CAN ALGAL BIOTECHNOLOGY BRING EFFECTIVE SOLUTION FOR CLOSING THE PHOSPHORUS CYCLE? USE OF ALGAE FOR NUTRIENT REMOVAL – REVIEW OF PAST TRENDS AND FUTURE PERSPECTIVES IN THE CONTEXT OF NUTRIENT RECOVERY

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ABSTRACT

Eutrophication of water by nutrient pollution is a global environmental issue. Biological methods for removing nutrients are environmentally friendly and sustainable. Therefore, this article summarizes main trends in the use of algae for removing nutrients from wastewater using both suspended and attached algal-based systems. A wide variety of algal species and experimental approaches has been tested to date. Researchers report that algae are able to effectively remove a variety of pollutants and nutrients. This review also discusses the potential of algal-based technology for nutrient, especially phosphorus, recovery. Despite the fact that effective nutrient removal has been demonstrated, there are still many challenges to be overcome in the development of succesfull technologies.

Keywords: wastewater treatment, algae, nutrients removal, phosphorus recovery

Introduction

Pollution of surface water due to high concentrations of nutrients is a global issue affecting all countries worldwide. Eutrophication is a term, which was used in limnology already at the beginning of the 20th century (Weber 1907). But this term was used only for describing the effects of pollution on ecosystems without a deeper understanding of the causes. Since the mid-20th century, mass presence of algal blooms in reservoirs, growth of macrophyta and periodic killing of fish were symptoms, which were not possible to ignore (Schindler 2006). R. A. Vollenweider was the first scientist who linked high nutrient input into lakes with eutrophication. He performed a comprehensive analysis of available data and of systematic scientific studies. From the results, he deduced that a reduction in the input of phosphorus (P) and in some cases also nitrogen (N) would avoid eutrophication and its symptoms in lakes (Vollenweider 1968). Vollenweider's results were supported by W. T. Edmonson, who published a six-year study of Lake Washington. He found a strong correlation between P concentrations and algal standing crops. When the nutrient load was reduced by diverting sewage away from the lake, it rapidly recovered (Edmonson 1970). In other studies P was also determined as the limiting factor for growth of phototrophic organisms in lakes and reservoirs (e.g. Schindler 1977; Ahlgren 1978; Holtan 1981). Numerous studies focused on causes and effects of high nutrient concentrations on water ecosystems were carried out starting from seventies of 20th century (e.g. Hutchinson 1973; Ahlgren 1978; Howarth 1988; Jeppesen et al. 2002).

The high input of P and N into surface water originated from variety of sources (Smith 1998). Main *point sources* of nutrient pollution are mostly wastewater effluent (municipal and industrial), runoff and leachate from waste disposal sites, runoff and infiltration from animal feedlots, runoff from mines and unsewered industrial sites and overflows of combined storm and sanitary sewers (Novotny and Olem 1994). *Point sources* are more easily monitored than *nonpoint sources,* which are diffuse. *Nonpoint sources* of nutrients include runoff from agriculture, runoff from unsewered areas, septic tank leachate and runoff from failed septic systems and atmospheric deposition over water surfaces (Carpenter et al. 1998). High concentration of P and N in water results in an abundant growth of algae, cyanobacteria and macrophytes. Nutrient pollution also causes shifts in the dominant species towards cyanobacteria, which are potential producers of toxic compounds (Skulberg et al. 1984). The abundant growth of algal biomass starts a cascade of negative processes in water ecosystems. Dense algal mats reduce the quality of the living conditions for other organisms such as invertebrates and fish. Decomposition of large amounts of algal biomass causes diel fluctuations in pH and in dissolved oxygen concentrations, which is harmful for fish. Decomposition of biomass can also cause taste and odour problems. Worse water quality also results in restrictions on recreation and swimming in polluted water (Quinn 1991).

