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Review

Mechanisms of removing pollutants from aqueous solutions by microorganisms and their aggregates: A review

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ABSTRACT

With the public's enhanced awareness of eco-safety, environmentally benign measures based on microorganisms and microbial aggregates have become more accepted as methods of removing pollutants from aquatic systems. In this review, the application of microorganisms and microbial aggregates for removing pollutants from aqueous solutions is introduced and described based on mechanisms such as assimilation, adsorption, and biodegradation. The advantages of and future studies regarding the use of microorganisms and microbial aggregates to remove pollutants are discussed. Due to the limitation of a single microorganism species in adapting to heterogeneous conditions, this review demonstrates that the application of microbial aggregates consisting of multiple photoautotrophic and heterotrophic microorganisms, is a promising method of removing multiple pollutants from complex wastewaters and warrants further research.

1. Introduction

Due to the worldwide increase in water pollution and aquatic ecosystem imbalance, as well as the increase in public awareness, more attention has been focused on environmentally friendly measures to combat these problems, such as the use of microorganisms and their aggregates (Wu et al., 2010a, 2011a).

To date, the use of technologies based on microorganisms and/or microbial aggregates has provided a wide range of useful and promising strategies to clean up many types of pollutants, such as cadmium, copper, lead (Choi et al., 2009) and microcystins (Wu et al., 2010a). A variety of environmentally benign technologies based on microorganism and microbial aggregates, such as periphyton and hybrid bioreactors are now used to remove pollutants from aquatic systems (Wu et al., 2010b, 2011a). These technologies have been applied worldwide and are generating an explosion of data on the pollutant removal process and improvements in removal efficiency, some of which will be discussed in this review. Little information however, focuses on describing the mechanisms of the technologies based on the use of microorganism and/or microbial aggregates to remove pollutants from water and wastewater.

Therefore, the major objective of this review is to explore the mechanisms through which microorganisms and microbial aggregates eliminate pollutants from aquatic ecosystems. This work may provide valuable information to optimize ecological engineering based on microorganism and microbial aggregate technologies and thus improve pollutant removal efficiency. This investigation may provide further insight into the interactions between microorganisms and/or microbial aggregates and pollutants.

2. Microorganisms and microbial aggregates

Microorganisms in aquatic ecosystems include bacteria, yeasts, molds, protozoa, algae, rickettsia, and viruses (Alexander, 1999). Microbial aggregates, such as river epilithic biofilms and periphytons, are composed of heterotrophic and/or autotrophic microorganisms, which exist in many ecosystems including water and soil systems.

Microbial aggregates in natural aquatic ecosystems are often complex microbial consortia of algae, bacteria and other micro- and meso-organisms that develop on solid substrata in aquatic environments (Wu et al., 2010a). Embedded in a mucilage matrix of microbially generated extracellular polymeric substances (EPS), these aggregates have relatively high mechanical stability and cell density (Boulêreau et al., 2011). In wastewater and water bio-treatment systems, microbial aggregates more often consist of heterotrophic microorganisms such as bacteria. Microbial aggregates constitute one of the important parts of activated sludge and particulate aggregates, which exhibit a superior ability to remove pollutants such as easily biodegraded organic matter (Suvilampi et al., 2003).

Microbial aggregates play a significant role in natural aquatic ecosystems and biological wastewater treatment systems by affecting primary production, food chains, organic matter and

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nutrient cycling, in addition to the accumulation of contaminants such as pesticides and toxic metals (Oller et al., 2011). These specific microbial aggregate functions are relevant to the aggregates' complex structure. The microbial aggregate structures formed from micro- and meso-microbes have different shapes, such as branched, filamentous, spherical, oval, mushroom, sheet, and some irregular shapes (Okabe et al., 1998). The configurations that microbial aggregates adopt depend on environmental conditions such as light, nutrient availability, water depth, and flow rate (Khatoon et al., 2007). Under favorable conditions, microbial aggregates will adopt compact structures and exhibit good performance with respect to both adsorption and sedimentation. These compact organizations in wastewater treatment systems can help improve pollutant removal efficiency. Conversely, microbial aggregates can become loose under unfavourable conditions and even form a single community, which will adversely affect wastewater treatment efficiency (George et al., 2005).

The extracellular polymeric substances (EPS) matrix is often considered the consolidating material of an entire microbial aggregate. The extracellular component can reach 98% of the total organic carbon fraction of microbial aggregates (Ras et al., 2011) with carbohydrates and proteins usually the major components. The spatial distribution of EPS is reportedly heterogeneous and can be observed using confocal laser scanning microscopy (CLSM) or fluorescence microscopy (Sheng et al., 2010). The contents of EPS crucially affect the properties of microbial aggregates, such as adsorption ability, surface characteristics, mass transfer stability, flocculation ability, settle ability, dewatering ability, stability, adhesion ability and the formation of microbial aggregates (Neu and Lawrence, 2010; Sheng et al., 2010).

3. Application to pollutant removal

Environmental pollution is one of the most urgent problems in some developing countries such as China due to the serious pollution that accompanies rapid economic development. Water pollution is one of the most prominent environmental issues and has become a global problem. Many water problems, such as eutrophication, harmful algal blooms, non-point source pollution and groundwater pollution threaten freshwater resources and the safety of drinking water. The outbreak of water pollution in surface and ground aquatic ecosystems poses a threat to human health, making it necessary to immediately treat water using environmentally benign measures.

