Chapter 6

Tertiary Treatment: Microalgae-based Wastewater Treatment

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6.1 Introduction

To address risks to environmental quality, nutrient limits in treated wastewater are mandated by many countries and regions, particularly where the final effluent is to be discharged into a water body. Various techniques are deployed to remove nutrients in centralised wastewater treatment systems, including chemical dosing (for phosphorus) and biological nutrient removal (for phosphorus and/or nitrogen). These techniques are generally unsuited to decentralised wastewater treatment, since they rely on process controls or chemical interventions that require specialist training and frequent maintenance.

Tertiary treatment in decentralised systems instead relies upon nutrient uptake by vegetation in constructed wetlands or swales (Al-Muyeed, 2017; Capodaglio, 2017), although phosphorus removal by adsorption in media filters is also possible (Bunce *et al.*, 2018). Microalgae offer the potential to intensify wetland treatment techniques, cultivated in bespoke reactors that maximise biomass productivity and nutrient removal. Excess biomass can be periodically harvested for supply to secondary value chains.

This chapter outlines the development of microalgal technologies – from potential protein sources in the mid-twentieth century, through to their potential roles in tertiary wastewater treatment, and barriers to their implementation in this application (Section 6.2). Section 6.3 goes on to explore the interactions between microalgae and bacteria in attached-growth communities, before Section 6.4 considers important operational aspects when exploiting the potential of these communities. Section 6.5 outlines the development of the INNOQUA Bio-Solar Purification (BSP) module from laboratory to pilot scale, presenting results from demonstration facilities in India, Peru and Spain.

6.2 The Potential of Microalgae Biotechnology

Microalgae biotechnology is a wide field of research with extended theoretical and practical applications in multiple sectors (e.g., agriculture, pharmaceutical, food industry, aquaculture, sanitation, bioenergy) and is gaining particular attention in environmental and circular bioeconomy applications (e.g., wastewater treatment, climate change mitigation) (Li *et al.*, 2019; Haarich *et al.*, 2017; Nagarajan *et al.*, 2020). Following initial market developments based on soil-grown crops and woody biomass, microalgal biomass may be considered a third-generation feedstock for the production of biofuels and bioproducts (Chowdhury and Loganathan, 2019). Microalgal biomass is increasingly seen as a feedstock that avoids many of the economic and environmental disadvantages associated with cultivation and processing of first and second generation feedstocks (Ubando *et al.*, 2020; Maity *et al.*, 2014).

Multiple applications for microalgal biomass have been demonstrated at bench and pilot scale, including biofuels, high-value chemicals (pharmaceuticals, cosmetics, etc.), food supplements, bioplastic, as well as fertilisers (Hayes *et al.*, 2017; Khan *et al.*, 2018; Javed *et al.*, 2019; Patil and Kaliwal, 2019). However, full-scale implementation of microalgae biotechnology has been limited, requiring further development to improve economic feasibility in many applications and in some cases to overcome social barriers regarding the origins of algal derivatives. Key aspects of economic feasibility in all applications include cultivation, harvesting and processing (de Carvalho *et al.*, 2020; Tang *et al.*, 2020). Social barriers relate to acceptability of novel practices such as the supply of wastewater-cultivated food-grade proteins (Matassa *et al.*, 2015) as well as multiple regulatory restrictions (Kehrein *et al.*, 2020).

6.2.1 From Opportunity to Implementation

Although the Venezuelan government developed the cultivation of phytoplankton for industrial purposes and extraction of carotene compounds since the early 1930s (Burlew, 1953; Jorgensen and Convit, 1953), it was during the second World War (1939–1945) that widespread interest in microalgae biotechnology was prompted by the need for new protein sources (Goldman, 1979). *Chlorella* spp. (mainly *C. pyrenoidosa*, *C. vulgaris* and *C. ellipsoidea*) and *Scenedesmus* spp. were among the first microalgae tested to show a high tolerance to varying environmental conditions and thus potential suitability for large-scale use. Despite the high proportion of target compounds in microalgae cells, lack of experience in microalgae cultivation meant that experimental studies were limited. However, by 1951, a pilot-scale cultivation system had been implemented by the Carnegie Institution in the USA. This specifically examined the influence of operational conditions (including those determined by local environments) on microalgae growth rates (Cook, 1951). Similar research investigations were also conducted in Germany, England, Israel and Japan where microalgae species were tested in open pond systems or even closed cultivation tubes (or 'photobioreactors') with increased biomass productivity being the focus of much of the work. This progression is shown in Fig. 6.1.

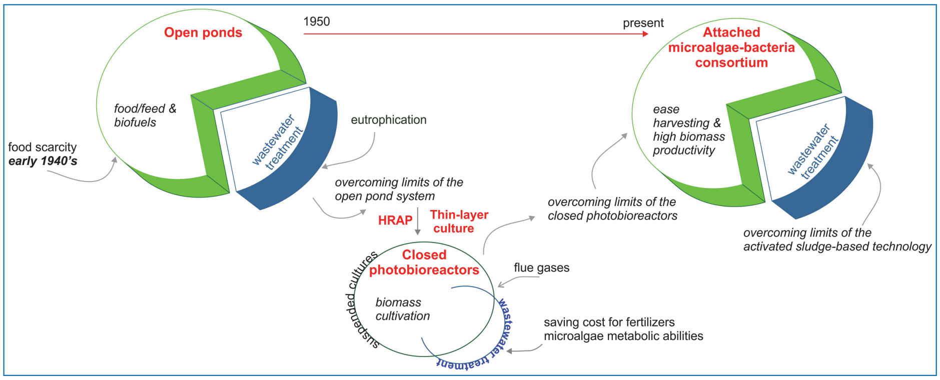

Figure 6.1. The technical evolution of microalgae cultivation systems.

Developments in algal cultivation techniques coincided with growth in the conventional agriculture sector, which caused a decline in interest in the food potential of microalgae. At the same time, their potential to remove nutrients as part of domestic wastewater treatment began to be explored, with cultivation in large-scale algal ponds (Oswald *et al.*, 1955). In addition, it was proposed that excess microalgal biomass from wastewater treatment processes could be used for methane generation via anaerobic digestion (Meier, 1955; Oswald and Golueke, 1960). The potential for positive impacts on both wastewater treatment and links to what is now called the circular economy has prompted ongoing cycles of research and commercial interest in this sector.

Concepts to use microalgae for wastewater treatment generally exploit two aspects:

- the symbiotic relationship between microalgae and bacteria, as primary producers in natural trophic networks; and
- the ability of microalgae to consume inorganic nitrogen and phosphorus from wastewater as an efficient mechanism to prevent or reduce downstream eutrophication.

The core of the symbiotic relationship harnessed in the wastewater treatment process is represented by a nutrient-support exchange between microalgae (which ensure oxygen supply by phototrophy) and aerobic heterotrophic bacteria (which provide macronutrients (mainly nitrogen and phosphorus) and inorganic carbon (CO_2) through degradation of organic matter) (Posadas *et al.*, 2017).

Despite being easy to design and operate, open pond cultivation systems are relatively deep (around 1 m) and host low microalgae concentrations (<500 mg/L) which result in increased costs for biomass harvesting and dewatering, and low organic matter removal efficiency (5–10 g BOD/m^2 pond surface area.day). This leads to relatively long hydraulic retention times (HRT) of between 10 and 40 days, and thus larger systems. In general, wastewater as a nutrient medium can deliver biomass productivity of up to 21 g/m^2.day in an open pond system (Ozkan *et al.*, 2012). The depth of open pond systems can create a downward gradient in O_2 concentrations, reducing algal efficiency and creating a requirement for mixing.

