *Chlorella* sp. were tested for the treatment of wastewater from the milk industry, tannery, and marine fish species aquaculture (Fig. 2A). The combination of bacteria and algae allowed not only to reduce 90% of the energy required for aeration but also to obtain algal biomass for the formulation of several products ranging from feed for piglets and fruit protective edible coatings to bio-composites for 3D printing (Cordis, 2019). This projected not only opened a window in the development of green technologies towards an efficient salted wastewater treatment but it was also innovative in the development of new green bio-materials obtained from an environmentally sustainable process.

Also, the disposal of wastewater from intensive aquaculture has a huge environmental impact worldwide. To find a solution to alleviate such environmental stress, Geng et al. (2022) developed a mussel/microalgae-bacteria system that could effectively reduce NH_3–N, TP, and COD in 94.7%, 92.9%, and 77.8%, respectively, after 6 d, due to the complementary ecosystem functions between the bacteria *B. subtilis* and *B. licheniformis*, and the microalga *C. vulgaris* (Fig. 2B). In batch experiments of 1000 L, it was possible to keep a self-maintained system for 35 d of operation, maintaining in the treated effluent COD, TN, NH_3–N, and TP concentrations of approximately 30, 0.8, 0.3, and 0.3 mg L^{-1}, at which the conventional pollutants in wastewater were assimilated by *C. vulgaris* cells, and microalgal biomass was fed to mussels (*Hyriopsis cumingii*) through continuous filter-feeding (Fig. 2B). Additionally, and besides their role in NH_3–N removal, *B. subtilis,* and *B. licheniformis* enhanced the digestive enzyme activities of the mussel. Furthermore, this system proved to be effective at large scale, in lagoon, for 7 months, this multitrophic microalgal system reached 90 thousand mussels per hectare, at which a mature mussel has a filtration capacity of 50 L per day. Moreover, the implementation costs of these systems at large scale are similar to the ones of traditional chemical WWTP, which makes this system very promising in terms of the development of aquaculture industry. However, studies on possible bioaccumulation of wastewater contaminants in mussel tissues were not carried out and this should be considered to fully validate its application at an industrial level.

Inspired by microbial mats and their effectiveness in bioremediation due to the biofilm organisation, a different approach was developed by Melnikova et al. (2022) for wastewater treatment. This system gathered wastewater treatment, the absence of microalgal harvesting requirements, and the use of microalgal biomass as fertilizer. Using the microalga *Chlorella sorokiniana* and natural materials such as alginate and cotton textile, the authors developed a novel nature-inspired biomaterial named AlgalTextile (AT), in which microalgae were immobilised in the alginate gel and attached to the organic textile (Fig. 3). Also, an open-type horizontal photobioreactor was developed, combining the increase of wastewater treatment efficiency (76% removal of total bound nitrogen and 99% of phosphate phosphorus - PO_4–P) with the convenience of simplified biomass harvesting. At the end of the treatment, the potential use of AT as a biofertilizer for cress *Lepidium sativum* was demonstrated, inducing a 35% greater length in comparison to the control. Besides, AT can also be used as an anti-erosion and anti-desertification agent, as well as a biofuel feedstock. Although this system was only tested at lab scale, it was developed to be tailored according to the needed proprieties in terms of type of microorganism, size, type of textile, type of gel and density. The portability of the finished mat was pointed out as an extra advantage in terms of transportation, it can be rolled up and easily carried to the application place.

All these case studies were funded by governmental entities from Asia (China, India and Russia) and by the European Union supporting the high interest in the implementation of microalgal systems for wastewater treatment. Despite the scale stage of each study, the economic and environmental benefits of these systems are highlighted in all scenarios, as well as their feasibility to be used to treat diverse types of wastewaters (such as from aquaculture, agriculture, municipal and

