**In-situ** Biological Water Treatment Technologies for Environmental Remediation: A Review

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**Abstract**

Water treatment technologies can be classified as *in-situ* or *ex-situ* technologies. *In-situ* biological techniques include the use of aquatic plants, aquatic animals, and microbial remediation. Approaches to alleviate surface water pollution should use bioremediation methods as a primary technique. These methods should be tested not only on rivers and lakes, but also on other polluted surface streams. Furthermore, bioremediation processes need to be optimized depending on flow condition and nutrient availability. This paper comprehensively reviews the latest surface water remediation techniques that are suitable for *in-situ* applications, focusing on bioremediation technologies as effective techniques to remedy polluted water.

**Keywords:** Biological treatment; Bioremediation; *In-situ* treatment; Removal mechanism; Surface water

**Introduction**

In developing countries, water pollution is a key problem, with high levels of contaminants being reported in many rivers [1]. Different pollution control and water treatment technologies methods can be applied to resolve this issue [2-4]. Water treatment technologies can be classified as physical, chemical, or biological treatment techniques. They can also be classified as *in-situ* or *ex-situ* technologies. *In-situ* remediation techniques involve treatment at the site, while *ex-situ* involves the removal of contaminants at a remote location. Understanding *in-situ* treatment systems is essential to maintaining and controlling hydraulic conditions in open streams [5,6].

Aeration, as a physical treatment approach, is used either as a stand-alone system [6,7] or as a support for other systems (e.g., wetlands) [8,9]. Other examples of physical treatment approaches are water diversion and sediment dredging. Water diversion, however, can be a large-scale, high-cost option while sediment dredging can cause the re-suspension of contaminated sediments [10,11]. Chemical water treatment methods are also an option, an example of which is flocculation. Flocculation is used for *in-situ* treatment of both surface water and groundwater [12]. However, caution should be taken when handling chemicals, as they are potentially hazardous and can be used in large quantities.

Biodegradation is the breakdown of organic compounds by living organisms resulting in the formation of carbon dioxide and water or methane [13]. These microorganisms are bacteria, fungi, and microfauna (e.g., protozoans, some worms, and some insects) [5]. *In-situ* bioremediation has many advantages when compared to other techniques, such as low costs, less adverse impacts on the environment, and no secondary production of pollutants [14].

Indeed, many *in-situ* remediation processes, such as ecological floating bed techniques and constructed wetlands, have been developed for the bioremediation of polluted surface water and have produced “satisfactory” results [15]. This paper provides a holistic review of the latest surface water remediation developments and technologies that can be applied *in-situ*.

**Remediation Techniques**

**Aquatic plants**

Plants with strong tolerance for pollutants can mitigate or fix water pollutants through adsorption, absorption, accumulation, and degradation [16,17]. Macrophytes such as water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*) have been used for upgrading effluent quality [18]. Whorl-leaf watermilfoil (*Myriophyllum verticillatum*), pondweed (*Potamogeton spp.*), common reed (*Phragmites communis*), cattail (*Typha latifolia*), duckweed (*Lemna gibba*) and canna (*Canna indica*) are also used for wastewater treatment purposes [19]. Aquatic plants can be introduced for *in-situ* surface water remediation in different treatment systems, such as in constructed wetlands and floating bed systems (e.g., Ruan et al. [20] or submerged systems using algae [21]).

These types of remediation techniques work either by aquatic plants assimilating pollutants directly into their tissues, or by increasing biodiversity in the rhizosphere, thereby increasing the variety of chemical and biochemical reactions that can enhance purification [22]. The primary characteristics of aquatic plants include their extensive root systems and rapid growth rate, which make them attractive biological support channels for bacteria [18]. Motility and chemotaxis enable the bacteria to move towards plant roots where they can benefit from root exudates as sources of carbon and energy, and may therefore contribute to the survival and colonization of the rhizosphere [23]. In addition to being able to mitigate organic pollutants, aquatic plants, especially algae, can be also used for the removal of nonconventional pollutants such as uranium from wastewater [21,24].