The efficiency of open pond systems was improved through the development of high rate algal ponds (HRAPs) in the 1970s and 1980s. HRAPs are characterised by a shallow depth (<0.5 m) and are equipped with a paddlewheel mixing system which increases photosynthetic activity and organic matter removal efficiency (around 35 g BOD/m^2.d) (Muñoz and Guieysse, 2006). These characteristics allowed HRT to drop below 10 days, while maintaining biomass productivity of between 15 and 25 g/m^2.d (Goldman, 1979). HRAPs also allowed the possibility for selected colony-forming microalgae to be cultured, thus allowing decreased harvesting costs (Mehrabadi *et al.*, 2015). However, despite proving their efficiency for both domestic and agricultural wastewater treatment (Hoffmann, 1998), HRAPs, as with open ponds, require a large land surface (ranging from around 6–10 m^2 per person equivalent) which is about 50 times higher than conventional activated sludge processes (Park *et al.*, 2011; Acién *et al.*, 2016).

Despite their relatively large land area requirements and low biomass productivity, cultivation ponds (open ponds and HRAPs) now account for more than 95% of commercial microalgae biomass cultivation systems, due to their simplicity of design and operation (Acién Fernández *et al.*, 2013).

The algal turf scrubber (ATS) is another configuration exploiting the potential for algae to remove nutrients from water flows. Craggs *et al.* (1996) demonstrated annual ATS removal of phosphorus from agricultural runoff and eutrophic lake water of 0.73 ± 0.28 g P/m^2.day, at an average periphyton productivity (microalgae and bacteria alike) of 35 g/m^2.day. Notably, the authors stressed the influence of the diurnal variation of light on ATS performance, recording significantly reduced nutrient removal efficiency at night.

Issues associated with mixing and oxygen gradients can also be overcome through the use of thin layer reactors, developed in the 1960s (Doucha and Lívanský, 2006). In comparison with open ponds, thin layer reactors typically operate at shallower water depths ($<$0.05 m) which promotes photosynthetic and respiratory efficiencies, leading to higher biomass productivity (up to 55 g/m^2.day) (Masojídek *et al.*, 2011) and lower hydraulic retention times (3–5 days) (Acién *et al.*, 2016).

The theoretical maximum photosynthetic efficiency[1] of microalgae is 9–10% (Vecchi *et al.*, 2020) compared to terrestrial plants of 1–2% (Peccia *et al.*, 2013). By using thin-layer reactors, a photosynthetic efficiency of 7% has been reported in outdoor conditions (Doucha and Lívanský, 2006), although maximum efficiencies of 9% are thought possible in such scenarios (Doucha and Lívanský, 2009; Morales-Amaral del Mar *et al.*, 2015a). One of the main features of these reactors is the slight slope applied to the surface ($<$3%) which prevents biomass settling and avoids the use of mixing equipment – reducing energy consumption (Acién Fernández *et al.*, 2013).

Other ‘thin layer’ adaptations of algae-based technology include algal rotating disk (ARD) and rotating algal biofilm reactor (RABR) systems. The RABR comprises rotating flexible belts that improve CO_2 diffusion and ensure uniform light exposure. One pilot scale study (8,000 L reactor) for photoautotrophic tertiary wastewater treatment reported average total nitrogen and total phosphorus removals of 14.1 g/m^2.day and 2.1 g/m^2.day, respectively, with biomass productivity (both microalgae and bacteria) of 31 g/m^2.day (Christenson and Sims, 2012).

Photobioreactors also perform well when compared with open pond systems, with biomass productivity of up to 47 g/m^2.day (Brennan and Owende, 2010). However, such reactors can have relatively high operational costs. Efficient mixing is necessary to minimise biomass attachment to vessel walls, and to ensure good nutrient and light distribution. This leads to energy demands that can be 15 times higher than required for mixing in open pond systems (Ozkan *et al.*, 2012). Other important factors include manufacturing/construction costs and system maintenance which will vary between technologies and applications.

1. That is, the proportion of energy in intercepted light that is converted to chemical energy via photosynthesis.

6.2.2 Microalgae and Tertiary Wastewater Treatment

Conventional (centralised) wastewater treatment technologies are increasingly energy intensive, and significant contributors of the GHG emissions within the water industry as a whole (Mamais *et al.*, 2015). Of the total energy consumption required for wastewater treatment, aeration is the most energy-intensive process, accounting for about 40–60% (Chae and Kang, 2013; Gu *et al.*, 2017). Furthermore, about 50% of the organic carbon from wastewater loadings is 'lost' as CO_2 during aerobic treatment (Muñoz and Guieysse, 2006) and there is growing concern around N_2O and CH_4 emissions from these processes.

Reliance on aeration during conventional wastewater treatment serves to signpost one of the key attractions of combined microalgal–bacterial systems, in which photoautotrophic metabolism can serve as an oxygen source for bacterial biomass. Photosynthesis ensures oxygen saturation of more than 100% during the light phase, and dissolved oxygen remains at between 30% and 50% saturation even during dark periods (Tiron *et al.*, 2015). Photosynthesis also acts as a temporary sink for CO_2, which can be accumulated by microalgae at an annual rate of around 2 kg CO_2/kg biomass (Xiaogang *et al.*, 2020).

Depending on influent loading, microalgae–bacteria biomass concentrations of up to 1 kg/m^3 wastewater can develop, around five times greater than could be expected in a conventional activated sludge process (Acién *et al.*, 2016). This leads to an excess of microalgal–bacterial biomass that can serve as a feedstock for other processes – whether bioenergy (through fermentative conversion to gaseous or liquid biofuels) or higher value products, supporting the commercial development of microalgae-based technologies and the wider bioeconomy.

The nutrient-accumulating characteristics of microalgae are another primary consideration and can be leveraged in tertiary wastewater treatment systems to reduce nutrient concentrations in final effluent more efficiently than conventional chemically or biologically-mediated approaches. Liu *et al.* (2017) report total nitrogen concentrations of less than 10 mg/L and total phosphorus concentrations of less than 1 mg/L in the effluent from a microalgal/bacterial system. Luxury uptake of phosphorus by microalgae has also been demonstrated, suggesting that lower discharge concentrations might also be possible, mirroring the pattern seen in phosphate-accumulating bacteria (Khanzada, 2020). Through techno-economic analysis, Chalivendra (2014) showed that the annual costs required for inorganic nutrient (N/P) and heavy metal (Cr and Cd) removal by conventional activated sludge processes could be decreased 7- and 26-fold, respectively, by using microalgae.

To date, the potential for microalgae-mediated treatment has been tested on a wide range of water and wastewater sources, including municipal wastewater;

industrial effluents from food processing, textile manufacture, aquaculture and livestock farming; acid mine drainage; centrate from anaerobic digestion; contaminated groundwater and contaminated surface waters (Wang *et al.*, 2012; Van Den Hende *et al.*, 2014; Garcia *et al.*, 2018; Zerrouki and Henni, 2019). Studies (mostly based on laboratory-scale work) have provided evidence for the utility of microalgae for pollutant/nutrient removal; the microalgal–bacterial interactions during wastewater treatment; economic advantages that could be obtained and the wider economic potential for using wastewater to produce microalgae as a feedstock for various high-value compounds and other commercial, industrial or agricultural purposes.