Monitoring and control of nutrient load is an essential part of water management (Daniel et al. 1994; EU Water Frame Directive 2000/60/EU). The problem of nutrient pressures on water resources are also included in the 7th Environment Action Programme (Decision No 1386/2013/EU of the European Parliament, 2013). Despite all efforts, control of nutrient pollution remains one of the most important environmental issues (Jarvie et al. 2013).

The next serious topic inseparably connected with eutrophication, is the P recycling. P is an essential ele-

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ment for all living organisms. P rocks are mined only in a few regions in the world. The currently known reserves are concentrated in few countries, particularly Morocco (Scholz et al. 2013). The resources of P rocks are very small in Europe, especially bearing in mind the high demand. The study "Phosphorus flows and balances of the European Union Member States" describe an unbalanced economy in terms of P. On the one hand European countries are fully dependent on imports of P rock, on the other hand there are a great losses of P to wastewater and food waste (van Dijk et al. 2016). Big losses of imported P to the environment cause serious environmental problems. This unsustainable management of non-renewable resources needs to be changed. For the above reasons, it is necessary to focus on the development of new technologies for phosphorus removal and recovery.

Biological methods of nutrient removal from wastewater are considered to be low cost and environmental-friendly technologies (Mantzavinos et al. 2005). Different groups of microorganisms can be used for removing nutrients (Bashan and Bashan 2004). Many studies demonstrate the high ability of microalgae to reduce the nutrient content of wastewater (Christenson and Sims 2011; Whitton et al. 2015). Moreover, this method can bring several benefits because it does not generate additional waste, such as activated sludge, does not require the use chemical substances for phosphorus reduction and provides an opportunity for efficiently recovering nutrients (Mantzavinos et al. 2005; Pittman et al. 2011). Therefore, the objectives of the present study are to summarize past development and recent progress in nutrient removal technologies using microalgae in the context of nutrient recovery.

The Algae and their Role in Biotechnology

The cyanobacteria and algae are highly heterogeneous groups of organisms including both small unicellular species and large freshwater and marine organisms of sizes more than 1 m with a multicellular organization. The algae, like plants, are photosynthetic organisms but have a simpler cellular organization. The algae have no roots, stems, leaves or complex vascular networks. They occur as single cells, multicellular colonies, simple or branched filamentous, leafy or blade forms without a high degree of cell differentiation (Barsanti and Gualtieri 2006). The cyanobacteria and algae can colonize all biotopes. They can live as planktonic organisms in the euphotic zone of lakes, water reservoirs or in the sea. They also colonize firm surfaces submerged in water and live attached on sediments, stones, plants etc. (Stevenson 1996). Microscopic species are called "microalgae". Large species with complex cellular organization are called "macroalgae". The term "microalgae" is used in a wide sense in applied phycology. It includes both prokaryotic cyanobacteria and eukaryotic algae (Masojídek and Prášil 2010).

Algae are used in several areas of biotechnology. Primarily, it is the commercial production of microalgae for dietary supplements, cosmetic products and nutrition for aquaculture (Becker 2004). Microalgae are characterized by a high content of valuable compounds such as proteins, amino acids, essential unsaturated fatty acids and vitamins. Commercially produced genera are mainly *Chlorella*, *Arthrospira* (*Spirulina*), *Dunaliella*, *Nannochloropsis* and *Haematococcus* (Spolaore et al. 2006; Mimouni et al. 2012).

Microalgae were identified as an important source of lipids with potential use as feedstock for biofuel production. Microalgae can produce different types of lipids, for example unsaturated fatty acids (eicosapentanoic acid or docosahexanoic acid) and neutral lipids including triacylglycerids (Markou and Nerantzis 2013). They can be a suitable feedstock for biofuel production after conversion of the lipids to fatty acid methyl esters.

Research on the potential for using algae for bioremediation is currently an important issue in microalgal biotechnology. Microalgae can be used in wastewater treatment for the removal of different pollutants. Reduction in chemical and biological oxygen demands are mainly studied together with the removal of N and P in agricultural, domestic or municipal wastewater (e.g. Shelef et al. 1980; Fallowfield and Garret 1985; Arcila and Buitrón 2016). Algae are also an effective bio-sorbent for removing heavy metals because their cell surfaces are negatively charged and they have large cell surface to volume ratios (Filip and Peters 1979; Wilde and Benemann 1993; Roberts et al. 2013; Li et al. 2015).