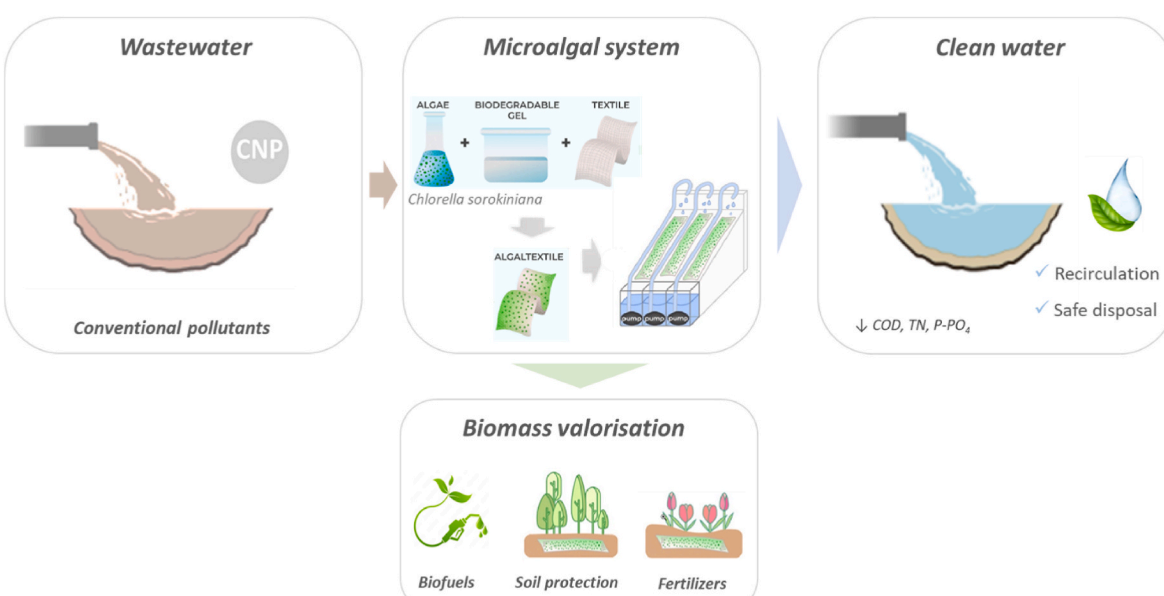

Fig. 3. Schematic representation of the integrated wastewater treatment, developed by Melnikova et al. (2022), for conventional pollutants removal, using the microalga *Chlorella sorokiniana*. Microalgae cells were immobilised in a biodegradable support composed of alginate and cotton textile, AlgalTextile (AT), and placed in an open-type horizontal photobioreactor. Treated wastewater was safely disposed and microalgal biomass had the potential to be used for biofuel production, as an anti-erosion and anti-desertification mat, and as a biofertilizer.

industrial sources), and to be used in a very diverse set-up (pond reactors, PBRS, lagoons). The conclusions of these case studies reinforce the high potential of application of microalgal systems at large scale, with the possibility of tailor the microalgal systems according to the specific needs and to build them on site.

5. Future work and challenges

As discussed throughout the review, many parameters affect microalgal systems' growth, productivity, and pollutant removal efficiencies. Several studies have shown the success of microalgal systems in pollutant uptake from several wastewaters; however, although some case-studies prove its applicability at large scales, most of them still are carried out on a laboratory scale, particularly the most fundamental ones, at which most of the variables are controlled (Yong et al., 2021). However, the truth is that on an industrial scale, the microalgal cultivation systems are exposed to fluctuating environmental conditions (e.g. nutrient availability, temperature, pH, light intensity and photoperiods). Thus, further research should focus on the optimisation of the operating conditions considering this real scenario at large scale. The knowledge regarding the cooperation and interactions between microalgae and bacteria is still scarce, and the relationship between the two types of microorganisms may affect the efficiency of the remediation process. This lack of knowledge threatens the development of a sustainable and cost-efficient microalgal-bacterial consortia system. Hence, more studies focusing on understanding the microalgal-bacterial interactions, such as the specific enzymatic stages and key metabolic pathways used, would be useful.

Selecting microalgal species and bacterial strains is critical to improve the efficiency and sustainability of microalgal systems in wastewater remediation. In a very specific scenario, such as the presence of very hazardous contaminants, and considering the use of closed microalgal cultivation systems, the development of genetically modified strains could also improve the symbiotic relations between microalgae and bacteria. The ideal microalgal species should exhibit certain features, such as (Jiang et al., 2021): (i) low sensitivity to the growth-inhibiting substances released by bacteria; (ii) resistance to harsh and severe environments; (iii) ability to regulate itself to suit fluctuating environmental conditions; and (iv) tendency to form flocs in cooperation with bacteria to facilitate harvesting. Nevertheless, few studies have investigated the benefits and potential of algae-fungi consortia (Li et al., 2022), and this potential could be explored in a near future.