Microalgal–bacterial biomass can be an efficient method for removal of cationic heavy metals and specific toxic compounds (phenols, cresols, nitrophenols, etc.) from wastewater (Surkatti and Al-Zuhair, 2018). Removal of dyes, polycyclic aromatic hydrocarbons and endocrine-disrupting compounds has been shown with microalgae (Zhuang *et al.*, 2020). According to a 2006 literature review (Muñoz and Guieysse, 2006) microalgae cells can accumulate significant concentrations of heavy metals (up to 192 $mg/g_{biomass}$) and sustain a high removal efficiency by adsorption mechanisms (up to 114.2 $mg/g_{biomass}\cdot d$), depending on species and the metal being targeted. For example, the microalga *Ulothrix* spp. was tested for heavy metal removal efficiency from acid mine wastewaters on a photo-rotating biological contactor with a 24 hour hydraulic residence time (Orandi *et al.*, 2012). Initial metal concentrations ranged between 80 and 100 mg/L (Cu), 2–3 mg/L (Ni), 35–45 mg/L (Mn), 18–20 mg/L (Zn), 0.005–0.007 mg/L (Sb), 0.03–0.04 mg/L (Se), 0.3–0.5 mg/L (Co) and 0.07–0.09 mg/L (Al). The study reported removal efficiencies ranging between 20% and 50% with the following selectivity Cu > Ni > Mn > Zn > Sb > Se > Co > Al. The tolerance of other species (*Chlorella* spp., *Scenedesmus* spp., *Oscillatoria* spp. and *Nitzchia* spp.) to heavy metals has also been demonstrated (Acién *et al.*, 2016).

Studies have also demonstrated that operational conditions impacted by photoautotrophy (such as the increase in pH, temperature and oxygen values) can prompt a decrease in populations of undesirable pathogens such as faecal coliforms (Ansa *et al.*, 2012). Replacing mechanical with 'biological' oxygenation also decreases risks of pollutant volatilisation (Muñoz and Guieysse, 2006).

6.2.3 Major Barriers to Market Adoption

Despite the many potential benefits of utilising microalgae in wastewater treatment, scaling-up has faced several technical and economic challenges. Cost and energy input linked to microalgae harvesting remains a significant issue – the economic impact of this downstream process, for open ponds, being about 20% of total cultivation costs (Davis *et al.*, 2011; Fasaei *et al.*, 2018).

The harvesting problem comes from microalgae cell particularities, the most commonly used species having a cell diameter lower than 30 μm and a cellular density similar to that of water (Granados *et al.*, 2012; Wang *et al.*, 2013). Centrifugation, chemical flocculation, filtration and dissolved air flotation are some of the most frequently applied harvesting techniques, each with their own advantages and disadvantages. However, even where two or more harvesting techniques are combined, microalgae removal efficiency rarely exceeds 95% (Tiron *et al.*, 2017). Furthermore, the remaining microalgae cells can impact effluent quality and compromise system functionality. Moreover, high levels of retained moisture in harvested biomass present challenges to downstream processing (Polizzi *et al.*, 2017; Khan *et al.*, 2018), and commercial microalgal applications tend to focus on high value functional characteristics or compounds (such as use in dietary supplements or pharmaceutical formulation) that preclude their cultivation in wastewater for reasons of perception, safety or quality management.

6.3 Microalgal–Bacterial Interactions

6.3.1 Overview

Microalgal–bacterial interactions within biofilms have gained significant recent research attention. In a wastewater treatment context, photoautotrophic microalgae, through photosynthesis, provide the necessary oxygen supply for aerobic processes (mainly organic matter degradation and nitrification). In turn, the macronutrients in the medium and/or supplied by bacteria (such as CO_2, NH_4^+, PO_4^{3-} and NO_3^-) are used by microalgae for cellular growth (Fig. 6.2). Inorganic nitrogen can also be provided for microalgae by nitrogen-fixing bacteria, while bacteria such as *Pseudomonas* spp., *Bacillus* spp. and *E. coli* are known to be excellent sources of inorganic phosphate for microalgae (Zhang *et al.*, 2020).

In addition to the bilateral exchange of macronutrients, growth-stimulatory compounds can also be exchanged. For instance, bacteria can provide essential microalgae cell growth compounds such as vitamins and hormones – while microalgae are producers of vitamins and hormones (Kiseleva *et al.*, 2012; Kim *et al.*, 2014) that are important for bacteria. Given that over half of the known microalgae species cannot synthesise essential vitamins required for their own cell development (Fulbright *et al.*, 2018), this interspecies relationship is essential. Indeed, relationships within the microalgal–bacterial phycosphere can be unidirectional (commensalism), bidirectional (mutualism) and/or parasitic (Yao *et al.*, 2019), exploiting three main pathways: stimulation/inhibition of growth; quorum sensing communication and gene transfer (Amin *et al.*, 2012; Kouzama *et al.*, 2015).

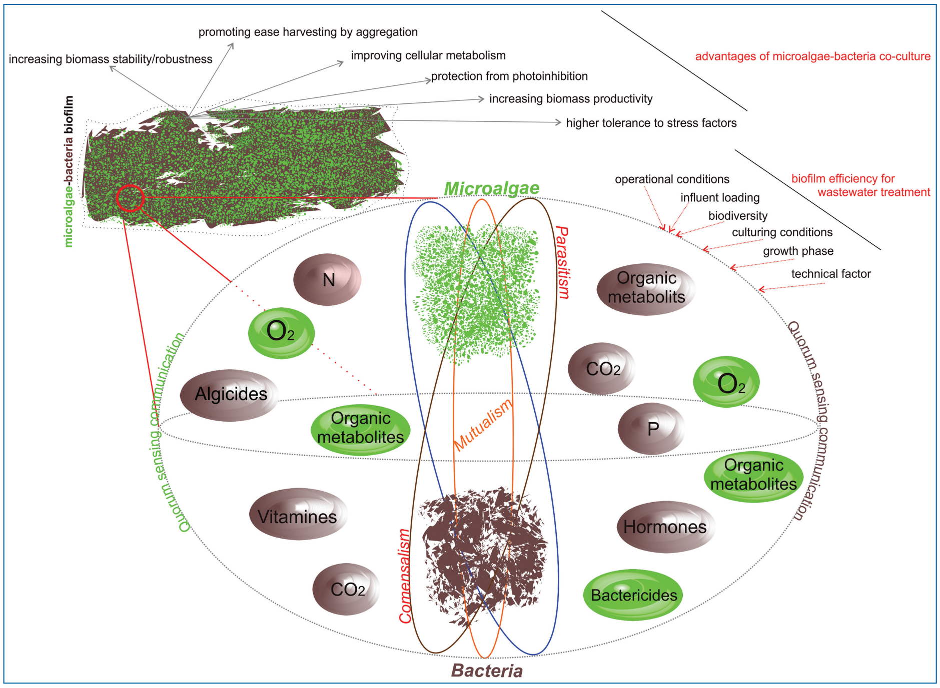

Figure 6.2. Possible microalgae–bacteria interactions occurring in the biofilm structure, the resulting effect on biofilm performance and stability, and factors influencing biofilm efficiency during wastewater treatment.

These interactions and interdependencies can benefit growth rates of both bacterial and algal populations. For example, the presence of 'microalgae-growth-promoting bacteria' can increase microalgal productivity by up to 70% (and vice versa) (Ramanan *et al.*, 2016). Kim *et al.* (2014) recorded an increase in the biomass productivity of microalgae *Chlorella vulgaris*, *C. reinhardtii*, *Scenedesmus* spp. and *Botryococcus braunii* by 70.3, 64.5, 92.7 and 59.6%, respectively, in co-culture with *Rhizobium* spp. In turn, the presence of green microalgae increased development of *Rhizobium* spp. by up to 7.8-fold. This positive effect is also seen in wastewater treatment. By using *Auxenonochlorella protothecoides* and *C. sorokoniana* in winery wastewater treatment, Higgins *et al.* (2018) recorded an up to 6-fold increase in bacterial productivity. Toyama *et al.* (2018) reported an increase in productivity of the microalgae *C. reinhardtii*, *C. vulgaris* and *Euglena gracilis* by 1.5, 1.8–2.8 and 2.1-fold, respectively, after 7 days of co-culturing with indigenous bacteria from a swine wastewater effluent. The counterpoint to this is that there is also a range of competitive effects between the different populations. These range from simple competition for essential micronutrients through to parasitism and even the secretion of algaecides or bactericides (Zhang *et al.*, 2020).