Environmentally friendly technologies based on microorganisms and/or microbial aggregates are usually used to remove pollutants from aquatic ecosystems. The main pollutant removal mechanisms include assimilation, adsorption, biodegradation, complexation, ion exchange, flocculation, precipitation, and predation by microorganisms and pollutant depletion through complex biochemical reactions during microorganism metabolism.

3.1. Assimilation

3.1.1. The ingestion of nutrients

Nutrients, such as carbon, nitrogen and phosphorus, are necessary for microorganism and microbial aggregate growth. The incorporation of nutrients within microbial biomass is assumed to be very efficient through the use of photosynthetic microorganisms. These microorganisms have the ability to utilize inorganic forms of nitrogen, such as nitrite, nitrate or ammonium, as the sole nitrogen source for growth (Yariv, 2001). In the first reactor of an intermittently aerated anaerobic–aerobic activated sludge process, nitrification and phosphorus uptake occur during the aeration period, followed by denitrification and phosphorus release during the agitation period (Sasaki et al., 1996). In the second reactor, nitrification and phosphorus uptake occur during aeration and denitrification and weak phosphorus uptake occur during agitation (Sasaki et al., 1996; Villaverde, 2004).

3.1.2. The role of organic materials

Microbial aggregates such as biofilms often provide the primary habitat for many neighboring suspended microorganisms (De Beer and Stoodley, 2006). The organic materials adsorbed and deposited onto microbial aggregates are often the main nutrient sources for these microorganisms. Most of these organic materials are absorbed and converted into cell materials such as cytoplasm, maintaining microorganism growth and fostering the formation of some active materials such as EPS. The rest of these organic materials are excreted. During the conversion process much ATP energy is released. This energy promotes microorganism growth and the formation of microbial aggregates (Adav et al., 2010; Neu and Lawrence, 2010; Sheng et al., 2010).

In addition, organic materials often 'envelop' and/or 'carry' nutrients that in turn supply the growth of microorganisms. It is well known that nutrient removal by microorganism assimilation is associated with microbial aggregate layers (Laspidou and Rittmann, 2002). It has been reported that there is a very thin water layer that adheres to a microbial aggregate surface. The particulates in this thin water layer move slowly, and the organic materials attached to the particulates are mostly absorbed by local microorganisms. As a result, nutrient concentrations in the thin water layer of microbial aggregates are lower than those in the inner layers of microbial aggregates. Nutrients carried by particulates in water then move to the thin water layer of microbial aggregates, resulting in greater nutrient ingestion by microorganisms (Badireddy et al., 2010; Laspidou and Rittmann, 2002; Sheng et al., 2010).

Not all organic materials that adhere to microbial aggregates can be converted into cell bioplasm; many are retained by microbial aggregates as "stored materials". These include dissolved and non-dissolved organic materials useful to microorganism metabolism during growth. Polysaccharides and polyhydroxy butyrate (PHB) are stored inside cells as easily degraded compounds, providing sources of carbon and energy for growth. They are also useful for the removal of nitrogen. Under anaerobic and anoxic conditions, these materials can be easily degraded during denitration (Badireddy et al., 2010; Laspidou and Rittmann, 2002; Sheng et al., 2010). Most easily degraded materials however, are stored in the extracellular matrix. Once the intracellular concentration of these compounds decreases to a certain degree, the materials in the extracellular matrix (microbial aggregate matrix) will become the carbon source for the denitration process (Badireddy et al., 2010; Laspidou and Rittmann, 2002; Sheng et al., 2010).

3.2. Adsorption

The potential for metal sorption by certain types of biomass provides the basis for the development of a new approach to remove low concentrations of heavy metals. Adsorption by microorganisms and/or microbial aggregates, often called biosorption, is a mechanism that can be used to remove pollutants from aqueous media. A variety of microbial materials are known to bind these pollutants, including bacteria (*Pseudomonas aeruginosa*) (Joo et al., 2010), fungi (*Aspergillus niger*), yeast (*Rhizopus oryzae*) and algae (*Chaetomorpha linum*) (Fu and Wang, 2011). The complex surface structures consist of the microorganisms mentioned above and feature some special properties, such as adhesion and flocculation abilities (Aksu, 2005), which enable microorganism communities to adsorb some heavy metals, dyes and toxic materials from solutions (Aksu, 2005; Sheng et al., 2010).

Furthermore, there is evidence that microbial aggregates such as biofilms maintain their structural heterogeneity by releasing EPS-degrading enzymes (Davies et al., 1998). Biosorption does not produce toxic metabolites, thereby providing a feasible way of treating and recycling wastewater. As the processes of complexation, ion exchange, flocculation and precipitation interact to remove pollutants from aqueous solutions they are discussed in conjunction with the adsorption process in this review.

3.2.1. The removal of heavy metals

Heavy metal removal is probably related to the structure of microbial aggregates. It is well known that the structure of microbial aggregates such as periphyton biofilm ranges from patchy monolayers to filamentous accretions during different phases of biofilm formation (Wu et al., 2010a). The basic structure of microbial aggregates includes at least three conceptual models; (i) heterogeneous-mosaic biofilm aggregations; (ii) penetrated water-channel biofilms; and (iii) dense confluent biofilms. Due to the special porous structure of microbial aggregates, the dynamics of pollutants adsorbed onto or desorbed from the active sites of an aggregate surface can occur concomitantly (Wu et al., 2010a); such is the case with cadmium, copper and lead ions that are freely shuttled into and out of *Ralstonia* sp. and *Bacillus* sp. aggregates (Choi et al., 2009).