As mentioned above, many studies have shown the ability of algae to grow in wastewater and to reduce nutrient concentrations in laboratory-scale studies (e.g. Proulx et al. 1993; Chevalier et al. 2000; Doria et al. 2012). For this purposes, the microalgae can be cultivated in suspension or attached to a firm surface. Much attention has been paid to the cultivation of algae in wastewater treatment ponds and natural attached algal-based systems (Adey et al. 2011; Park et al. 2011). The possibility

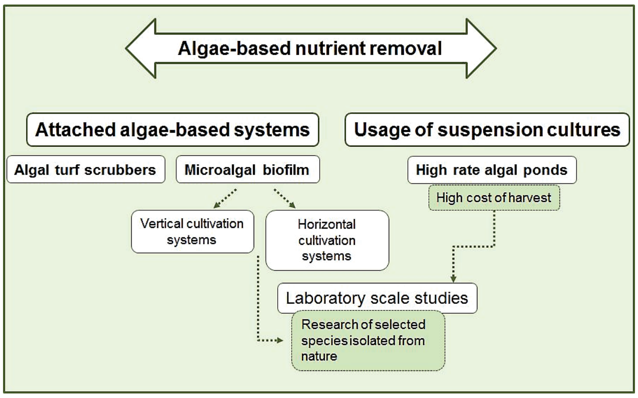

Fig. 1 Summary of main trends in nutrient removal technologies using algae.

of nutrient removal coupled with production of biofuels has been studied. The connection of these two processes would bring important economic benefits, including a reduction in the cost producing biofuels (Chinnasamy et al. 2010; Christenson and Sims 2011).

Wastewater Treatment Using Suspended Algae

Wastewater Treatment of High Rate Algal Ponds (WWT HRAP)

The first studies that focused on the potential of algae for wastewater treatment were published in the middle of the 20th century (Oswald and Gotaas 1957; Bogan et al. 1961). At that time, the concept of WWT HRAP was established. High rate algal ponds (HRAP) are shallow oxidation ponds mixed by means of a paddle wheel. They are used for the treatment of municipal, industrial and agricultural wastewater (Park et al. 2011). This technology was tested in South Africa in the 70s. Its efficiency in treating industrial wastewater with high concentrations of N was studied over a long period of time. Parameters of HRAP were optimized to achieve better light conditions in cultivation suspension and to minimize evaporation of water from the HRAP. Density of cell culture was set at 0.5 g DW l^{-1}. Simultaneously, a sufficient reduction in nitrogenous substances was achieved (Bossman and Hendricks 1980). HRAP was tested for different kinds of wastewater, but the most often investigated treatment of liquid wastes was those from agriculture. Repeated reductions in BOD, concentrations of N and P are recorded in agriculture wastewater (Shelef et al. 1980; Picot et al. 1991). Reduction of N substances in municipal wastewater was studied in Spain. During a pilot study, algal cultures in HRAP was able to remove about 70% of the N (Garcia et al. 2000). Predictive models of algal growth, oxygen production and reduction in pollutants were developed based on long-term studies (Kroon et al. 1989; Sukenik et al. 1991). Typical biomass production in these systems ranged from 8–35 g m^{-2} d^{-1} (Shelef et al. 1980).

No special algal species were selected for inoculating HRAP, instead the algal assemblage developed naturally in the ponds. Therefore, many studies were focused on the ecology and succession of these specific algal assemblages. Palmer (1974) studied species composition in WWT HRAP. He found that the most frequently recorded species were green algae, especially *Chlorella*, *Ankistrodesmus*, *Scenedesmus*, *Chlamydomonas*, *Micractinium*, *Euglena* and Cyanobacteria, genus *Oscillatoria*. Erganshev and Tajiev (1986) report similar species in six lagoons in central Asia. Sim and Goh (1988) also report an algal assemblage dominated by green algae in HRAP containing agriculture wastewater in Singapore.