Other interactions that can occur during microalgae–bacteria system development – for example cell-to-cell interactions through quorum sensing communication and gene transfers – are less studied. Quorum sensing communication refers to intercellular communication sustained by an exchange of signalling compounds (such as lipid-based molecules, bacterial signalling molecules n-Acyl-homoserine lactones (AHLs) and microalgae secondary metabolites (allelochemicals)) which influence specific gene expression (Zhang *et al.*, 2020) and have a stimulatory, regulatory or inhibitory effect (Gross, 2009). This type of intercellular communication influences a population's abundance and richness – and impacts on practical operational aspects relevant to such systems. For example, while Ramanan *et al.* (2016) reported uncertainties around the mechanisms by which bacteria promote microalgal bioflocculation, Zhou *et al.* (2017) subsequently observed that in the presence of the bacterial signalling molecules AHLs (extracted from activated sludge), the microalga *Chlorophyta* spp. was stimulated to secrete specific aromatic proteins which promoted self-aggregation of the biomass in flocs and increased the efficiency of biomass settling by up to 41%.

Another possible interaction that could arise between microalgae and bacteria populations is horizontal gene transfer. This kind of exchange is more likely under stressful conditions, with transfers occurring from bacteria to microalgae. The stability of such transfers varies tremendously, along with the eventual location of transferred material and mechanism of expression within the receiving cell, but there is evidence that such transfers have allowed eukaryotes to adapt to changing environments – for example, where nutrients are limiting Husnik and McCutcheon (2018). Although they provide insights into the adaptability mechanisms of microalgae and bacteria, the practical implications and applicability of such transfers are as yet unknown, but could eventually support niche wastewater treatment applications.

6.3.2 Advantages of Attached Growth Communities

Algae-based biofilms are characterised by dense, multi-layer biological structures comprising a mixed population of microalgae and bacteria. Biofilm development mainly occurs in two steps. The first step consists of biomass attachment onto a support material through physico-chemical interactions (hydrophobic/hydrophilic, acid–base interactions, etc.), the second step is characterised by the intervention of secreted polymeric substances. The presence of bacteria increases the rate of microalgal adherence through these two mechanisms, decreasing biofilm establishment times and improving the stability of the biomass structure.

Attachment of microalgae–bacteria systems (defined as immobilisation on the surface of the support material) leads to more complex biologic and metabolic

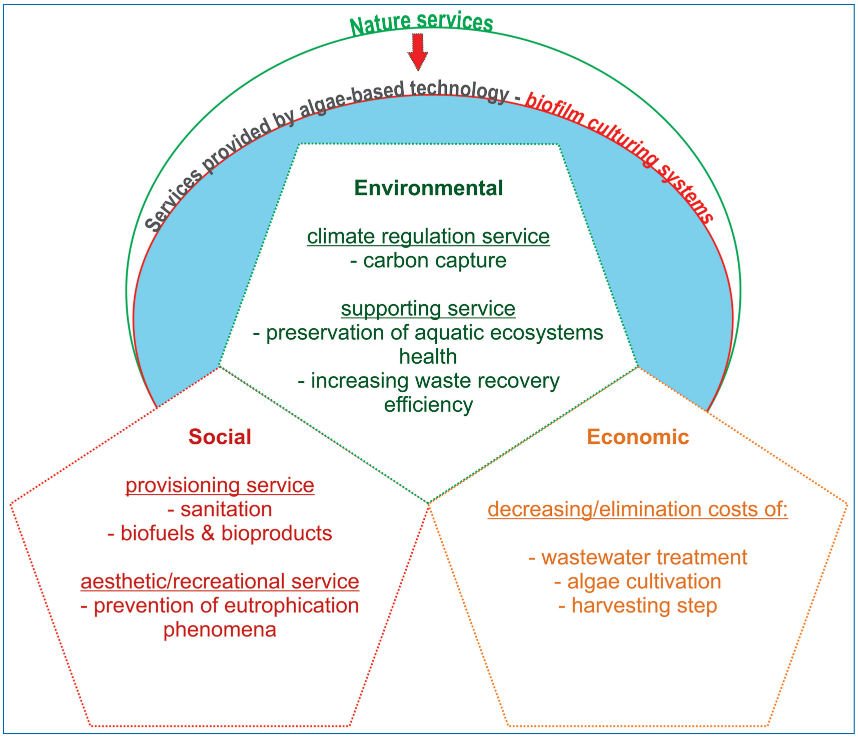

Figure 6.3. Services provided by microalgae-based biofilm culturing systems.

networks compared with suspended biomass. Community composition, extracellular physico-chemical attachment mechanisms and a wide range of operational factors (technical, influent loading, laboratory/outdoor conditions, etc.), lead to systems with different behaviours than those observed in suspended systems with similar community composition. Hence, there is a need for advanced research on interspecies relations, the influence of excreted metabolites on biomass functional activities and treatment performance (involving physico-chemical mechanisms that in turn sustain other biological functions). It is believed that quorum sensing communication could have a high influence on biofilm development and stability, even from the start-up stage, influencing both population size and species richness (Irie and Parsek, 2008). Difficulties in comparing behaviours across previous studies are further compounded by system design and operational differences that also influence these parameters. However, it is unquestionable that attached-growth microalgal–bacterial biofilms can deliver a number of services (Fig. 6.3), including wastewater treatment, which are considered hereunder.

Chlorella spp. and *Scenedesmus* spp. are the most studied microalgae in biofilm systems, being noted from around 40% of published studies in a recent review (Zhuang *et al.*, 2018). Compared with suspended growth cultures, the density of attached growth cultures can significantly decrease the operating land area required (Christenson and Sims, 2012). This has important impacts on the economic analysis of biofilm reactors over (often more easily) operated (low-cost) open pond systems. As an example, in a study on microalgae cultivation Morales-Amaral del Mar *et al.* (2015a) emphasised that even though costs for the operation of thin layer attached reactors are almost four times higher than raceway suspended-growth reactors, the difference between reactor productivity (45 g/m^2.day vs. 24 g/m^2.day) substantially decreased the price for biomass cultured in thin layer attached reactors. Biomass productivity per plan surface area can be further optimised by using multiple layer reactors (Roostaei *et al.*, 2018), applying different operational approaches or different support material configurations. For example, Gross and Wen (2014) obtained biofilm productivity of up to 18.9 g/m^2.day by using a pilot-scale RAB cultivation system in a vertical configuration.

Increases in biomass density can also improve photosynthetic efficiencies (particularly in the surface layers of a mixed community biofilm). When comparing photosynthetic efficiencies of the microalga *Scenedesmus* spp. cultivated in diluted centrate from anaerobic digestion under similar operational conditions, maximum values of 9% were demonstrated in the thin-film attached-growth reactor, compared with 5% in the suspended-growth raceway reactor (Morales-Amaral del Mar *et al.*, 2015a).

Harvested biofilm biomass also has lower water content than harvested suspended biomass. Polizzi *et al.* (2017) report moisture levels of between 80% and 90% in scraped biofilm, which is comparable to that of centrifuged suspended culture. This point was also highlighted by Johnson and Wen (2010), who harvested *Chlorella* spp. biofilm from a dairy wastewater treatment system and determined its water content at around 94% – which was sufficient to avoid the use of a centrifuge for preliminary dewatering. In culturing a *Botryococcus braunii* biofilm, Ozkan *et al.* (2012) with a biomass productivity of 0.71 g/m^2.day and a biomass density of 96.4 kg/m^3, achieved a decrease in required dewatering energy requirements of 99.7% during harvesting and post-harvesting steps.