Complexation plays an important role in removing heavy metals by microbial aggregates. Many functional groups in the EPS, such as carboxyl, phosphoric, sulfhydryl, phenolic and hydroxyl groups, can complex with heavy metals (Sheng et al., 2010). To date, many studies have shown that there is a significantly practical potential to remove heavy metals from aqueous solutions using microorganisms and microbial aggregates (Joo et al., 2010; Kao et al., 2006, 2008; Tsuruta, 2004). For example, *P. aeruginosa ASU 6a* (Gram-negative) aggregates and *Bacillus cereus AUMC B52* (Gram-positive) aggregates are inexpensive and efficient biosorbents for Zn(II) removal from aqueous solutions (Joo et al., 2010). Some Gram-negative bacterial strains, such as *Acinetobacter calcoaceticus*, *Erwinia herbicola*, *P. aeruginosa* and *Pseudomonas maltophilia*, have a high affinity for gold biosorption, as do *P. maltophilia* cells immobilized with polyacrylamide gel (Tsuruta, 2004). *Escherichia coli* is an effective bacterial biosorbent used for the removal of multiple heavy metals, such as lead (Pb), copper (Cu), cadmium (Cd), and zinc (Zn) (Kao et al., 2006). The studies mentioned above indicate that the removal of heavy metals is related to the composition of the microorganisms and/or microbial aggregates employed.

The ion exchange mechanism is the main mode of interaction between some divalent cations and the EPS (Sheng et al., 2010). It has been reported that the binding between the EPS and divalent cations, such as Ca^{2+} and Mg^{2+}, is one of the main intermolecular interactions supporting microbial aggregate structures. During the removal of metals by microbial aggregates, Ca^{2+} and Mg^{2+} are simultaneously released into solution, indicating that ion exchange is involved (Yuncu et al., 2006).

Solid-liquid separation mechanisms, such as flocculation and/or precipitation, are important processes employed by microbial aggregates in removing heavy metal ions from wastewater (Choi et al., 2009). For example, a brewer's yeast strain (*Saccharomyces cerevisiae*) was used to remove heavy metals (Cu^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+} and Cr^{3+}) from a synthetic effluent. The solid–liquid separation process was carried out using the flocculation ability of the strain. The results demonstrated that flocculation by yeast strains can be used as an inexpensive and natural separation process to remove heavy metals for a wide range of industrial effluents (Machado et al., 2008).

During heavy metal adsorption, ions can be isolated by adsorption onto EPS from microorganisms and microbial aggregates (Choi et al., 2009). Natural and extreme acidic eukaryotic biofilms have a strong binding capacity for heavy metals, such as Hg(II), Zn, Cu, Co, Ni, As, Cd, Cr and Pb, by releasing colloid materials such as protein, or affecting the ion value (e.g., the transformation of Hg^{2+} to Hg^{0}) (Choi et al., 2009; George et al., 2005; Neu and Lawrence, 2010). This indicates that EPS plays an important role during the removal of heavy metals by microbial aggregate adsorption. The EPS characteristics may significantly affect the chemical forms, mobility, bioavailability and ecotoxicity of heavy metals in aqueous solutions.

The efficiency of heavy metal adsorption by microbial aggregates is affected by many factors including biological composition, chemical composition, functional groups and pH. For example, mammalian and fish metallothioneins (MTs) expression in *E. coli* aggregates leads to a significant increase (5–210%) in the overall efficiency of biosorption of Pb, Cu, Cd and Zn (Kao et al., 2006). The biosorption of gold from a solution containing hydrogen tetrachloroaurate (III) using *P. maltophilia* with a high affinity for gold adsorption was very rapid and affected by the pH of the solution, external gold concentration, and cell amounts (Tsuruta, 2004). The presence of amino, carboxyl, hydroxyl, and carbonyl groups led to greater zinc biosorption by a Gram-negative bacterium (*P. aeruginosa*) with respect to that of a Gram-positive bacterium (*B. cereus*) (Joo et al., 2010).

The major advantages of biosorption by microbial aggregates are their high effectiveness in reducing heavy metal ions and the use of inexpensive biosorbents. Microbial aggregate biosorption processes are particularly suitable for treating dilute heavy metal wastewater (Fu and Wang, 2011) and may be used under various conditions due to the complex composition of heterotrophic and photoautotrophic microorganisms.

Microbial biomass heavy metal biosorbents characteristically exhibit environmental safety, broad sources, low cost, short maturation and acclimation periods, and rapid adsorption. These microbial aggregates have vast potential in removing heavy metals under various conditions because of the hierarchical and self-maintained micro-ecosystem that is established in microbial aggregates. Moreover, these microbial biomasses can be cultivated and fostered in wastewater treatment systems such as bioreactors, which not only improves heavy metal removal efficiency but also maintains the microbial aggregate micro-ecosystems in a steady state (Wu et al., 2011b).