The main disadvantage of HRAP is that it is difficult and expensive to harvest the algal biomass, which is necessary for effective wastewater treatment (Cromar et al. 1992). HRAP is mostly criticized because of their low productivity due to light limitation, high dissolved oxygen levels and loss of biomass to grazers (Chisti 2007; Mata et al. 2010; Park et al. 2011). Recently, researchers have focused on optimizing the operating parameters such as hydraulic retention time, mixing, CO_2 availability and cultivation mode and controlling grazers (Park and Craggs 2011; Park et al. 2013). New progress was recently presented in the potential usage of WWT HRAP for low-cost biofuel production (Mehrabadi et al. 2015; Arcila and Buitrón 2016).

Laboratory-Scale Studies Using Suspension Cultures

While HRAP are naturally colonized by algal assemblages, many studies since the 90s have evaluated the effectiveness of particular species of algae for N and P removal. In particular, great attention was paid to those species that were easy to harvest in order to reduce the costs of harvesting. Proulx et al. (1993) studied the growth of the cyanobacterium *Phormidium bohneri* in secondary effluent. These species are able to remove 83% of the N and 81% of the P from municipal wastewater, moreover, they also have a high ability to aggregate and settle in ponds. Several evaluations of the nutrient removal capacity under different conditions for several benthic cyanobacteria are published. Arctic species *Phormidium tenue* and *Oscillatoria sp.* were tested to develop technology suitable for the cool climate in Canada (Talbot and de la Noüe 1993; Chevalier et al. 2000).

Doria et al. (2012) isolated the microalga *Scenedesmus acutus* from municipal wastewater and recorded its biomass production coupled with reduction in nutrients during growth in a tubular bioreactor (50 l). She reports a biomass production of 0.24 g DW l^{-1} d^{-1} and complete removal of N from wastewater. The disadvantage was that it was necessary to add microelements (Fe, Mg) to the wastewater. Different species of green microalgae are repeatedly used in various types of wastewater. The genus *Scenedesmus* is able to remove 94% of organic N and 66% of P from municipal wastewater (de Alva et al. 2013). Similarly, Ren et al. (2015) report that *Scenedesmus* isolated from soil reduced the concentration of COD and nutrients in starch wastewater. Other species used are for example *Monoraphidium sp.*, *Chlorella ellipsoidea*, *Chlorella vulgaris*, *Neochloris oleoabundans* or *Desmodesmus sp*. All these species are able to effectively remove nutrients from wastewater (Wang et al. 2011; Arbib et al. 2014; Holbrook et al. 2014; Fang et al. 2015).

In addition to monocultures, algal consortia were also tested. Chinnasamy et al. (2010) developed algal consortia and determined their capacity for nutrient absorption. A consortium including the green microalgae *Chlorella sp.*, *Chlamydomonas sp.*, *Scenedesmus sp.*, *Gloeocystis sp.* and cyanobacteria *Anabaena sp*. and *Limnothrix sp.* was cultivated in wastewater mainly from carpet mills. This consortium removed 96% of the nutrients. Similarly, Renuka et al. (2013) used four consortia dominated by *Chlorella sp.*, *Scenedesmus sp.*, *Chlorococcum sp.* and cyanobacteria *Phormidium sp.*, *Limnothrix sp*. and *Anabaena sp*. and report that the highest nutrient removal was achieved by a consortium dominated by filamentous cyanobacteria. Generally, algal consortia are able to survive environmental fluctuations and are resistent to invasion by other species (Subashchandrabose et al. 2011).

Recently, the research on the use of algae for wastewater treatment has included an evaluation of the energy content of the algal biomass produced, lipid production and production of biofuels (Fang et al. 2015; Kim et al. 2015; Ren et al. 2015). This topic is also connected with the development of new technologies and new devices for cultivating algae.