In antibiotics removal, even though the biodegradation mechanisms of antibiotics have been widely investigated, the mechanisms of removal are not fully understood, especially on a molecular level. For example, there is strong evidence that EPS play an important role in the bioadsorption and extracellular biodegradation of antibiotics; however, the underlying mechanisms associated with the complex interactions between EPS and antibiotics remain unclear. Additionally, apart from the presented microalgal mechanisms for antibiotics removal, other active mechanisms can occur that are not yet disclosed, hence future research should explore that possibility (Li et al., 2022). Microalgae exhibit a high potential to be converted into biochar, and this material can be applied to treat wastewater; however, its potential for antibiotics removal has not been yet explored. Some fungi can produce extracellular enzymes to effectively degrade a wide variety of antibiotics. Hence, consortia of microalgae and fungi may be a good alternative for the treatment of these chemical pollutants.

Knowledge about the impact of microalgal systems on wastewater disinfection is still scarce. It was reported that operating conditions, such as pH, DO, dissolved CO_2, and light exposure, are the prime factors affecting pathogens removal. However, the mechanisms of their synergetic or antagonistic effects are not yet fully understood. Besides, it was observed that the effects of some abiotic factors are strain-specific, like DO and dissolved CO_2. Light is crucial for photosynthesis and thus for the effectiveness of microalgal growth, and as discussed before, it is also a key factor in disinfection, however, few studies have focused on the effect of light intensity on both microalgal growth and disinfection. On the other hand, some researchers indicated that microalgae could have a role in the increase of faecal coliforms, which is sometimes associated with the increased organic matter due to microalgae cell death. This reinforces the importance of future studies focusing on strategies to overcome such constraints and optimise operating conditions towards optimal microalgae cell growth and disinfection effects. Nonetheless, due to the antagonistic abiotic factors requirements, the big challenge is to combine microalgal growth (and thus high conventional pollutants uptake) with high disinfection rates, hence as proposed, the development of a two-stage bioprocess could meet both microalgal systems application.

Furthermore, some environmental sustainability issues of the use of algal systems for wastewater treatment technologies can be questioned (Yong et al., 2021): (i) algae and bacteria could produce the greenhouse N_2O, which has a global warming potential 298 times higher than CO_2; (ii) the use of open photobioreactors could increase water footprint in water-stressed areas due to the high evaporation rates, and (iii) algae can accumulate pesticides, toxins and heavy metals, hence biomass use should be fully scrutinised in terms of their content before its use for other purposes. Also, the use of algal biomass from wastewater treatment to produce algae-based biofuels and bioenergy has been widely reported, however, it must be ascertained case-by-case if the process is cost-competitive and feasible for production at an economic level (Yong et al., 2021).

6. Conclusions

This review aimed to provide a critical and updated overview of the potential use of microalgal systems as wastewater bioremediation agents towards the simultaneous removal of conventional pollutants, antibiotics and/or pathogens. It was concluded that microalgal-bacterial systems could enhance biomass production, facilitate biomass harvesting, and improve pollutant removal from wastewater. However, species selection and their ratios require particular attention. Considering recent studies on wastewater treatment at lab-scale, species from the *Chlorella* genus seem to be the most promising for conventional pollutants and antibiotics removal. However, the feasibility to keep a monoculture at large scale seems unreasonable. Hence, the most proper strategy is the use of autochthonous microalgal polycultures in consortia with bacteria. Biotic and abiotic factors strongly affect microalgal systems' efficiency, but different purposes might require opposite conditions. While high light intensity could play a negative role, particularly due to the inhibition of nitrite-oxidizing bacteria activity leading to NO_2–N accumulation, it also showed to have a main role in pathogens removal. Furthermore, light exposure can induce direct and indirect photodegradation of some antibiotics, such as tetracycline and disinfection due to the formation of ROS species. HRAPs are the most used reactors for microalgal wastewater bioremediation due to their low costs associated. However, the area/volume ratio and light intensity exposure/fluctuation are sensitive parameters that can affect the treatment efficiency, particularly in disinfection. The inoculum size and the HRT of the photobioreactor must be adjusted according to the algal species and contaminants concentration for an efficient bioremediation. High pH showed to significantly contribute to the reduction of faecal coliform in wastewater stabilisation ponds and the hydrolysis of pH sensible antibiotics, such as tetracycline, improving their removal efficiency. However, controversial results were observed in CO_2 supplementation in HRAPs, while it increases microalgal growth and the removal efficiency of *E. coli*, *P. aeruginosa* and conventional pollutants, it has the opposite effect on *Ent. Faecalis* removal. Nonetheless, and despite some challenges being discussed, the presented case studies point out the efficiency of microalgal systems in wastewater remediation along with the generation of biomass that can be converted into products with high commercial value. In summary, all the evidence gathered in this review supports the importance and the potential of using microalgal systems as a tertiary wastewater treatment to promote a circular bioeconomy.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2023.117678.

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