3.2.2. The removal of organic matter

EPS in microbial aggregates have many available sites for the adsorption of metals and non-biodegradable and persistent organic matter, such as dyes, aromatics, aliphatics in proteins and hydrophobic regions in carbohydrates (Sheng et al., 2010). For example, the color removal rate of the azo-dye, Congo Red by *Basidiomycete* biosorption in an agitated batch system reached 90% (Tatarko and Bumpus, 1998). Sivasamy and Sundarabal (2011) used *A. niger* and *Trichoderma* sp. as biosorbents for the biosorption of another azo dye, Orange G. They found that the maximum biosorption occurred at pH 2, and compared with *Trichoderma* sp., the biomass obtained from *A. niger* was a better biosorbent. Wu et al. (2010a) demonstrated that biosorption by microorganisms and microbial aggregates was the main mechanism for the removal of Microcystin-RR from aquatic solutions during the adaption period.

The adsorption of organic pollutants by microbial aggregates may be attributed to the fact that there are some hydrophobic regions in EPS (Spath et al., 1998). More than 60% of benzene, toluene and m-xylene was reportedly adsorbed by EPS with only a small fraction of these pollutants adsorbed by cells (Spath et al., 1998). EPS with negative charges is capable of binding with positively charged organic pollutants via electrostatic interaction (Neu and Lawrence, 2010; Sheng et al., 2010). Moreover, proteins have a higher binding strength and better binding capability than humic substances. Soluble EPS has a higher fraction of proteins than bound EPS; thus, it may have a greater binding capacity than bound EPS (Pan et al., 2010; Sheng et al., 2010).

3.3. Biodegradation

Biodegradation is the chemical disbanding of organic materials by microorganisms or other biological agents. Microbial degradation of chemicals in the environment is an important route for the removal of these compounds. The types of compounds range from plastics to organic chemicals (both industrial chemicals used in large quantities and trace chemicals such as endocrine disruptors) to organometallics such as methylmercury (Fu and Wang, 2011; Sheng et al., 2010). The biodegradation of these compounds often involves a complex series of biochemical reactions and usually varies with the microorganisms involved. Compounds can be degraded aerobically and/or anaerobically. A term related to biodegradation is biomineralization, which is the conversion of organic matter to minerals (Diaz, 2008).

Currently, most efforts are directed toward the removal of specific contaminants such as nutrients (N and P) and sulfurous compounds because they are of great concern due to their significant impact on water quality (Villaverde, 2004; Wu et al., 2011a,b,c). The most important practical use of microbial aggregates such as biofilm is in biological wastewater treatment, while many emerging technologies utilize microbial aggregates for biodegradation and bioremediation in bioreactors (Liong, 2011). Owing to the inclusion of specific microorganisms with special degradation functions in microbial aggregates (e.g. microcystins-degrading bacteria *Sphingpoyxis* sp. and *Sphingomonas* sp.), microbial aggregates as an ensemble are also often used to remove specific compounds such as microsytin-RR (Wu et al., 2010a), aliphatic homopolyesters and aliphatic-aromatic copolyesters (Abou-Zeid et al., 2004).

3.3.1. The removal of N, P and sulfurous compounds

The biodegradation of nitrogenous compounds by microorganisms and microbial aggregates has been well described. This process often features two predominant processes: autotrophic nitrification and heterotrophic denitrification. Some minor processes such as heterotrophic nitrification and aerobic denitrification are also involved (van Loosdrecht and Jetten, 1998). Organisms or aggregates that degrade nitrogenous compounds can be divided into three main groups according to the biological nitrogen removal processes they conduct: (i) degradation of nitrogen-organic matter and the release of ammonia by various microorganisms; (ii) conversion of ammonia to nitrate by certain autotrophic microorganisms and; (iii) conversion of nitrate to nitrogen gas by a mixed culture that uses nitrate as an electron acceptor (as opposed to free oxygen used by the nitrifiers) in the metabolism of organic carbon (Villaverde, 2004; Wu et al., 2011b,c).

Another major route for the removal of biological nitrogen from waste liquids involves the removal of nitrogen by nitrification-denitrification processes (Yariv, 2001). The use of a multi-level bioreactor fosters the coexistence of photoautotrophic and heterotrophic microorganisms, which provide environments for the combination of oxidative and reductive processes (Wu et al., 2011b). Dos Santos et al. (1996) investigated a system that uses the oxidative and reductive environments within polymer beads to remove nitrogen via nitrification-denitrification processes. Their results showed that high nitrogen removal rates (up to 5.1 $\text{mmol N m}^{-3}\text{ polymer s}^{-1}$) were achieved under continuous flow and aerobic conditions because the nitrifier *Nitrosomonas europaea* and either of the denitrifiers *Pseudomonas denitrificans* or *Paracoccus denitrificans* were co-immobilized in this system.

It is well known that the introduction of excessive phosphorus accelerates the eutrophication process of closed water areas. Phosphorus is often regarded as the limiting factor for phytoplankton growth, thereby accelerating the growth of harmful algal blooms (Smith et al., 1999). Thus, the removal of phosphorus, especially biological phosphorus, has recently been a subject of great concern. In microbial aggregate systems such as wastewater treatment plants using the activated sludge biofilm method, biological phosphate removal is based on the capacity of some microorganisms to store ortho-phosphate intracellularly as poly-phosphate. These microorganisms store polyhydroxybutyrate (PHB) anaerobically, which is oxidized in a phase with an electron acceptor such as oxygen or nitrate present (Villaverde, 2004).