Of course, there are certain disadvantages of using the planted floating-bed in lake restoration. First, it is difficult to control the hydraulic retention time (HRT) and the pollutants loading rate when this treatment system is applied at real field sites and secondly, these systems in tropical and sub-tropical areas are especially vulnerable to natural disasters such as hurricanes or typhoons [25]. Moreover,

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problem facing plant-based systems is being sensitive to nutrient availability, pollutants load and seasonally changes, as a result of the change of natural metabolic activities [2,26]. Therefore, some treatment systems were invented to simulate the natural aquatic plants and to overcome the disadvantages of the living plants. Aqua Mats, for instance, are a type of artificial seaweed with a high surface area that is designed to encourage colonization and growth of anaerobic bacteria, aerobic bacteria, algae, zooplankton and other aquatic organisms [27]. Further, removal of pollutants by bacteria in the system can be enhanced by methods such as immobilized bacteria [28] and/or by utilizing biofilm carrier [25]. Increasing the plant coverage plays an important in enhancing the removal efficiency as well [29]. Plus, the choice of appropriate plant species has been shown to generally improve pollutant removal and this seems an important avenue to explore for optimizing treatment system efficiency [16].

Nevertheless, plant-based systems are regarded as a low-cost, solar-energy-based, eco-friendly technology for in-situ purification of surface water, and an important ecological remediation to control water eutrophication [30]. Thus, more studies should consider optimizing these systems depending on the flow conditions and nutrient availability.

Aquatic animals

Aquatic animals such as clams, snails and other filter-feeding shellfish have a prominent effect on nutrient removal from eutrophic water bodies [6]. The biological treatment of stocking filter-feeding silver carp (Hypophthalmichthys molitrix) in eutrophic water bodies has been widely applied to control excessive phytoplankton levels and improve the quality of water bodies [27,31].

Silver carp has a long lifespan in natural water bodies (6-10 years, even 20 years in some instances) [32]. Commonly stocked in water reservoirs in developing countries, silver carp is an omnivorous filter-feeder that can filter particles >10 μm, including zooplankton and phytoplankton [27].

Filter-feeding fish such as silver carp have been shown to select zooplankton on the basis of prey escape ability; for instance, cladocerans are more vulnerable than copepods to fish predation because they have lower escape ability [33]. An example of aquatic animal usage to improve water quality is the introduction of Asiatic clam (Corbicula fluminea) into the Potomac River, United States of America. This was done in the early 1980s, when chlorophyll-a concentration in the Potomac River appeared to be strongly depleted, at levels of less than 1 g/L [25]. The Asiatic clam can also promote nutrient regeneration. Therefore, the species imposes simultaneous top-down and bottom-up effects on the ecosystem [27].

Experiments have shown that filter-feeding fish are able to reduce phytoplankton biomass to a certain degree, although the final efficiency depends on the characteristics of the given ecosystem. However, the application of such biomanipulation may lead to different effects depending on the composition of the initial plankton community (i.e., zooplankton and phytoplankton), the species and stocking density of fish, and the water temperature [27].

Silver carp usage to control algal biomasses remains controversial. For instance, several studies have shown that stocking silver carp fails to reduce phytoplankton biomass in the presence of large herbivorous cladocerans [27,33]. A key reason for this was the reduction of grazing pressure on phytoplankton by zooplankton as a result of fish predation [33]. Moreover, inorganic or organic pollutants present in untreated water and some bio-toxins released by Microcystis spp. are harmful to silver carp, which therefore affects the efficiency of this biological treatment. Further studies in the toxicity and security of water quality should be conducted [32] with an increased focus on understanding the effects of bioremediation on local ecosystems and biodiversity.

Microorganisms

Microorganism-based technologies are used to decompose, transform, and absorb water pollutants. Results to date generally confirm the existence of the appropriate microbial functional groups responsible for removing specific pollutants from wastewater [34]. Practically, two microorganism-based methods are used for in-situ surface water remediation. The first method is microbial dosing and the second utilizes biofilms [35].

Microbial dosing: Microbial dosing uses specific and efficient microorganisms to remove pollutants present in the water. Commercial products, such as FLO-1200, could achieve remarkable results in the river pollution control under the conditions of river aeration. Bioenergizer, combined water mixing was added and strengthened the ability of microbial degradation artificially for water purification [6].

Sheng [36] utilized two kinds of microbial reagents to remediate a heavily polluted river in Fangcun District, China, which became a black and odorous river. The dominant microbes in these reagents were photosynthetic bacteria and Bacillus subtilis. HRT was around 20 h. The reagents were directly diluted with river water before inoculation. The results of the small-scale experiment indicated that the removal rate increased with the increase of photosynthetic bacteria (PSB) concentration. The chemical oxygen demand (COD) and NH3-N removal (corresponding removal rate are all over 60%). Furthermore, Field-scale test was undertaken. Except for suspended solids (SS), the total removal rates for each pollutant all exceeded 70%. Eventually, they recommended applying this method to remediate other heavily polluted rivers.

