INNOQUA is demonstrating how nature-based solutions can treat wastewater to a standard at which can be safely discharged back to the environment or used for irrigation purposes. This technical bulletin comprises a mini review of published data on the fundamental operating principles and performance microalgal / bacterial biofilms, as used in the INNOQUA Bio-Solar Purification (BSP) treatment process.

INTRODUCTION

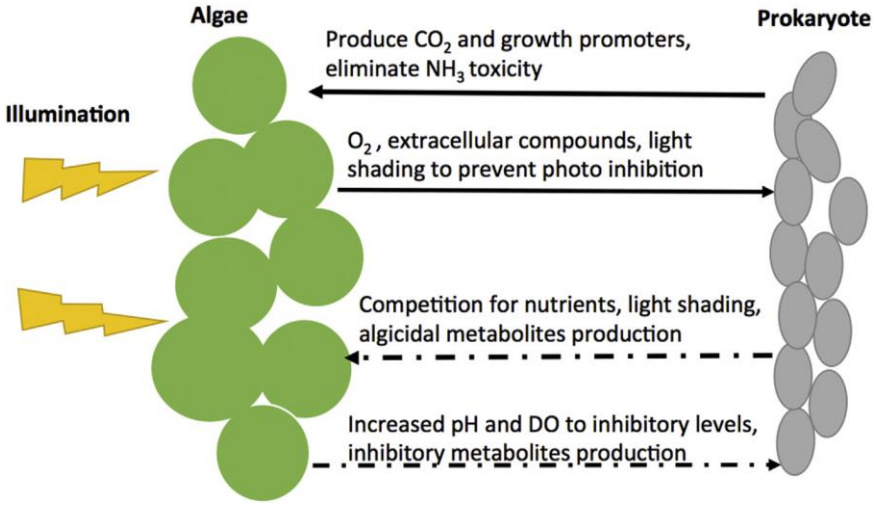
The first applications of microalgae for domestic wastewater treatment were implemented in the mid 1950s in California in high-rate-algal-ponds (HRAPs) (Oswald & Gotass, 1957). About two decades later, McGriff & McKinney (1972) outlined a novel approach to wastewater treatment in the form of 'activated algae', in which symbiotic relationships between microalgae and bacteria were examined as possible alternatives to conventional activated sludge treatment processes.

Although consistent nutrient removal has been demonstrated in both HRAPs and photobioreactor systems (Hoh, Watson, & Kan, 2016; Zhang et al., 2018), such microalgae-based approaches face several drawbacks, with harvesting / removal of the microalgae constituting a major operational limitation (Coward, Lee, & Caldwell, 2013; Okoro et al., 2019). Inadequate separation of suspended (algal) biomass can cause final effluents to exceed regulatory requirements for suspended solids (TSS) (Boelee et al., 2014) – but this limitation can be overcome through the use of attached-growth systems (Miranda et al., 2017), where algal-bacterial biofilms are intentionally established on surfaces over which wastewater passes. Such systems offer a number of advantages, and are currently the subject of intensive research activity (Wollmann et al., 2019).

Algal–bacterial processes are based on a number of basic symbiotic interactions between microalgae and bacteria (*Figure 1*). Oxygen photosynthetically produced by microalgae in the presence of light and CO_2 is used by heterotrophic bacteria to oxidize the organic pollutants present in the wastewater, producing in turn the CO_2 required for microalgal photosynthesis. Hence, biofilm photobioreactors allow for the simultaneous production of a biomass-free effluent and an easily harvestable biomass (Posadas et al., 2013). It has been claimed that this "free" oxygenation function (as compared with fine bubble diffusion, membrane and other types of energy-intensive oxygenation), together with the low capital investment for photobioreactor installation and the simplicity of their operation and maintenance, make microalgae-bacteria systems cheaper than activated sludge processes (Posadas et al., 2014). However, microalgal requirements for photosynthetic light can restrict the application of such technologies to specific locations[1].