Harvesting frequency is another important factor, with Johnson and Wen (2010) reporting increased productivity and nutrient removal with shorter harvest intervals (Table 6.1). They treated dairy manure wastewater with *Chlorella* spp. biomass in laboratory conditions, with light irradiance of between 110 and 120 $\mu mol/s.m^2$.

In terms of performance obtained for wastewater treatment, organic matter and N and P removal efficiencies vary depending on factors such as the reactor design, microalgae and bacteria species, influent loadings, harvesting period, etc. Although it has been suggested that attached-growth microalgae–bacteria systems can sustain higher wastewater treatment efficiencies when compared to suspended cultures (Zhang *et al.*, 2020), a recent review highlighted that only 10% of relevant

Table 6.1. Biomass and nutrient removals at various harvest intervals, as reported by Johnson and Wen (2010).

Harvest Intervals	Days	6	10	15
Nitrogen (N)	$g/m^2.d$	0.77	0.59	0.39
Ammonium (NH_4-N)	$g/m^2.d$	0.74	0.45	0.3
Phosphorus (P)	$g/m^2.d$	1.45	0.8	0.47
Biomass	$g/m^2.d$	3.5	3	2

publications included performance data from operations outside laboratory environments (Zhuang *et al.*, 2018). According to Zerrouki and Henni (2019), several pilot-scale systems (only) using attached microalgae technology for wastewater treatment are known to have been successfully implemented worldwide.

According to Acién *et al.* (2016), the theoretical maximum mean nitrogen removal rate that can be achieved by microalgae biomass is 3.5 g/m^2.d with maximum mean biomass productivity of 50 g/m^2.day. Boelee *et al.* (2014a) recorded a removal rate of 3.2 g NH_4-N/m^2.day, 0.41 g PO_4-P/m^2.day and 43 g COD/m^2.day at an HRT of 4.5 hours when treating synthetic municipal wastewater. Treating municipal wastewater in a 32 m^2 pilot-scale thin-layer cascade photobioreactor (0.02 m water depth), Sánchez Zurano *et al.* (2020) recorded daily removal rates of between 15 and 30.6 mg/L NH_4-N, 1.8 and 5.6 PO_4-P mg/L, and 81 to 178.3 mg/L COD depending on seasonal variations; biomass productivity ranged between 28.3 and 47.3 g/m^2.day. This equates to removals of 1.0–2.0 g NH_4-N/m^2.day, 0.12–0.37 g PO_4-P/m^2.day and 5.4–11.9 g COD/m^2.day.

Various supporting matrices have been trialled for algal biofilm wastewater treatment processes – Adey *et al.* (1993) reported highest microalgae productivity (15–27g/m^2.d) when treating agricultural run-off wastewater with biomass attached to plastic screens – while Zhuang *et al.* (2018) found that cotton, polycarbonate and cellulose acetate were the most commonly used supports. Melo *et al.* (2018) tested borosilicate glass, polyurethane foam, polyvinyl chloride, stainless steel, polyethylene and polypropylene, reporting that PVC was the most appropriate for culturing *C. vulgaris* on a rotating flat plate photobioreactor (RFPPB).

6.4 Operational Aspects

6.4.1 Biofilm Community Structures

Biodiversity (referring to community share and species richness) is an important functional parameter that helps define biofilm characteristics and performance.

A high microbiological diversity is associated with complex metabolic and inter-species relations at the cellular level, and can increase biomass value, wastewater treatment performance outcomes and economic and environmental impacts. In other cases selected or engineered biodiversity (e.g., through inoculation) can enhance removals of targeted pollutants, increase biomass value (for further use as a feedstock for energy production or co-product extraction), or even harvesting efficiency (by using self-aggregating/colonial species or large cell size microalgae). As is the case in suspended microalgae–bacteria cultures, filamentous microalgae play an important role in biomass attachment efficiency, density and harvesting – and are specifically targeted for implementation of ATS technologies (Adey *et al.*, 2011).

Identifying factors that can limit the presence of undesirable communities is important in ensuring biofilm efficiency. These factors include (Doucha and Lívanský, 2006):

- Pathogenic viruses and bacteria
- Fungi
- Grazers (protozoa and rotifers) and other predators
- Undesirable microalgae species and some cyanobacteria

Compared to suspended-growth microalgae–bacteria cultures, high physical density within biofilm structures can decrease the risk of culture contamination with unwanted communities (Doucha and Lívanský, 2006). In general, it is far more complex and challenging to maintain 'desired' populations in uncontrolled large-scale applications, as community structures undergo significant changes over time due to the impacts of wastewater physico-chemical and biological characteristics, and wider environmental conditions (Carney *et al.*, 2014).

Diverse biofilm structures contain microalgae species with different trophic patterns (photoautotrophic, heterotrophic and mixotrophic) (Roostaei *et al.*, 2018). Depending on insolation levels, conditions suited to autotrophic and mixotrophic microalgae can occur. Under such conditions, the carbon source (e.g., wastewater) can be assimilated by both heterotrophic bacteria and mixotrophic microalgae, potentially increasing wastewater treatment efficiency. Mixotrophic growth also has a positive effect on microalgae cell lipid content, which can be important when this is a target for downstream processing/valorisation (Zhan *et al.*, 2017). Mixotrophic growth has been found in a large number of microalgae, including *C. vulgaris*, *C. regularis*, *Spirulina platensis*, *Haematococcus pluvialis*, *E. gracilis*, *Nannochloropsis* spp., *Arthrospira* spp., *Synechococcus* spp., *Anabaena* spp., *Phaeodactylum* spp., *Botryococcus braunii*, *Tetraselmis* spp., *Scenedesmus* spp. and *Desmodesmus* spp.

It is also important to note that some microalgae (e.g., *Chlorella* spp.) are capable of nitrification (Gerardi, 2002). Between ammonium and nitrate nitrogen sources, it is assumed that microalgae will assimilate ammonium rather than nitrate compounds due to lower energy requirements for synthesis. However, the preference for one or another nitrogen source varies even within the same genus (Liu and Chen, 2016).

Several methods are commonly used to identify and quantify microorganisms (such as microscopy, fluorescence and PCR/qPCR). In recent years, alternative methods have been proposed for rapid *in vivo* assessment of microalgae communities (and their competitors/predators), such as spectroradiometric monitoring (Reichardt *et al.*, 2020). These emerging techniques should allow biofilm structure and characteristics to be more carefully monitored and controlled, allowing wastewater treatment processes to be further optimised.

6.4.2 Light Irradiance

Alongside other operational parameters, light represents an important driving factor in autotrophic cultivation as it controls photosynthetic activity, and thus oxygen supply – and is closely linked to biofilm productivity.

Photosynthetically active radiation varies with season and latitude but an average of around 1800 μmol/m^2.s reaches the surface of the earth on sunny days (Masojídek *et al.*, 2014). However, photosynthetic activity only increases with light intensity to a certain level (light saturation level) which is around 1/10th of maximum irradiance (Torzillo *et al.*, 2010). Prolonged exposure to excessive irradiance can lead to photoinhibition, through damage to photosynthetic structures within algae (Nikolaou *et al.*, 2015). Different microalgal species have different strategies to counter photoinhibition – including lipid accumulation and secretion of carbohydrates – allowing them to become photo-acclimatised (Nikolaou *et al.*, 2016) and (Ramanan *et al.*, 2016).

Determining mechanisms of photo-acclimatisation in biofilm structures is complicated by the physical arrangement of mixed microalgal and bacterial communities – which can lead to physical shading of algae by bacteria (Schnurr *et al.*, 2016), although the mechanisms of light distribution within biofilms require further research (Wang *et al.*, 2015). Although provision of uniform irradiance across and within biofilms might be perceived as useful for maximising photosynthetic productivity, this may not be required or desirable in wastewater treatment applications where chemolithotrophic bacteria use nitrate as an energy source. Indeed, relationships between irradiance, photoperiod and biofilm community and physical structure must all be considered in the context of the wastewater being treated – and the objectives of that treatment.