Wastewater Treatment Using Attached Algae-based Systems

The harvest of algal biomass from suspension in wastewater is technically difficult and accounts for 20–30% of the costs connected with cultivating algae (Liu and Vyverman 2015). This led to a greater interest in solutions using algae attached to submerged surfaces (Hoffmann 1998).

Attached algal communities are traditionally called "periphyton", a term that was introduced for the first time in 1928 (Sládečková 1962). Later, the names "phytobentos" or "microphytobenthos" were adopted by hydrobiologists. Over the last few decades, the term "algal biofilm" for attached algae has become more widely accepted in algal biotechnology (Wetzel 2001). Research on periphyton was conducted intensively from 60s. Special interest was particularly directed to studies on the community structure and primary productivity in streams and rivers. Artificial shallow channels were also used for research on nutritional conditions (e.g. McIntire 1968). The effect of high concentrations of P and N on the primary productivity of algae is reported in many publications (e.g. Whitford and Schumacher 1961; Lowe et al. 1986; Davison 1991; McCormick 1996). As one of the first, Bush et al. (1963) report using algae attached in a raceway pond for removing nutrients. Hemens and Mason (1968) evaluate wastewater tertiary treatment in an outdoor shallow stream. Sládečková et al. (1983) proposed using artificial streams fitted with nylon mesh to remove nutrients from polluted water. Vymazal et al. (1988) further continued this concept and tested periphyton growth and rate of nutrient uptake in an outdoor artificial channel (5 m long) with artificial substrates for algal growth. The algal assemblage that developed spontaneously on the substrata came from the upper part of stream, which served as a source of water for the channel. In both experiments there were reductions in the concentrations of phosphorus and nitrogen together with an abundance of algal growth.

The Algal Turf Scrubbers (ATS) – Ecologically Engineered, Algal Based System

Simultaneously with Czech researchers, the American scientist Walter Adey and co-workers examined options for improving the artificial channel concept (Adey et al. 1993). They were inspired by coral reefs. The algal turfs growing on coral reefs are characterized by high primary production due to regular flooding by waves. Scientists designed pulsing hydraulic system to mimic the wave action on coral reefs (Adey et al. 2011). This ATS system consisted of an attached algal community in the form of a "turf" growing on polyethylene screens. The algal turfs grew in a shallow slopping raceway into which water was pumped from a water body. After the biological uptake of nutrients by algae, the water was released at the end of the raceway back into the water body. The algal biomass was regularly harvested (Craggs et al. 1996). The algal assemblage on turfs consisted mostly of filamentous green algae *Spirogyra sp.*, *Microspora sp.*, *Ulothrix sp.*, *Rhizoclonium sp.* and *Oedogonium sp*. These dominant species were accompanied by the cyanobacteria *Phormidium sp.* and *Oscillatoria sp.* and benthic diatoms. These algal turfs are a heterogeneous community with a high growth rate and high ability of regenerating (Craggs et al. 1996; Mulbry et al. 2008; Sandefur et al. 2011). Maximum values of the rate of P and N uptake were 0.73 and 1.58 g m^{-2} d^{-1} respectively. Harvests (including trapped organic particulates) varied from 5 to 60 g DW m^{-2} d^{-1} (Craggs et al. 1996; Mulbry et al. 2008; Kangas and Mulbry 2014).

This ecologically engineered, algal-based technology has been developed for more than 30 years in USA and was patented as an Algal Turf Scrubber (ATS™). The nutrient reduction potential of ATS systems have been assessed for both point and non-point sources of pollution. For example, the treatment of dairy manure effluent in central Maryland (USA) and agricultural wastewater in the Florida Everglades (Adey et al. 2011). Commercialization of this technology is under active development by HydroMentia Inc., which builds and operates ATS mainly in Florida. Recently, research to improve the performance of ATS has continued, with tests involving new applications and evaluations of the harvested biomass (Adey et al. 2011; Valeta and Verdegem 2015).