Mingjun [14] carried out a field trial of bioremediation in 60 m² of eutrophic water body in a local park for four months. A little amount of natural humic acid was added to speed up flocculation and deposition of the superfluous algae. Thus, the multiple microbial preparations used were composed of nitrobacteria, mixed bacteria and humic acid. The following conclusions were drawn: Pollution indexes of total nitrogen (TN), total phosphorus (TP), NH3-N, COD and turbidity were declined different, and the rates were 77.8, 72.2, 94.2, 60.0 and 85.6%, respectively. After bioremediation, the color of lake turned light green from dark green and clearer. The turbidity declined and DO increased. The water environment improved. Thus the problem of Lake Eutrophication can be solved radically by bioremediation.

Bio-film: The bio-film technology utilizes bio-membrane attached to the natural river bed and micro-carrier to move the pollutants in the river through adsorption, degradation and filtration under the conditions of artificial aeration or dissolved oxygen. Gravel contact oxidation method, artificial packing contact oxidation method, thin layer flow method, underground stream purification method, etc. The strengthening purification technology of The bio-film technology for river purification in Japan and South Korea and other countries were river researched by Japanese were mainly indirect purification, which was to build the purification facilities on the side of the river [6].

Ref. [37] evaluated the role of biofilm attached on streambed in linear alkylbenzene sulfonates (LAS) degradation in the stream using Environmental observations and laboratory biodegradation experiments using biofilm collected from Nogawa river bed located in
southern part of Tokyo, Japan. Three batch culture experiments and one continuous culture experiment were conducted. For most observations, greater than 80% of the LAS were removed within 2-3 h of the travelling time. The batch culture experiments clearly indicate that the existence of the biofilm accelerates the biodegradation of LAS [38].

For the same river (Nogawa river), gravel contact oxidation was utilized, the packing was gravel, and the removal rates of biological oxygen demand (BOD) and SS were 72.3% and 84.9% respectively. With new non-woven fabric as packing, the drainage ditch facilities in Chiba County was set on the side of the ditch, and the removal efficiency of SS reached 97%, the removal rates of BOD and COD were 88% and 70% respectively [6]. Moreover, Ruan et al. [20] used Plant-biofilm oxidation ditch for in-situ treatment of polluted water. The system was designed for in-situ treatment of municipal sewage or polluted lake water in combination with plant biofilms for performing N and P removal. And running experiments at pilot scale for about 1.5 years resulted in the following observations:

1) The system was quite satisfactory and stable for treatment of municipal sewage and polluted lake water in removing COD, NH₃-N and PO₄-P.
2) The direct uptake of nitrogen and phosphorus by plants was negligible in comparison with the total removal by the system, but indirect mechanisms via plant root exudates and biofilms merit further studies.

The proposed process could dramatically reduce the costs of sewage collection, the land-space requirement and the construction costs compared with conventional sewage treatment plants; might be suitable for treatment of both municipal sewage and polluted lake water; and could lead to the promotion of wastewater treatment in many developing countries.

Further, biofilm processes, such as aerated bio-filter biological fluidized bed, suspended carrier biofilm reactors (SCBR), etc., are commonly used in surface water remediation. Immobilization of biomass in the form of biofilms is an efficient method to retain slow growing microorganisms in continuous flow reactors. These systems operated as aerobic or anaerobic phases with freely moving buoyant plastic biofilm carriers [6]. More specifically, microorganisms grow attached on small carrier elements that are kept in constant motion throughout the entire volume of the reactor, resulting in uniform, highly effective treatment [39].

The moving bed reactors provide distinct advantages, including being simple in operation, at low risk of losing the biomass and less temperature dependent. In addition, they have better control of biofilm thickness, higher mass transfer characteristics, they are not subject to clogging and they have a lower pressure drop [39,40].

Given its specific advantages, moving bed reactors are the most common activated sludge modifications used for industrial wastewater treatment [40], secondary effluent from sewage treatment plant [41], and river water [35,42] investigated the removal of organic matter from agriculture drainage water using MBBR. It was concluded that COD removal could reach up to 95% when the biofilm was acclimated to the same salinity level.

