



Review

In situ bioremediation of surface waters by periphytonsYonghong Wu^{a,*}, Lizhong Xia^a, Zhiqiang Yu^b, Sadaf Shabbir^{a,c}, Philip G. Kerr^d^a State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, No. 71 East Beijing Road, Nanjing 210008, PR China^b Center for BioEnergetics, Biodesign Institute at Arizona State University, P.O. Box 875701, Tempe, AZ 85287-5701, United States^c Department of Microbiology, Quaid-i-Azam University, 3rd Avenue, 45320 Islamabad, Pakistan^d School of Biomedical Sciences, Charles Sturt University, Wagga Wagga, NSW 2678, Australia

HIGHLIGHTS

- Periphyton communities are capable of removing miscellaneous contaminants.
- Cell immobilization technology displays immense potential for periphyton formation.
- The application of periphyton is feasible for bioremediation of surface waters.

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ABSTRACT

Environmentally benign and sustainable biomeasures have become attractive options for the *in situ* remediation of polluted surface waters. In this paper, we review the current state of reported experiments utilizing naturally occurring periphyton. These are microbial communities consisting of heterotrophic and photoautotrophic microorganisms that are reportedly capable of remediating surface waters which suffer from pollution due to a variety of contaminants. In our review, we focus on four aspects of bioremediation: multiple contaminant removal, the processes involved in contaminant removal, successful cell immobilization technologies and finally, the consideration of safety in aquaculture. It has been noted that recent developments in immobilization technologies offer a fresh approach facilitating the application of periphyton. The use of periphyton biofilm overcomes several disadvantages of single species microbial aggregates. The inclusion of periphyton, as a stable micro-ecosystem, is a promising *in situ* strategy to restore decimated surface water ecosystems.

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1. Introduction

Bioremediation refers to the use of microorganisms to eliminate or reduce the concentrations of hazardous wastes at a contaminated site (Boopathy, 2000; de Lorenzo, 2008). One important characteristic of bioremediation is that it is carried out in non-sterile open environments comprising of a variety of microorganisms (Huang et al., 2013; Sivakumar et al., 2012). Out of this diversified group of microorganisms, the central role towards degradation of contaminants is being accomplished by bacteria (Huang et al., 2013). A biological treatment system comprising of these microorganisms has various applications such as the rehabilitation of contaminated sites, e.g., water, soils, sludges, and waste streams (Boopathy, 2000; de Lorenzo, 2008; Wu et al., 2012).

Boopathy (2000) has categorized bioremediation methods into *ex situ* and *in situ* bioremediation (Boopathy, 2000; de Lorenzo, 2008) but addresses primarily *in situ* methods (e.g., composting (Jørgensen et al., 2000; Peng et al., 2013), bioreactors (Wijekoon

et al., 2013; Wu et al., 2011a,c; Yan et al., 2011), biofilters (Gómez-Silván et al., 2010; Jing et al., 2012), bioaugmentation (Mrozik and Piotrowska-Seget, 2010; Schauer-Gimenez et al., 2010), biostimulation (McGlashan et al., 2012). Although *in situ* bioremediation methods have been in use for two to three decades, they have not yet yielded the expected results. Their limited success has been attributed to reduced ecological sustainability under environmental conditions. Therefore, it is necessary that a stable microbial eco-system including the balance of structure and composition should be obtained during the implementation of a bioremediation engineering project.

de Lorenzo (2008) discusses a biological systematic approach giving an excellent overview of bioremediation involving algal species. It is wanted to focus a little more precisely on periphyton communities which are an important ecological constituent in surface waters consisting mainly of phototrophic benthic microbial communities that plays a major role in primary productivity and food source biomasses (Larned, 2010). The periphyton mainly comprised of algae, diatoms, fungi, bacteria and the associated protozoa as well as small multicellular animals (Wu et al., 2010a,b). Periphyton can be found virtually anywhere under

* Corresponding author. Tel.: +86 25 86881330; fax: +86 25 86881000.

E-mail address: yhwu@issas.ac.cn (Y. Wu).

water; it plays an important role in nutrient cycling and trophic transfer of nutrients, and affects the length or structure food chain (or web) in water ecosystems (Arnon et al., 2007; Azim, 2009). These factors, and the immobilization nature of periphyton communities, make periphyton a potentially important curator of contaminants in aquatic ecosystems (Kanavillil et al., 2012; Small et al., 2008).

Periphyton filtration is a renowned method of bioremediation of polluted water (Bradac et al., 2010). Moreover, periphyton communities can be easily contrived and/or incorporated into bioreactors, resulting in efficiently operating and running bioremediation engineering projects (Wu et al., 2010b). In addition, the microbial composition of the periphyton communities has a relatively robust nature, which tends to resist change under variable conditions (Flynn et al., 2013; Larned, 2010; Stenger-Kovács et al., 2013). Due to their properties, the application of periphyton does not need a long acclimation phase for adapting to local environmental conditions.

2. Current treatments

Diverse measures have been developed to treat domestic wastewater, groundwater, and specific industrial wastewater and/or contaminants, such as the treatment of domestic wastewater by an activated sludge process (Kassab et al., 2010), the bioremediation of groundwater by aeration (Jechalke et al., 2010), the use of bacterial H₂ reduction via a membrane biofilm reactor for the removal of oxidized contaminants (Xia et al., 2013), and the methylene blue dye removal bioadsorbent (*Sesamum indicum* L.) (Feng et al., 2011). These measures have shown obvious benefits of periphytons in purifying wastewater. However, surface waters often suffer from multiple contaminants carried by point and non-point source fluxes (Bosch et al., 2013; Wu et al., 2011b,d,e; Yan et al., 2011). Thus the development of a multi-functional water treatment system has practical significance for simultaneous removal of the diverse array of contaminants from drinking water and wastewater.