The group of microorganisms in microbial aggregates that are largely responsible for P removal are known as the polyphosphate accumulating organisms (PAOs) (Oehmen et al., 2007). PAOs take up readily biodegradable chemical oxygen demand substrates and store them as polyhydroxyalkanoates (PHAs). The energy required for this anaerobic process is derived from the hydrolysis of intracellular polyphosphate. In the subsequent aerobic stage, PAOs use PHAs to generate energy for growth and phosphorus uptake. In this process the PAOs take up more phosphorus than that released during the anaerobic stage (luxury uptake) (Zhou et al., 2010). Removing phosphate using nitrate instead of oxygen has the advantage of saving energy (oxygen input) and using less organic carbon. It has been shown that it is possible to accumulate denitrifying P-removing bacteria (DPB), which can remove P and N simultaneously in microbial aggregate systems (Tsuneda et al., 2006).

Sulfate is a common constituent of many industrial wastewaters (Lens and Hulshoff Pol, 2000) and its reduction is dominated by two stages of inhibition. Primary inhibition is due to competition for common organic and inorganic substrates between sulfate-reducing bacteria, which suppresses methane production. Secondary inhibition results from the toxicity of sulfide manifested in various bacteria groups (Chen et al., 2008). Two major groups of sulfate-reducing bacteria (incomplete and complete oxidizers) dominate the sulfate reduction process. Incomplete oxidizers reduce compounds such as lactate to acetate and CO_2. Complete oxidizers completely convert acetate to CO_2 and HCO_3^- (Chen et al., 2008).

The microbial conversion of sulfurous compounds involves the metabolism of several different specific groups of bacteria, such as sulfate-reducing bacteria, sulfur- and sulfide-oxidizing bacteria, and phototrophic sulfur bacteria (Lens and Hulshoff Pol, 2000). Moreover, some of these microorganisms can simultaneously use nitrate in what has been reported as autotrophic denitrification by sulfur- and sulfide-oxidizing microorganisms (Villaverde, 2004). In the anaerobic part of microbial aggregates such as biofilms, sulfate reduction contributes considerably to the mineralization process (Zhang et al., 2009). The other important internal cycle in microbial aggregates such as biofilms is sulfate reduction coupled to sulfide oxidation (Villaverde, 2004).

3.3.2. The removal of phenols

Phenols are an important industrial chemical widely and commonly used in explosives, medicine, pesticides, dyes, wood preservatives and rubber production as raw materials or intermediates. Phenols are toxic, carcinogenic, mutagenic and teratogenic and are regarded as priority pollutants in the USEPA list (Veeresh et al., 2005). In the natural environment, the biodegradation rate of phenol is slow. Consequently, phenols accumulate in the environment and persist for a long time, threatening the safety of flora and fauna as well as human beings (Dosta et al., 2011). From a practical standpoint, it is therefore important to study phenol removal.

Many phenol-degrading microorganisms, including bacteria, fungi, yeast, and periphyton, have been identified in aqueous solutions (Kang et al., 2006; Kurzbaum et al., 2010; Wang et al., 2007; Yan et al., 2006). For example, the immobilized bacterium *Acinetobacter* sp. has good potential for the treatment of phenol -containing wastewater (Wang et al., 2007). Fungi strains (*Graphium* sp. and *Fusarium* sp.) have high percentages of phenol degradation with 75% degradation of 10 mM phenol in 168 h (Santos and Linardi, 2004). The biodegradation of phenol and m-cresol using a pure culture of yeast (*Candida tropicalis*) demonstrated that *C. tropicalis* alone could degrade 2000 $mg\,l^{-1}$ phenol within 66 h (Yan et al., 2006). Planktonic *Pseudomonas pseudoalcaligenes* cells exhibited a high phenol removal rate in constructed wetland systems, especially those with sub-surface flow, suggesting that surface-associated microorganisms (biofilms) can provide a much higher contribution to the removal of phenol and other organics due to their greater bacterial biomass (Kurzbaum et al., 2010). Although olive mill wastewater has a high polluting power and concentrations of phenols as well as high antibacterial activity (Bleve et al., 2011), some bacteria such as *Pleurotus* spp. strains have the ability to remove phenolic compounds from this wastewater stream (Tsioulpas et al., 2002).

The biodegradation of phenol (i.e., Bisphenol A or BPA) by microorganisms is mainly carried out by lignin-degrading enzymes such as manganese peroxidase (MnP) and laccase, which are produced by white rot basidiomycetes microorganisms (Kang et al., 2006). MnP is a heme peroxidase that oxidizes phenolic compounds in the presence of Mn(II) and H_2O_2 while laccase is a multicopper oxidase that catalyzes the one-electron oxidation of phenolic compounds by reducing oxygen to water (Kang et al., 2006; Reinhammar, 1984). MnP and laccase can degrade BPA and disrupt its estrogenic activity (Kang et al., 2006). In the case of laccase, BPA metabolism is faster in the presence of mediators, such as 1-hydroxybenzotriaxzole (HBT) and 2,2′-azo-bis(3-ethylbenzthiazoline-6-sulfonate), than in laccase alone.