6.4.3 Flow Velocity and Turbulence

Another important parameter to be considered during biofilm development is the flow velocity and turbulence as this influences shear stress, diffusion processes and biofilm stability. The impacts of flow velocity and turbulence can be technology-specific but in one study which looked at this issue González *et al.* (2008) reported that a flow velocity of 0.4 m/s resulted in biofilm disintegration and compromised treatment performance, particularly in terms of COD removal efficiency. In this study a flow velocity of less than 0.1 m/s was required to ensure biofilm stability.

6.4.4 pH

One of the challenges that arises during microalgae cultivation is control of pH, which is impacted by photosynthetic phenomena (as well as biochemical wastewater treatment processes). In general, organic carbon degradation and nitrification processes will reduce pH through destruction of alkalinity and production of CO_2 whereas denitrification processes or nitrate uptake by microalgae will restore some of this lost alkalinity (for example, through simultaneous cellular OH^- release). However, nitrification may be reduced where microalgae compete with nitrifying bacteria for ammonium.

The impacts of pH vary between microalgae cultivation systems. For example, axenic microalgal cultures can sustain a high increase in pH value due to the absence of bacterial activity. Meanwhile, the presence of bacterial populations can have a strong buffering effect on pH, limiting its increase during microalgae activity. A high pH value (mainly higher than 9) can lead to increased ammonium removal through volatilisation, while higher pH values also favour phosphate precipitation with Ca/Mg or autoflocculation phenomena (Muñoz and Guieysse, 2006).

6.4.5 Wastewater Nutrient Loads

One of the factors which influences nutrient removal efficiency is the ratio between nitrogen and phosphorus concentrations (N:P) in the influent. Although developed during studies on marine phytoplankton, the Redfield ratio (C:N:P – 106:16:1) is commonly used as the basis for microalgal cultivation (Smith *et al.*, 2017). However, the N:P ratio of microalgae biomass is species-specific, and ranges from 8N:1P to 45N:1P (Hecky *et al.*, 1993). Wastewater influent can comprise wide variations in nutrient concentration (both within treatment plants and between various wastewater types) with 'ideal' compositions rarely occurring. This drives the general adoption of nutrient-specific processes. Microalgae have been shown to alter their nutrient composition at a cellular level in response to varying nutrient concentrations in their host environment (Whitton *et al.*, 2016), a feature which can be leveraged in wastewater treatment applications. Where the composition of influent is stable, correlations between nitrogen and phosphorus removal efficiency and biomass productivity can be established (Morales-Amaral del Mar *et al.*, 2015a). However, if the target of a tertiary treatment process is to deliver final effluent of particular nutrient characteristics (for example, to meet regulatory limits), then tailoring of the influent nutrient load and/or optimisation of the biofilm community structure may be required (Sadatshojaei *et al.*, 2020).

Zhuang *et al.* (2020) observed that attached microalgae biomass responds less to the presence of nitrate sources than to organic carbon, phosphate and ammonium (they also reported removal efficiencies ranging between 78.2% and 93.2% for all of the measured parameters: COD, TN, TP, NH_4-N and PO_4-P). At a certain concentration (usually above 100 mg NH_4-N/L, although the precise response is species-specific), ammonium-nitrogen can be toxic to microalgae biomass. For example, Morales-Amaral del Mar *et al.* (2015) found concentrations above 192 mg NH_4-N/L decreased *Scenedesmus* spp. productivity when cultured in diluted centrate from anaerobic digestion.

6.4.6 Environmental Conditions

The performance of attached microalgae–bacteria cultures used for wastewater treatment under laboratory conditions will generally be different from onsite pilot or full-scale tests and thus it is being important to test the biofilm behaviour in environmental conditions that are as similar as possible to the intended final application. For instance, decreases in nutrient removal efficiency (of between 1- and 3-fold) and biomass productivity (of between 10- and 13-fold) as well as modification of the community structure were noted by Van Den Hende *et al.* (2014) when upscaling a novel wastewater treatment technology from indoor lab-scale reactors to outdoor conditions. Boelee *et al.* (2014a) also noted differences in process efficiency when municipal wastewater was treated in a pilot-scale biofilm reactor under real conditions, as compared with treatment at bench scale. They speculated that light and temperature were the possible limiting factors. Bacterial communities can also undergo changes during scaling-up, even between small to medium and large systems operated under the same outdoor conditions (Fulbright *et al.*, 2018).

Even where inoculated with target biomass, biofilm community diversity can undergo multiple changes during the start-up stage. As a result, it is encouraged to use microalgae species with a high tolerance to stressful operational factors such as *Dunaliella salina*, which is found frequently in open pond systems (Xiaogang *et al.*, 2020). Similar shifts in community structure and diversity have also been noted from systems inoculated with native biomass (Sekar *et al.*, 2004) significantly increasing start-up periods (Liu *et al.*, 2017).

Recently, a tool based on photo-respirometry models has been proposed which can enable estimation of algae–bacterial growth rates in different environmental conditions (Rossi *et al.*, 2020). The method proposed is designed to allow for fast and reliable calibration of algae–bacterial growth models as a function of environmental conditions, and optimal growth conditions can be identified for different algae strains. The tool leverages a standard photo-respirometric model calibration protocol and has been validated in an HRAP system treating digestates.

6.4.7 Harvesting Frequency

In contrast to the various methods available for harvesting biomass from suspended-growth systems, published literature suggest that a simple scraping approach is the only applicable method for biofilm harvesting. Questions then arise as to optimal harvest frequency. If biofilm harvesting occurs after the exponential growth phase, treatment performance can decrease significantly as a result of senescence within the film. Dead cells and a destabilised phycosphere can contribute to increased organic carbon and phosphorus concentrations (Jiang *et al.*, 2007). Other consequences of late biomass harvesting are an increase in biofilm thickness and density with a negative effect on photosynthetic activity, increased ash content, biofilm detachment that can negatively impact effluent quality (by increasing turbidity) and the immigration of predators, with their direct impacts on biofilm biodiversity and stability. Furthermore, bacteria have different growth rates to microalgae, meaning that in a mixed culture system – even where microalgae have entered a stationary or decline phase – a significant increase of the bacterial growth rate can still occur through consumption of metabolites and other nutrients released from the microalgal biomass. This can lead, in turn, to destabilisation of the trophic network and compromise system functionality. On the other hand, harvesting immature biomass that has yet to reach its metabolic peak will reduce treatment performance.

Although optimum biomass harvesting frequency is species-dependent, the usual rule of thumb is to use an interval of between 7 and 14 days (Siville *et al.*, 2020).

6.5 INNOQUA Microalgae-based Module

Within the INNOQUA system, the microalgae-based bio-solar purification (BSP) unit is the module designed for polishing lumbrifilter (primary and secondary treated) effluent in mild climates with high insolation. It can provide an addition or an alternative to other tertiary treatment technologies, before disinfection and/or wastewater discharge and reuse.

The focus of the INNOQUA project was decentralised wastewater treatment applications, and thus cost and maintenance efficiency were key considerations. The module developed was an open thin layer cascading photobioreactor, whereby the influent was fed to the reactor during daytime and a recirculation pump was employed to ensure the required exposure of wastewater to sunlight with an overflow mechanism for effluent from the recirculation tank.

6.5.1 Laboratory Scale Testing

This concept was developed and tested at laboratory scale in the Environmental Technology department of ECOIND (a public research and development organisation based in Romania). The laboratory systems were developed to:

(i) Assess the influence of platform design (cascade vs. single platform – Figs. 6.4 and 6.5) on performance;
(ii) Assess the influence of water depth (1 cm and 5 cm) on reactor performance;
(iii) Establish stable operation and assess potential treatment performances of this treatment step prior to pilot scale development and
(iv) Evaluate biomass-specific growth and its impact on effluent suspended solids.