3. The potential of periphyton

It is noticeable that biological methods have become precedence in water and wastewater treatment due to their environmentally benign and sustainable characteristics. Thus, periphytons comprising of diverse species will be discussed in this review so as to highlight their potential to remediate surface waters that have been polluted by 'heterogeneous water' such as non-point source runoff or complex industrial wastewater.

The objective of this survey of the literature is to examine whether the hypothesis that the technologies based on periphyton are capable of remediating surface water that suffers from pollution by multiple contaminants. Although the use of periphyton in bioremediation it is still in its infancy, understanding how it may be used in surface water and wastewater management will represent a step forward. In this review, the microorganisms in the periphyton were considered as an ensemble, calling periphyton biofilm, ignoring the interactions among microorganisms in periphytons because it is impossible to understand the interactions among microorganisms and/or contaminants in such complex surface waters under the current technological level.

4. The removal of various contaminants

4.1. Heavy metal removal

Metal accumulation by periphyton has been relatively well-investigated. (Serra et al., 2009) recently reported their studies of

copper exposure and its effects on the periphyton community in fluvial ecosystems, albeit under controlled conditions. Bere et al. (2012) found that Cr (III) and Pb (II), under field conditions, influence accumulation and toxicity of Cd (II) in tropical periphyton communities. Concentration and speciation were observed to vary dynamically in a small stream during rain events. Analysis revealed that the Cd (II) content in periphyton closely followed Cd (II) concentrations in water, despite being in the presence of higher concentrations of Zn (II) and Mn (II). Decrease of the Cd (II) content in periphyton after the rain events was slower than its decrease in water and is suggestive of metal accumulation (Bradac et al., 2010). Many photosynthetic species such as the green alga *Chlamydomonas reinhardtii* also known to possess the immense capacity to absorb metals, and so have great potential for removing metals from wastewaters (Fortin et al., 2007; Mehta and Gaur, 2005).

Periphyton accumulate heavy metals by three main mechanisms (Holding et al., 2003): adsorption in extracellular polymeric substances, cell surface adsorption, and intracellular uptake (or absorption). Metal uptake in periphyton by adsorption and absorption have been evaluated by measuring total and intracellular metal content (Meylan et al., 2003; Serra et al., 2009). Further research has revealed that inactive/dead microbial biomass also passively binds metal ions via various physicochemical mechanisms (Chien et al., 2013; Wang and Chen, 2009).

4.2. The degradation of organic compounds

Periphyton biofilm is a complex symbiotic system consisting of both photosynthetic and non-photosynthetic microorganisms. A number of species in the periphyton community degrade a range of organic compounds. It has been found that most of the organic matter produced by periphyton is degraded microbially within the periphytic community (Azim, 2009). That being said, it should be noted that the rapid removal of *Microcystis aeruginosa* toxin, microcystin-RR (MCRR) by periphyton in the so-called latent adaptation period is largely due to adsorption, after which time biodegradation plays the key role (Wu et al., 2010a).

Phenols are a common contaminant and their degradation pathways have been extensively investigated. Generally, the metabolic degradation of these organic compounds is associated with oxidative enzymes in microorganisms (Olaniran and Igbinsosa, 2011). For example, the degradation pathway of 2,4,6-trichlorophenol (2,4,6-TCP) in the well-known chloroaromatic compound-degrading aerobic bacterium *Cupriavidus necator* JMP134 (pJP4) (formerly *Ralstonia eutropha* JMP134) takes place in the presence of a array of enzymes encoded by the *tcpRXABCYD* gene cluster (Sánchez and González, 2007). Studies have also reported that the *tcpABC* genes from *C. necator* JMP134 (pJP4) (Clément et al., 1995; Olaniran and Igbinsosa, 2011; Padilla et al., 2000), encode enzymes that convert 2,4,6-TCP to 2-chloromaleylacetate (2-CMA) (Louie et al., 2002; Olaniran and Igbinsosa, 2011). The *tcpABC* genes in *C. necator* are adjacent to four other open reading frames (ORFs) (*tcpY*, *tcpD*, *tcpR*, and *tcpX*), thus forming a putative catabolic operon (Olaniran and Igbinsosa, 2011; Sánchez and González, 2007). The *tcpR* gene carries a significant identity to the *pcpR* gene, which encodes a LysR-type regulator involved in the degradation of pentachlorophenol (PCP) in *Sphingobium chlorophenolicum* (Cai and Xun, 2002; Olaniran and Igbinsosa, 2011). The *tcpX* protein is supposed to provide FADH₂ to *tcpA* because it has high identity to the TftC protein, which executes this function in the degradation of 2,4,5-TCP in *Burkholderia cepacia* AC1100 (Gisi and Xun, 2003; Olaniran and Igbinsosa, 2011). This flavin reductase activity would also be encoded by the *tcpB* gene, which shows sequence similarity to genes coding for nitroreductases. However, a *tcpB* mutant still degrades 2,4,6-TCP (Louie et al., 2002; Olaniran and Igbinsosa, 2011).

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