Environmental conditions, i.e. whether aerobic or anaerobic, can significantly affect the efficiency of phenol biodegradation. Bisphenol A in river waters is biodegraded under aerobic conditions but not under anaerobic conditions (Kang et al., 2006). Bisphenol A in spiked samples was rapidly biodegraded under aerobic conditions (>90%), with little decrease in BPA observed under anaerobic conditions (<10%) over 10 days (Kang and Kondo, 2002a). Under anaerobic conditions, such as those in anaerobic marine sediment, BPA was not biodegraded even after 3 months of incubation. These results suggest that anaerobic bacteria have little or no ability to degrade BPA (Kang et al., 2006; Voordeckers et al., 2002).

Phenol biodegradation by microorganisms is also influenced by temperature and microbe counts. The half-lives for phenol biodegradation in 15 river water samples averaged 4 and 7 days at 30 and 20 °C, respectively, but only about 20% (0.04 mg/l) of the spiked phenol was biodegraded at 4 °C over a period of 20 days (Kang and Kondo, 2002b). With respect to bacterial counts, due to the greater bacterial biomass in the subsurface flow of constructed wetlands, the phenol removal rate is higher than that at the surface (Kurzbaum et al., 2010). It has also been reported that phenol biodegradation does not correlate with bacterial counts (Klecka et al., 2001). These differences may be due to the size of bacterial populations that can execute fast and complete phenol biodegradation or mineralization (Klecka et al., 2001).

3.3.3. The removal of quinoline

Quinoline is a heterocyclic aromatic organic compound, which is mainly used to synthesize pharmaceuticals, dyes, pesticides and many chemical additives (Padoley et al., 2008). Due to its toxicity and nauseating odor, discharging quinoline-containing waste does great damage to human health and environmental quality. The study of quinoline-degrading bacteria not only helps to reveal the metabolic mechanism of quinoline but also benefits the bio-treatment of quinoline-containing wastewater (Sun et al., 2009).

Data show that the decomposition of quinoline and its derivatives have recently been enhanced either by using the free or immobilized cells of degrading microorganisms such as *Burkholderia pickettii* (Wang et al., 2002) and *Pseudomonas* sp. BW003 (Sun et al., 2009). In many cases, the biodegradation of quinoline by microorganisms is well described by some mathematic models (Wang et al., 2002). For example, *B. pickettii* immobilized on a hybrid carrier could be used to degrade quinoline, with the subsequent degradation process described by a zero-order reaction rate equation when the initial quinoline concentration was in the range of 50–500 $mg\,l^{-1}$ (Wang et al., 2002).

Although different genera of bacteria may produce different intermediates, almost all of them transform quinoline into 2-hydroxyquinoline as a first step under anaerobic conditions (Kaiser et al., 1996). During the following transformation step, a new degradation product of quinoline 3,4-dihydro-2-quinoline accumulates and is further transformed into unidentified products (Johansen et al., 1997). The transformation of quinoline by *Pseudomonas* sp. under anaerobic conditions has been reported. The first intermediate metabolite of quinoline catabolism was identified as 3-hydroxy coumarin (Padoley et al., 2008). In general, degradation rates including quinoline degradation, are significantly faster under aerobic conditions (Sun et al., 2009). This is consistent with the accepted view that microbial populations are generally larger and more metabolically active under aerobic conditions.

Because quinoline is one of the most important compounds containing N as a heteroatom (Padoley et al., 2008), this property leads to the reration of quinoline with N transformation during quinoline decomposition. In the aforementioned study, if quinoline was the sole C and N source, N was transformed primarily into ammonia-N, which was then utilized to synthesize cells; moreover, less than 6% of ammonia-N was transformed into nitrate through heterotrophic nitrification. When glucose was added, more ammonia-N was utilized by BW003 such that the concentration of ammonia-N was clearly reduced. Therefore, by controlling the C/N ratio, ammonia-N as well as quinoline and its metabolic products can be completely eliminated (Sun et al., 2009).

Endocrine disrupting compounds (EDCs) are chemicals with the potential to elicit negative effects on the endocrine systems of humans and wildlife (Liu et al., 2009). In the past few decades, many methods for EDC elimination from the aquatic ecosystem have been developed such as activated sludge wastewater treatment systems. During the removal of EDCs by activated sludge wastewater treatment systems and other similar systems, the major mechanisms involved are adsorption and biodegradtion (Liu et al., 2009). Considering the EDCs's toxicity to microorganisms, the combination of physical and chemical methods such as activated carbon absorption and advanced oxidation may improve the EDC removal efficiency during the application of technologies based on microbial aggregates.

3.4. The conjoint action of assimilation, adsorption, biodegradation

The diverse metabolic capabilities of microorganisms and their interactions with hazardous organic and inorganic compounds have long been recognized. In many practical cases, assimilation, biodegradation and biosorption (including complexation, ion exchange, flocculation and/or precipitation) occur at the same time during the removal of pollutants. This conjunct action may be described as shown in Fig. 1.