The Algal Biofilms

The ATS technology is an ecologically engineered design of a controlled ecosystem for nutrient removal. The success of this technology has depended mainly on the construction of hydraulic system with a specific water regime (Adey et al. 2011). But the assemblage of periphytic organisms was not manipulated to favour more desirable species with a higher ability to remove nutrients. A novel approach is to use microalgal biofilms consisting of selected species. These species are selected based on a high ability to reduce nutrients (Sukačová et al. 2015). This approach is a shift from ecological engineering to the design of biotechnology applications.

The term microalgal biofilm was introduced for microalgal assemblages that consist of microalgae that colonize illuminated surfaces submerged in water (Jarvie et al. 2002). The use of term microalgal biofilm overlaps with name periphyton in hydrobiology as described above. In aquatic ecosystems, the growth of microalgal biofilms starts with the colonization of a submerged surface by pioneer species. At first, the surface is colonized by diatoms, which are followed by coccal green microalgae. The growth of filamentous microalgae and cyanobacteria after one month is the last phase of species succession (McIntire 1968; Komárek and Sukačová 2004). Development of microalgal biofilms depend mainly on water temperature and trophic conditions (Johnson et al. 1997). The growth of algal biomass is exponential at the beginning and then decreases depending on the thickness of the biofilm. Biomass losses are caused by respiration, cell death, parasitism and grazing by invertebrates (Biggs 1996). In watercourses, the algal biofilm plays a key role in biogeochemical cycles and transformation of carbon, N and P (Allan and Castillo 2007).

In the context of algal biotechnology, the research on algal biofilms is motivated by two factors. The first is the cultivation of biotechnologically important species in the form of a biofilm in order to reduce the cost of harvesting the biomass. The evaluation of the potential of algal biofilms for nutrient removal is the second reason. The research is closely connected with the design of different cultivation devices. The microalgal biofilm cultivation systems can be constructed as panels from different materials placed vertically or horizontally with a slight slope.

The vertically constructed system "Twin Layer" bioreactor using filter paper attached to a glass plate as the area for biofilm growth. Cultivation medium flows down the glass plate and keeps the filter paper moist. This system is placed in an aquarium and is aerated with air enriched with CO_2. Production potential of such a bioreactor ranges from 3 to 18 g DW m^{-2} d^{-1} depending on the intensity of illumination (Liu et al. 2013). A similar system was used for nutrient removal from municipal wastewater. *Hallochlorella rubescens* CCAC 0126 growing on a nylon membrane fixed to a metallic frame was situated vertically and wastewater from different treatment stages flowed down the membrane. The average uptake rate of PO_4-P varied from 0.8 to 1.5 mg l^{-1} d^{-1}, the removal of P from wastewater during a two-day cycle of bioreactor operation was 78.9% and 85%, respectively, and the average microalgal growth was 6.3 g DW m^{-2} d^{-1} (Shi et al. 2014).

Other carrier materials used for biofilm cultivation are radially flexible PVC fillers placed in plexiglass chambers. Biofilm consisted of a mixture of several species: *Chlorella pyrenoidosa*, *Scenedesmus obliquus*, *Anabaena flos-aquae*, *Synechococcus elongatus* and *Microcystis aeruginosa*. P and N removal efficiency was about 95% and 84%, respectively from simulated wastewater during a four-day cycle.

Guzzon et al. (2008) focus on basic research on P removal using biofilms. The growth was measured in a horizontal incubator with four separated lanes under different conditions (Zippel et al. 2007). Polycarbonate slides inside lanes served as cultivation areas. Guzzon and co-workers describe the influence of different parameters such as light intensity, temperature and flow rate on biofilm growth and P removal. They report positive correlations between algal biomass production and its P content with light intensity. They also describe the occurrence of polyphosphate granules inside the cells of the algae on the biofilms.