[1] Note that several technology providers have recently combined algal reactors with photosynthetic LED illumination, allowing such systems to be deployed anywhere, irrespective of ambient illumination. Such systems do – of course – require additional energy when compared with reactors powered by ambient light

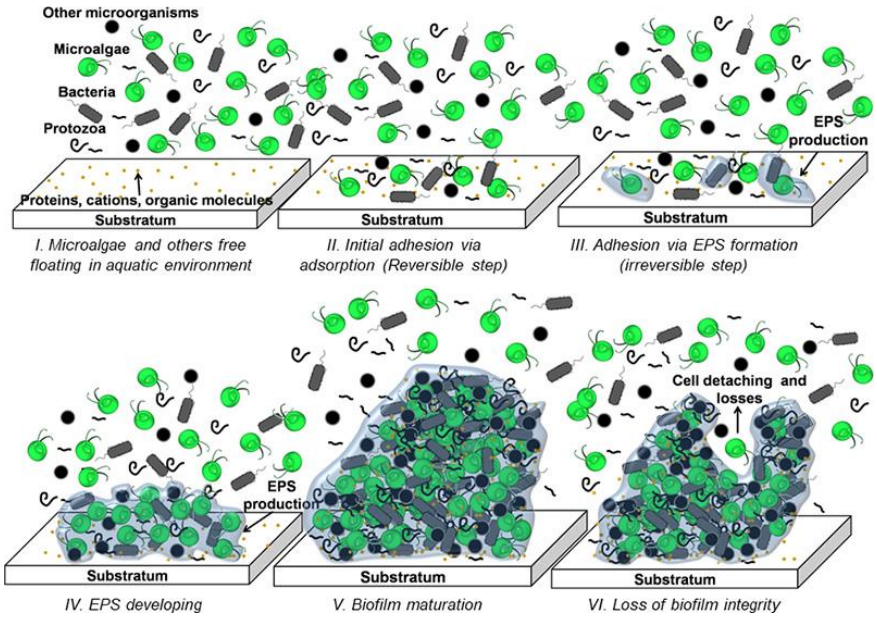
Figure 1 Algal-prokaryotic (bacterial) interactions. Solids lines indicate positive impacts and dashed lines indicate negative impacts (from Wang et al., 2018).



Biofilms are complex communities of microorganisms surrounded by an extracellular matrix that helps them survive and thrive by creating an efficient and stable trophic network. In the main, biofilms form when free-swimming microorganisms such as bacteria produce sticky extracellular polymeric substance (EPS), in response to environmental stresses (*Figure 2*). EPS comprises mainly a mix of sugars, proteins and nucleic acids (Watnick & Kolter, 2000).

Algal-bacterial biofilms are communities dominated by microalgae that colonize illuminated surfaces in the presence of moisture and nutrients. Like bacterial biofilms, algal-bacterial biofilms have the ability to adapt to changes in the environment, sustain colonies on a surface, and dissociate from a surface as a single colony or in clumps. The extent and specificity of algal–bacterial interactions in biofilm communities is not well understood (Kesaano & Sims, 2014).

Figure 2 Stages in biofilm formation. From Mantzorou & Ververidis, 2019.



In engineered algal-bacterial biofilm systems, wastewater passes through the bioreactor while the biomass remains attached to a stationary or moving support medium; therefore, the residence time of the algae and bacteria (or mean cell residence time (MCRT)) is much longer than the hydraulic retention time (HRT). This allows algal-bacterial biofilm reactors to be operated at higher organic and ammonium loading rates and shorter HRT than suspended growth systems because communities with slow growth rates (such as nitrifying bacteria) are retained in the reactor. In addition, the attached growth system has the potential to be operated with greater wastewater depth, which decreases reactor footprint (Wang et al., 2018). Biomass in these systems must be harvested by scraping from the support medium (Kesaano & Sims, 2014). Several bench-, pilot- and full-scale studies have been carried out with algal-bacterial biofilm reactors, although the absence of long-term pilot or demonstration studies has been highlighted by Wang et al. (2018) and other authors.

DESIGN CONCEPTS

Designs for algal-bacterial biofilm reactors include constantly submerged systems in a liquid culture, intermittently submerged systems with liquid and gaseous phases, permeated biofilm systems and systems which utilise immobilisation of the microalgae in a resin/gel-like matrix which allows for the diffusion of nutrients (Mantzorou & Ververidis, 2019). Designs for algal-bacterial biofilm treatment processes are different to those used for suspended-growth microalgae cultivation (for example, as illustrated in Wang et al., 2018) and include biofilms established on stationary or mixed substrata (*Figure 3*). A schematic of a typical laboratory-scale cascade system is provided in Figure 4. This basic configuration has been used for the INNOQUA BSP unit (Figure 5).