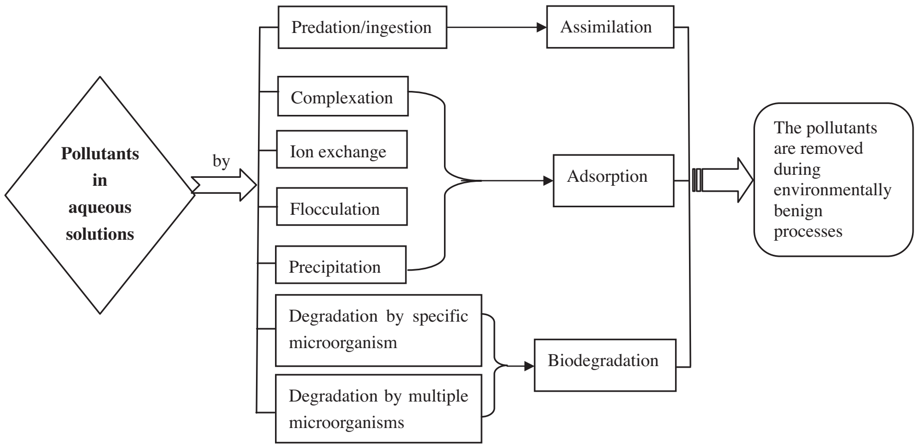

Fig. 1. The process of pollutant removal from aqueous solutions by the conjunct mechanisms of assimilation, adsorption and biodegradation.

Many studies on the conjunct action of assimilation, adsorption and biodegradation have been conducted in recent years. Some typical cases are summarized as follows.

3.4.1. The biosorption and biodegradation of trichloroethylene (TCE)

The experimental results showed that at 25 °C the adsorption equilibrium of TCE at concentrations ranging from 10 mg/L to 200 mg/L could be described by the Freundlich isotherm with adsorption completed within 15 min. The results further indicated that glucose could serve as a co-substrate and enhance TCE biodegradation through co-metabolism. The TCE biodegradation conformed to first-order reaction kinetics, and the rate constant was 0.3212 day^{-1} at 25 °C (Yang et al., 2009). A similar study revealed the effectiveness of microcystin-RR removal by periphyton in surface waters using the dual removal mechanisms of adsorption and biodegradation (Wu et al., 2010a).

3.4.2. The biosorption and biodegradation of pentachlorophenol (PCP)

Ye and Li (2007) investigated the biosorption and biodegradation of PCP by anaerobic microbial aggregates to better understand the fate of PCP in an upflow anaerobic sludge blanket reactor (UASB). Their results demonstrated that the main mechanism leading to the removal of PCP in anaerobic microbial aggregates was biodegradation, while adsorption was an accessorial process.

3.4.3. The removal of N, P and organic pollutants from water using seeding type immobilized microorganisms

Ten strains of dominant heterotrophic bacteria belonging to *Pseudomonas*, *Coccus*, *Aeromonas*, *Bacillus*, and *Enterobateriaceae* were isolated. The rates of TOC, TP, and TN removal were 80.2%, 81.6%, and 86.8%, respectively (Wang et al., 2008). The removal mechanisms simultaneously employed assimilation, adsorption and biodegradation.

To date, many devices based on the combined mechanisms of assimilation, adsorption and biodegradation to remove pollutants have been developed in which various robust microorganisms have been cultivated and cultured. These complex microorganisms, which exhibit strong activities, foster the improvement of pollutant removal efficiencies and meet the demand for the treatment of heterogeneous wastewater. Wu et al. (2011b) utilized a hybrid bioreactor featuring sequential anaerobic, anoxic and aerobic (A^2/O) processes to treat industrial wastewater and domestic sewage. The removal process included assimilation, adsorption and biodegradation, and the removal efficiencies of the nutrients were 81% for TP, 74% for TDP, 82% for TN, 79% for NO_3-N and 86% for NH_4-N. In addition, a photobioreactor-wetland (Wu et al., 2011c) and a multi-level bioreactor (Wu et al., 2011b) have been employed to remove UV_{254} matter, metals and nutrients from non-point-source wastewater.

The individual contributions of assimilation, adsorption and biodegradation to pollutant removal are not equal. During the interaction between microorganisms and pollutants, the rate of assimilation is typically low. Moreover, most pollutants are adsorbed and biodegraded by the unique metabolic activity of microorganisms and microbial aggregates. Microbial processes are compatible with various environments and can create environmental contaminants transform, decompose and degrade in some degree (Singh and Ward, 2004).

The mechanisms of assimilation, adsorption and biodegradation may affect each other, thus limiting or stimulating pollutant removal. For example, adsorption might restrict biodegradation, while nutrient limitation and the presence of organic contaminants might stimulate biodegradation (Chen et al., 2010). Adsorption and non-biodegradation have been observed to be the most common fate of tetracycline entering a biological process (Prado et al., 2009).

4. Advantages of removing pollutants from aqueous solutions

The process based on integrated assimilation, biodegradation and biosorption mechanisms can be defined as micro-remediation, which mainly exploits the unique metabolic activities of microorganisms and microbial aggregates to remove pollutants from polluted environments. The most common microbial materials used for micro-remediation include bacteria, algae, yeasts and fungi. Most of these microorganisms are environmentally benign and can be isolated from natural ecosystems. The use of technologies based on microorganisms and/or microbial aggregates offers the following benefits.

(i) Micro-remediation not only reduces pollutant concentrations effectively but can also transform end-products into nontoxic, harmless and stable substances, e.g. carbon dioxide, water and nitrogen (Alexander, 1999). Compared to physical remediation, which leads to secondary pollution and requires high operating costs, micro-remediation is safer, cleaner, and more economic (Wu et al., 2010b). Biological instead of chemical processes can reduce the levels of pollution created by human activities, helping realize ecological processes and non-waste production and ultimately achieve the goals of cleaner production and the sustainable development of resources and the environment.