In several studies, polystyrene foam was found to be a suitable material for algal biofilm cultivation. Johnson and Wen (2010) determined the growth of *Chlorella* sp. attached to polystyrene foam in dairy manure wastewater and evaluated the algal biomass for producing biodiesel. This revealed that this technology potentially can provide a less expensive method of growing and harvesting algal production. Posadas et al. (2013) constructed a horizontal cultivation system with polystyrene foam as the carrier material for the biofilm, which was inoculated with a microalgal-bacterial assemblage from HRAP treated municipal wastewater. They compared the removal efficiency of N, P and organic compounds by the microalgal biofilm with that of a bacterial biofilm in the same cultivation system. The microalgal biofilm was the most effective in nutrient removal and the reduction of organic compounds was the same in both systems.

The comprehensive research on new wastewater treatment technologies using microalgal biofilm was done in the European center of excellence for sustainable technology (WETSUS) in the Netherlands. Boelee et al. (2011) determined whether microalgal biofilms were suitable for the post-treatment of municipal wastewater. Reduction in P and N was measured in a laboratory-scale system. Microalgal biofilm dominated by filamentous cyanobacteria (*Phormidium* and *Pseudanabaena*) and coccal green algae (*Scenedesmus sp.*) were cultivated on PVC plastic sheets. Wastewater circulated over the biofilm, which absorbed nutrients. Maximum rate of P and N uptake under continuous illumination was 0.13 g m^{-2} d^{-1} and 1 g m^{-2} d^{-1}, respectively. Subsequent studies of Boelee and co-workers focused on the evaluation of the frequency of harvesting in relation to biomass production and nutrient reduction. They report that the same biomass was harvested on the second, fourth and seventh day. The premise of this study that there would be a reduction in the biomass produced as the biofilm thickened was not confirmed (Boelee et al. 2014).

The work mentioned above was done in a laboratory. There are very few pilot studies that evaluate removal efficiency of biofilms. Sukačová et al. (2015) report the rate of uptake of P by an algal biofilm assemblage that

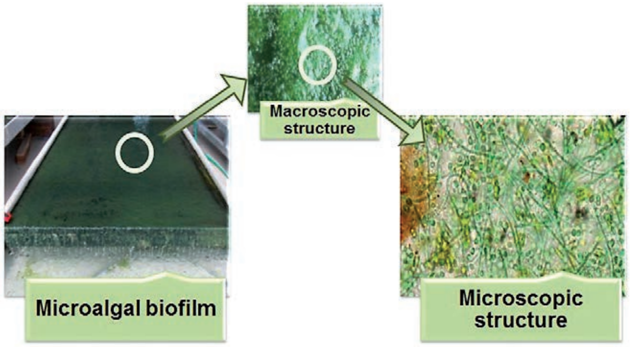

Fig. 2 Macroscopic and microscopic structure of algal biofilm. The filamentous cyanobacteria form a net that traps unicellular green algae.

consisted of filamentous cyanobacteria and coccal green algae, which grew on concrete panels with total area of 8 m^2 inclined at a slight angle. The wastewater was retained within the system for 24 hours. The average rate of uptake of P was about 0.16 g m^{-2} d^{-1}. The algal biofilm removed about 97% of total P from municipal wastewater after 24 hours.

The studies cited indicate that algal biofilms are very efficient at removing nutrients. Compared with ATS, the use of phototrophic biofilms on a large-scale is still uncommon and needs further improvement before it can be used for treating wastewater (Kesaano and Sims 2014; Whitton et al. 2015).

Matrix-Immobilized Algae

There are several laboratory studies on phycoremediation technologies using immobilized algae (e.g. Lau et al. 1997; de Bashan et al. 2002; Zhang et al. 2008). The main advantages of this technique is the separation of microalgae from wastewater and the production of a usable biomass. The immobilization method involves encapsulation of microalgae in beads. The material used for the immobilization must be very permeable, of low toxicity and highly transparent. The most often used material is alginate (Lau et al. 1997; Zhang et al. 2008). Various strains of microalgae have been immobilized, including the green algae *Chlorella vulgaris*, *C. pyrenoidosa*, *C. sorokiniana*, *Scenedesmus bicellularis*, *S. quadricauda* and cyanoprocaryota *Phormidium* (De la Noüe and Proulx 1988; Kaya and Picard 1996; Filippino et al. 2015). Immobilized microalgae are highly efficient at removing nitrogen and phosphorus from secondary effluents (De la Noüe and Proulx 1988; Kaya and Picard 1996). However, over the last few decades there has been little attention given to developing algal immobilization techniques for the treatment of tertiary wastewater (Filippino et al. 2015). Recently, research has started to focus on the optimization of growth of immobilized algae and increasing the efficiency of nutrient removal in the laboratory (Filippino et al. 2015).