6.5.1.1 Materials and methods

The experimental apparatus comprised two photobioreactor configurations (with one configuration run simultaneously at two different water levels) each with the same total platform area and capacity of 10 L. All reactors were run in duplicate and all run in parallel:

– Double cascade reactors with two platforms each with an average water layer depth on the platform of approximately 5 cm (Fig. 6.5a);
– Thin layer cascade reactors with two platforms each with an average water layer depth on the platform of approximately 1 cm (Fig. 6.5b) and
– Single platform reactors with an average water layer depth of approximately 5 cm (Fig. 6.5c).

All experiments were performed at laboratory scale and used synthetic wastewater designed to replicate secondary treated municipal or domestic wastewater. The reactors were fitted with individual feeding pump and recirculation pumps and illuminated artificially using LED photosynthetic light sources. The experiment used photoperiodicity of 12 hours light and 12 hours darkness at 12,000 lumens/m^2 and a hydraulic loading rate of 250 L/m^2.day. All experiments were performed at room temperature ($22 \pm 5°C$).

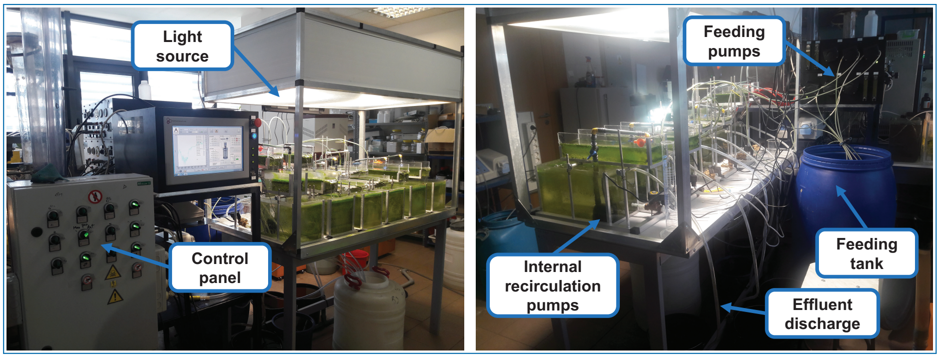

Figure 6.4. Laboratory set-up (front view – left; back view – right).

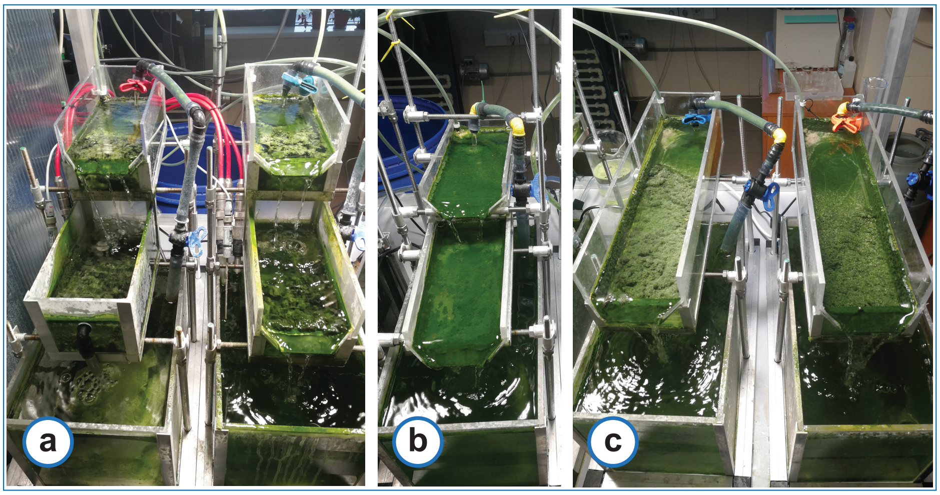

Figure 6.5. Laboratory reactors (a) double cascade photobioreactors, (b) thin layer cascade photobioreactors and (c) single platform photobioreactors.

6.5.1.2 Results and discussion

During steady-state operation, removal efficiencies averaged 70% COD, 80% TN and 60% TP with limited influence of platform design. However, the average specific surface removal performances varied between each reactor type and are summarised in Table 6.2. In all cases the remaining effluent COD was mainly associated with biomass washout (i.e., related to effluent TSS) as the residual COD in filtered samples was below 20 mg/L.

As shown in Fig. 6.6, during the first two weeks the effluent COD concentrations were relatively high which corresponds to suspended growth of microalgae and partial biomass washout in the effluent – reflected as particulate COD. However, after these two weeks, the biomass developed as a mixed microalgae–bacteria biofilm on the platforms and effluent TSS concentrations were relatively low (Fig. 6.7).

Table 6.2. Specific average mass removal of main contaminants (g/m^2.day).

Total Nitrogen Removal			Total Phosphorus Removal			COD Removal		
Double cascade	Thin layer cascade	Single platform	Double cascade	Thin layer cascade	Single platform	Double cascade	Thin layer cascade	Single platform
12.0	12.5	13.4	1.1	1.2	1.3	30.0	29.2	31.2

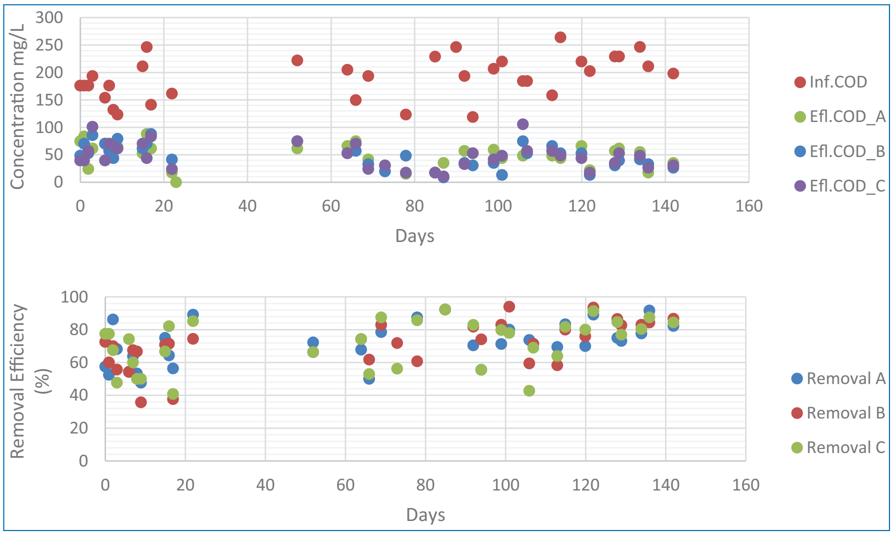

Figure 6.6. COD concentrations and removal performances for each of the tested platforms designs. Note: A is the average data from the double cascade photobioreactors, B the thin layer cascade photobioreactors and C the single platform photobioreactors.

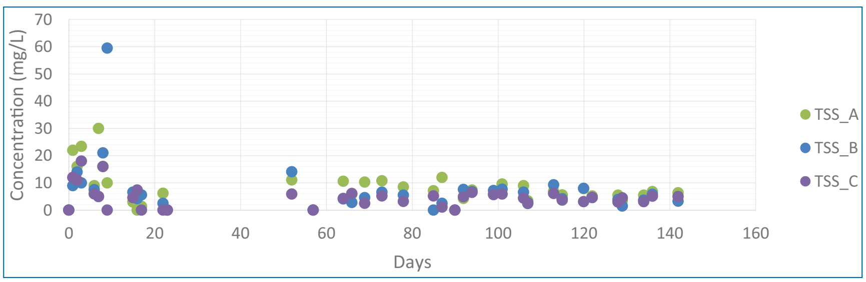

Figure 6.7. TSS concentrations in the effluent of laboratory scale units. Note: A is the average data from the double cascade photobioreactors, B the thin layer cascade photobioreactors and C the single platform photobioreactors.