(ii) The removal of pollutants based on micro-remediation often involves enzymatic reactions (Sheng et al., 2010). An enzyme is a type of active protein that has a high specificity to a certain substrate; this allows for highly efficient biodegradation with fewer by-products. Compared with chemical processes, which often require high temperatures and pressures, biodegradation is an economical investment as evidenced by its low cost, low consumption, better results, more stable processes and simple operation.

(iii) Based on the principle of micro-remediation, a variety of commercial bioremediation agents has been developed, such as Oil Spill Eater II® (OSE II), which can be rapidly decomposed after removing pollutants and be utilized as a nutrient source by microorganisms at the same time (Zhu et al., 2004). There are two advantages when using microbial agents. Firstly, using microbial agents to replace all alternative chemical medicaments, fossil energy, synthetics, etc. can reduce environmental pollution to a minimum degree and lead to the economic development of sustainable pollutant treatment. Secondly, the technologies based on micro-remediation, as a model of commercial microbial agents, may easily enter several different markets and be adopted worldwide.

(iv) The last, but not the least, advantage of using the technologies based on microorganisms and/or microbial aggregates is that the microorganisms involved are the preliminary research subjects of gene engineering, cellular engineering, enzyme engineering and other biotechnology studies. With the development of novel experimental techniques, these modern technologies may be integrated into the micro-remediation process, hence improving pollutant removal efficiency, reducing operating costs, expanding into more application areas, and securing a higher degree of safety.

Overall, the application of environmentally friendly microorganisms and microbial aggregates in the prevention and control of environmental pollution is a promising prospect. The research findings discussed herein should encourage us to further investigate the mechanisms of pollutant removal and develop more effective and cost-efficient microorganisms to control aqueous environmental pollution.

5. Future perspectives and conclusions

To date, many micro-remediation technologies, such as those discussed in this review, have been reported to remove pollutants (Aksu, 2005). These methods, however, may not be highly effective when applied to the treatment of surface waters and non-point-source wastewater due to a number of limitations. First, surface water and non-point-source wastewater samples are often more heterogeneous than daily sewage or specific industrial wastewater samples (Wu et al., 2011a). In addition, technologies based on the activated sludge method generally require a maturation phase, which is the time required for active sludge to be established. This maturation phase may take weeks or even months. Similarly, the methods employing specific pollutant-removing microorganisms may require an acclimation period prior to pollutant removal, during which the microorganisms isolated from other systems are incubated under appropriate conditions. The longer the acclimation phase the greater the risk of exposing animals and humans to these toxins. Moreover, some pollutant-removing microorganisms are unable to entirely adapt to the actual conditions of surface waters and non-point-source wastewater and eventually die (Wu et al., 2010a). At the same time, the use of photosynthetic microorganisms requires efficient illumination. This energy investment cannot be omitted (Yariv, 2001).

With the multifarious development of people's lives, the composition of water and wastewater that enters downstream surface waters or non-point-source wastewater becomes more complex. It is necessary and practical to develop an integrated technology to remove multiple pollutants simultaneously. Therefore, the development of pollutant-removing microbial aggregates with multiple compositions is suggested as a subject of future study.

From an ecological point of view, complex microbial aggregate compositions may be robust to variable surface water conditions, making it easy to form a stable and self-recycling microsystem. In recent years, several adopted biological technologies based on multiple composition microbial aggregates have been tested by using specific bioreactors (Wu et al., 2011a,b). Another possible solution is the use of immobilized microorganisms (dos Santos et al., 1996; Wang et al., 2002, 2010b). The literature shows that hybrid bioreactors can culture complex microbial aggregates composed of photoautotrophic and heterotrophic microorganisms. Hybrid bioreactors could be used to remove metals (Cu, Zn, Fe, Ca and Hg), nutrients (N and P), COD and UV_{254}nm-matter from heterogeneous non-point-source wastewater (Wu et al., 2011a,b). These positive results imply that the development of complex microbial aggregate compositions to simultaneously treat multiple pollutants is feasible.

Correspondingly, the conjunct mechanisms of assimilation, adsorption and biodegradation of microbial aggregates should be investigated to explore the processes of pollutant removal, thus enhancing removal efficiencies. In addition, other potential mechanisms might occur during pollutant removal by multiple-composition microbial aggregates. Recent studies have also demonstrated that the presence, abundance, composition, and growth of microbial aggregates are influenced by disturbances, stressors, resources, hydraulic conditions, and biotic interactions (Wu et al., 2011d). Multifaceted investigative approaches that integrate the functional modification of microbial aggregate ecology according to these five factors will be required for the development of systematically integrated technologies to remove pollutants and improve the health of aquatic ecosystems (Wu et al., 2011d).

In summary, this paper has reviewed the application of microorganisms and microbial aggregates to pollutant removal. From the data reported in the wide collection of papers discussed in this review, we conclude that microbial treatment is sensitive, easily available, easily implemented, rapid, flexible and inexpensive compared to other conventional treatments. The data also indicate however, that the study of microorganisms and microbial aggregates for removing pollutants is limited and such processes can only remove and monitor certain pollutants on a few types of microorganisms. Thus, the development of complex microbial strains (microbial aggregates) consisting of bacteria, fungi, yeast etc. for pollutant removal from "real-world" aqueous solutions will be the focal point of future research.

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