Figure 3 Examples of reactor configurations for algal-biofilm wastewater treatment. [a] Permanently immersed biofilms; [b] Biofilm between two phases; [c] Permeated biofilm system. Adapted from Mantzorou & Ververidis, 2019.

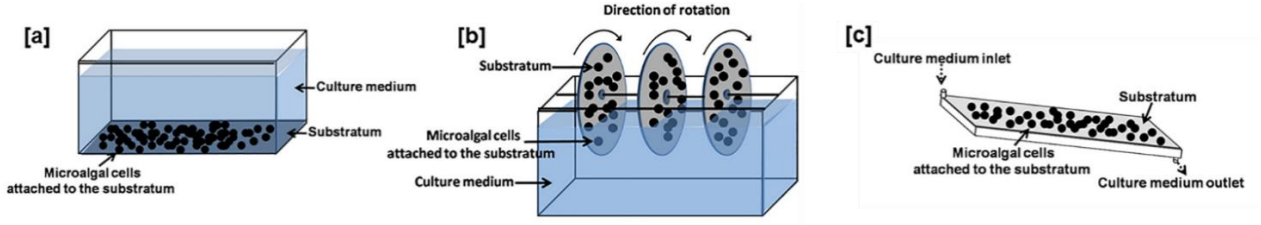
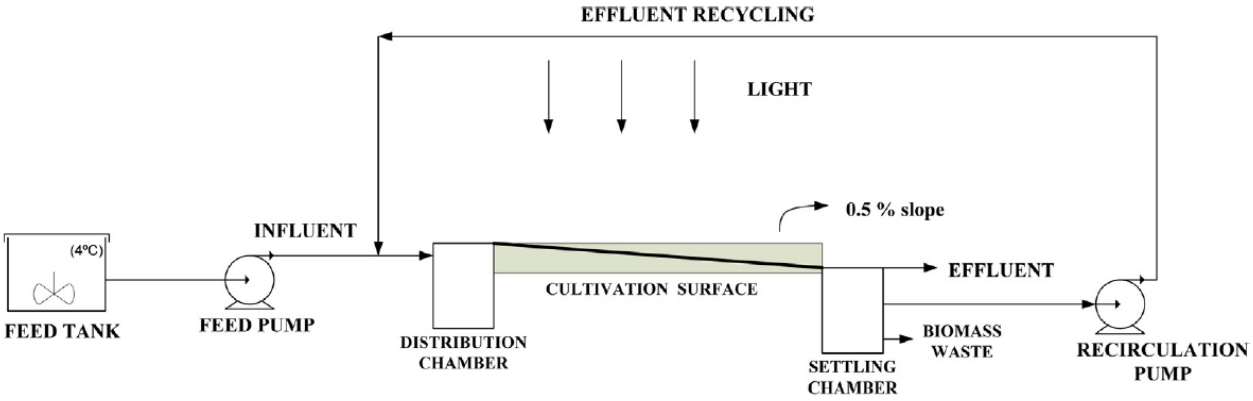


Figure 4 Schematic diagram of laboratory-scale biofilm photobioreactor. From Posadas et al., 2014.



Factors to be considered in the design and use of algal-bacterial biofilm reactors include: influent quality and quantity, treatment requirements, expected species mixes of microalgae/bacteria, nutrient

concentration gradients, oxygen diffusion gradients, CO_2 levels. Environmental conditions such as pH, temperature and light source as well as the type of attachment material used to stimulate biofilm development are also important. Some strains and species of microalgae grow better on different surfaces, while the nature of the wastewater will also all play a role in the community structure (Mantzorou & Ververidis, 2019). Flow rates also need to be considered carefully. A high flow velocity within the system can cause shear stress, damaging the biofilm and reducing treatment performance – while very low flow velocities can result in uneconomically slow treatment rates, although moderate flows are required during start-up to allow biofilm to attach to the support matrix (Whitton et al., 2015).