Nutrient Recovery Potential

Considering the future need to recover nutrients, the utilization of nutrients by microalgae is an important issue in the nutrient removal process. The algal biomass that develops in wastewater can be utilised in several ways (Pittman et al. 2011). However, the presence of heavy metals, micropollutants or pathogens can reduce the possibility of reusing the nutrients.

One option is to use the algal biomass as a biofertilizers. The use of blue green algae for soil conditioning and as a biofertilizer in rice production is reported (Metting et al. 1990; Metting 1996). Mulbry et al. (2005) have used the algal biomass that developed during the treatment of cow manure treatment as a slow release fertilizer. They compared seedling growth using a commercial potting soil amended with either ATS biomass or a roughly comparable commercial fertilizer and report that plant growth was similar in both. Roberts et al. (2015) report that algae growing in bioremediation ponds at a coal-fired power station sequester metals from the wastewater. The algal biomass, which consists of the filamentous alga *Oedogonium,* can be converted to algal biochar for soil amelioration. When this biochar is added to a low-quality soil, it improves its retention of nutrients from fertilizer, which resulted in a better growth of radishes of 35–40% (Roberts et al. 2015). Although biochar is currently used to improve soil by restoring the carbon pool and providing essential trace elements, we hypothesize that algal biomass rich in phosphorus can also be effectively converted to biochar for enriching soils with phosphorus.

Algae are a good supplementary feed for livestock because they have a high protein content (Spolaore et al. 2006). However, the potential for using algae produced during wastewater treatment for feeding animals has not yet been studied. The algal biomass would have to meet the standards required for animal feed, which means that the feed source has to be free of pathogens and harmful substances.

Nutrient recovery using the algal biomass from wastewater treatment presents many challenges that remain to be overcome. Many algal species have been successfully used for removing nutrients in laboratory cultivation systems (e.g. Chinnasamy et al. 2010; Johnson and Wen 2010; Boelee et al. 2011; Fang et al. 2015), however, there are very few large-scale applications (Craggs et al. 1996). The lack of large-scale systems is limiting research on its potential for producing a phosphorus rich algal biomass. One of the few studies on the production of algal biomass as a biofertlizer is still that of Mulbry et al. (2005), which was published more than ten years ago. However, the results of this research indicates that algal biomass produced during the treatment of wastewater has very high potential for use as a biofertilizer.

Conclusions

The recovery of nutrients, especially P, seems to be necessary for the sustainable development of agriculture and the environment in the future. Many studies demonstrate the high ability of algae to remove nutrients from wastewater. Fewer studies have also shown the high potential of algae for nutrient recycling. Several steps are needed to overcome the problem of successfully developing algal biotechnologies for nutrient recycling. Primarily, it is the optimization of current technologies for more efficient sequestration of nutrients. These efforts should be focused especially on the traditional usage of HRAP for wastewater treatment. The adaptation of new methods developed in the laboratory for large scale use is also important. This step includes selection of suitable cultivation systems for specific species with a high ability of nutrient removal. The large-scale cultivation of microalgae in wastewater using closed photobioreactors is rarely reported. However, the optimization of energy inputs into the cultivation process and new technologies for harvesting could bring progress in this area. The next stage of the research will be the utilization of nutrient rich algal biomass obtained during the wastewater treatment process. The application of different kinds of biomass to soil connected with the investigation of nutrient release and the utilization by plants are only a few of the issues, but they are very complex. An effective solution could close the nutrient cycle. Despite the high potential of microalgae for nutrient recovery, there is still little attention paid to their use for nutrient removal in water management.

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