In general, the cascading platform approach with recirculation was conducive to conditions for good biomass growth and good treatment performance. However, in these configurations biomass accumulation requires the need for regular harvesting and maintenance. For the laboratory scale experiments we observed that

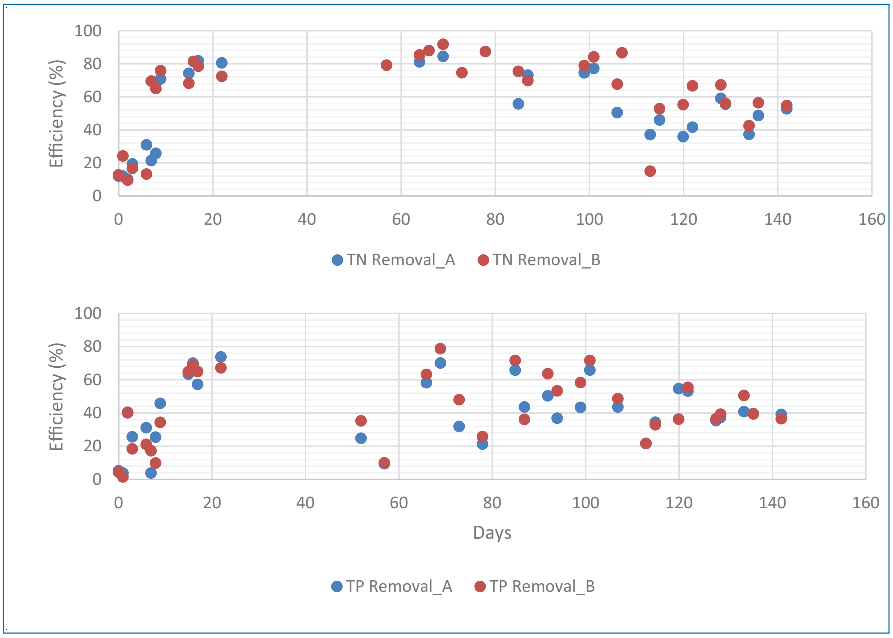

Figure 6.8. Nitrogen and phosphorus removal efficiencies in the lab-scale BSP units. A = double cascade photobioreactors; B = thin layer cascade photobioreactors.

the excess biofilm which detached and accumulated on the platforms needed to be removed periodically to avoid biomass decay and nutrient re-solubilisation within the system (Fig. 6.8). The frequency of biofilm detachment and biomass harvesting was dependent on biomass growth rate, which was in turn dependent on the quality of influent (quantities of nutrients introduced). Other authors have suggested that in phototrophic biofilm photobioreactors used for effluent polishing as part of wastewater treatment, the average biomass production rate is approximately 7 g dry weight m^{-2} day^{-1} while the harvesting frequency should be at least twice a month – as the biofilm starts to spontaneously detach after two weeks (Boelee *et al.*, 2014).

Phosphorus removal performance was most affected by biofilm accumulation and decay – and harvesting twice a month had to be considered to avoid excess biomass accumulation resulting in decay and unstable phosphorus removal performance (Fig. 6.8).

6.5.2 Pilot-scale Testing

Based on lab-scale performance data, the BSP system was designed as a cascading photobioreactor. In addition to performance, the choice of the double platform system also had the advantage of simple and rapid manufacturing (allowing local materials to be used to create an easily handled system that was simple to maintain and adapt).

Pilot-scale BSP units were tested in Spain by the University of Girona, in India by BORDA (Bengaluru) and in Peru by the Catholic University of Santa Maria (Arequipa) (Fig. 6.9).

Each installed system comprised two platforms (each of which had a 2 m^2 surface area) in a cascade sequence. Each system comprised the platforms (constructed from polypropylene), a recirculation tank, a feeding pump and a corresponding recirculation pump. The module was designed to treat 1 m^3 of effluent from a lumbrifilter per day. The modules were self-inoculated with local microflora – either microalgae from ponds/lakes/rivers or local cultures. The start-up duration for each site varied from 1 week to 1 month depending on inoculation technique and amount of inoculum used.

Figure 6.9. Pilot-scale BSP modules of the INNOQUA system installed in India (left) and Peru during inoculation (right).

Table 6.3. Average concentrations of Lumbrifilter influent and effluent and BSP effluent, and the process efficiencies for each treatment step and global efficiency of an integrated LF+BSP system.

	TSS	BOD	COD	NH_4-N	TP
			Average concentrations (mg/L)		
LF influent	2,190	1,165	2,242	104	23.8*
LF effluent	271	90	371	15.2	15.1*
BSP effluent	224	30	183	3.2	6.48
			Process efficiency (%)		
LF	87.63	92.27	83.45	85.38	36.55
BSP	17.34	66.67	50.67	78.95	57.09
LF+BSP	89.77	97.42	91.84	96.92	72.77

*Composite sample on the 8th of September 2020.

In each case, the removal rates for key parameters by lumbrifiltration averaged about 80% for TSS, COD, BOD and NH_4^+-N (Table 6.3). The addition of the

BSP polishing step increased average performances close to 90% for COD and BOD, and above 90% for TSS and ammonium nitrogen. At these sites, excess biomass removal was required approximately once every two weeks.

As a general conclusion, the BSP module as developed within the INNOQUA system requires regular biomass harvesting and maintenance, making it unlikely to be suitable for low-intervention decentralised applications. However, the concept as developed here could be readily adapted for tertiary treatment at a suitably staffed centralised or semi-centralised wastewater treatment facility.

6.6 Conclusions

For more than half a century, microalgal biomass has been investigated as a potential feedstock for advanced generation of biofuels and high-value compounds, and also as an important contributor to sustainable solutions for emerging problems derived from human activities such as climate change, ecosystem pollution and sanitation. During this period, microalgae-based technology has undergone a constant evolution, with improved knowledge of the performance of different cultivation approaches and selection of target species, as well as identification of those economic sectors with high environmental impact and energy consumption (such as wastewater treatment) where microalgae could make an important contribution. However, technological limits – such as those related to costs, biomass harvesting and cultivation – and the fact that most studies have been conducted at the laboratory level, mean that research remains necessary.

The characteristics of photoautotrophic microalgae make them suitable for a number of wastewater applications – their ability to utilise dissolved nutrients supports rapid development of biomass while reducing nutrient loads in final effluents, and their productivity generates oxygen that can be utilised by bacteria to break down dissolved organic pollutants. When combined, these attributes can (potentially) lead to significant reductions in energy and chemical usage in wastewater treatment, whilst simultaneously delivering valuable ecosystem services, increasing sustainability and (even) produce biomass with significant potential in the wider bioeconomy.

Both suspended and attached-growth microalgal–bacterial cultures have been examined, with the latter offering simpler/cheaper opportunities for biomass harvesting that could lend themselves to decentralised wastewater treatment approaches. Microalgal biofilms were at the heart of the INNOQUA BSP module, targeted at treatment of secondary wastewaters from lumbrifilter systems. The BSP module has been demonstrated to be a feasible polishing step for this effluent at both laboratory and pilot scales, using real wastewater with site-specific characteristics. However, before it can be considered ready for commercial deployment, the BSP module requires further optimisation and field testing to better understand maintenance requirements and thoroughly assess its suitability for use in decentralised applications. Although developed within INNOQUA as a tertiary treatment solution, microalgal–bacterial communities also have potential for delivery of primary or secondary wastewater treatment. Current research is exploring the fundamental aspects to support such applications.

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