TREATMENT EFFICIENCY

Algal-bacterial biofilms have been shown to be suitable for removal of various wastewater contaminants, including COD and nutrients. *Table 1* shows various wastewater characteristics and their removal by biofilms. *Table 2* reports treatment efficiencies from a number of previously published trials (as summarised by Kesaano & Sims, 2014) and includes retention times.

Table 1 Treatment performances in an algal biofilm reactor for three different wastewaters (calculated from data reported by Choudhary, et al., 2017). [1] Domestic greywater; [2] Livestock wastewater; [3] Anaerobically digested livestock slurry. COD = Chemical Oxygen Demand; TDP = Total Dissolved Phosphorus; TAN=total ammoniacal nitrogen); TSS = Total Suspended Solids; TDS = Total Dissolved Solids

Wastewater parameters	Influent loadings (mg / litre)			Treatment performances (% removal)		
	1	2	3	1	2	3
COD	235 ± 2.12	1720 ± 35.3	2200 ± 141.4	69.7	89.5	86.2
Nitrate-N	6.2 ± 0.21	125 ± 1.41	72.6 ± 0.28	100	91.7	70.9
TDP	24.5 ± 1.06	131 ± 0.70	256.8 ± 2.26	90.1	93.5	88.4
TAN	29.8 ± 0.56	137.5 ± 0.35	253.5 ± 0.35	94.2	98.1	93.2
TSS	270 ± 8.00	121.1 ± 5.26	340 ± 2.1	62.0	27.3	71.2
TDS	1940 ± 6.00	4480 ± 29	8900 ± 141.12	47.4	66.5	77.5

Posadas et al. (2013) demonstrated the superior nutrient removal capacity of algal biofilms as compared with bacterial biofilms when operated at equivalent hydraulic retention times. Nitrogen removal in mixed algal-bacterial systems may be a result of various concurrent mechanisms including nitrification/denitrification, nutrient uptake into biomass or even stripping (at pH values above 8.5) (*Tiron, et al., 2015*). For high strength ammoniacal wastewaters, the increase of pH above 8.5 (as a result of dissolved CO_2 uptake by microalgae) may cause volatilisation of ammonia with negative impacts for compliance with local air quality or other environmental standards.

Algal-bacterial biofilms are also of interest when considering emerging contaminants (EC) and have been shown to enhance the removal of antibiotics through production of reactive oxygen species as a by-product of algal photosynthesis (Wang et al., 2018). Microalgae can remediate ECs via several pathways, including bioadsorption, bio-uptake and biodegradation. When paired with nutrient removal, removal or attenuation of ECs could be a key factor in favour of future wastewater treatment processes.

Table 2 Nutrient removal efficiencies reported for laboratory and pilot trials of municipal wastewater/effluents in algal biofilm systems operated in continuous flow mode. From various published sources, as summarised by Kesaano & Sims (2014)

Parameter	Influent (mg/l)	Effluent (mg/l)	% removal	Retention time (d)
TP	1.3	0.4	69.7	2
	2.1	1.6	23.8	0.25*
TN	18.5	11.0	40.5	2
	91.1	34.1	62.5	5.2
	4.5	1.1	75.6	0.25*
NH_4-N	5.4	3.0	44.4	2
	32.0	1.5	95.3	4
PO_4-P	0.97	0.2	88.5	0.7
	7.0	5.0	28.6	3.1
	2.2	0.8	63.6	4
				(*Pilot studies)

BSP DEMONSTRATION AND SITE VISITS

High rate algal-bacterial cascade photobioreactors (referred to as BSP or Bio-Solar Purification units within the INNOQUA project) have been installed at demonstration sites in three countries, to test their performance under different conditions as a polishing step after preliminary treatment (Figure 5).

Figure 5 The INNOQUA BSP, as installed after the lumbrifilter at the demonstration site in Peru

A series of open days and training events are planned for each of these sites. If you would like to take part or arrange a visit, then please contact the relevant site manager:

Country	Site manager	Contact details
India	Tatjana Schellenberg	schellenberg@borda.org
Peru	Joshelyn Paredes-Zavala	joshelyn.pz@gmail.com
Spain	Victoria Salvado	Victoria.Salvado@udg.edu

Further details of the INNOQUA project can be found at: www.innoqua-project.eu

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