The biological contact oxidation process (BCOP), also called submerged biological filter or contact aeration system, is a hybrid wastewater treatment system, taking the advantages of both activated sludge process and biofilm process, e.g., no bed clogging and sludge bulking. Previous studies reported two types of biological contact oxidation processes (BCOP). Step-feed (SBCOP) unit and Inter-recycle (IBCOP) unit were designed to investigate the treatment of heavily polluted river water. When spring dry season arrived, considering the lower substrate concentration of the raw water and positive effect of temperature rise on biological treatment, the total influent of each unit was 71.3 L/h with an HRT of 2 h. During the summer rainy season, in order to enhance the nitrification in the two biological treatment units, the total influent of each unit was recovered to 26.4 L/h with an HRT of 5.4 h. Further, the recycling ratio was 200% for the IBCOP. The results showed that the SBCOP unit had higher adaptability and better performance in the reduction of pollutants, i.e., with the average removal efficiency for COD, TN, and TP of 58.0%, 9.7%, and 40.4% in the winter, 46.4%, 24.7%, and 45.1% in the spring, and 66.5%, 27.2%, and 47.3% in the summer, respectively. Therefore, SBCOP is more applicable for the treatment of river water.

Yu and Tsao [43] studied the treatment efficiency of a gravel contact oxidation treatment system located in Guanfu, Taiwan. This system was constructed at the riverside. The river water was inducted into an influent well by piping, and then pumped to a storage tower by submersible pumps. Finally, the river water flew into the system by gravity. They reported that the removal rates of BOD, TSS and NH₃-N with an average of 46%, 71% and 24%, respectively. HRT for better removal of SS was 15-20 h, 13-17 h for BOD, and 10-15 h for NH₃-N.

Ref. [44] evaluated the treatment efficiency of a gravel contact oxidation treatment system which was newly constructed under the riverbed of Nan-men Stream located at the Shin Chu City of Taiwan. The design flow rate of this system was 10,000 CMD (m³/day) and the HRT ranged between 1.5 ~ 3 h. River water flew through the whole system by gravity; During wet days, if the river flow rate is higher than the design flow rate, the superfloosur flow will directly pass through the treatment system to the downstream of the river. The results showed that the average removal rates of five-day biological oxygen demand, total suspended solids and NH₃-N were 33.6%, 56.3% and 10.7%, respectively. And they reported that since the river water flew through this system by gravity, no power was consumed in the whole treatment process and the operation and maintenance cost was apparently reduced. Plus, further studies might be required to confirm whether higher HRT will improve the treatment efficiency of this gravel contact oxidation system.

Bio-ceramics were used as the carrier to treat a polluted river in Shenzhen, and the average removal rates of NO₃-N, NO₂-N, COD, turbidity, color, Mn and alga were 90.8%, 84%, 21.4%, 62%, 47%, 89% and 68% respectively. Based on the use of sewage treatment technology by rubber packing inner loop fluidized bed bio-film, the average removal rates of COD and ammonia were 88.16% and 91.8%, and the highest removal rates were 94.64% and 94.08% respectively. Wang Shu mei installed aerators, bio-film and added special bacteria in the river, and the removal rates of COD, BOD, NH₃-N, TN, TP and SS were 67.4%, 87.7%, 34.3%, 30.3%, 53.3% and 39.7%, the dissolved oxygen and transparency in the river increased from 0.9 mg/L and 12.5 cm to 7.6 mg/L and 137.5 cm respectively. Yang Tao laid the biological filter media on the river surface, and the average removal rates of COD, ammonia nitrogen and total phosphorus were 40.00%, 36.43% and 43.02% respectively [6].

Biofilm carrier can be either artificial or biological media [15]. Cao and Zhang [15] used filamentous bamboo as a biofilm carrier (Biocarrier) for bioremediation of polluted river water. Besides, evaluating the system under continuous flow conditions, they assessed the COD bioremediation efficiency when glucose was added to the river...
water in a hybrid batch reactor. Raw water was taken from a polluted river and poured into a wastewater tank. The flow rate was regulated using a peristaltic pump, and the column was operated in up-flow mode. In addition, air was supplied into the reactor from the bottom. The microorganisms used in the experiments were cultivated in the reactor, which was a hybrid system composed of filamentous bamboo and suspended activated sludge. The continuous flow reactor kept the same packing of filamentous bamboo used in the batch experiment, and had HRT of 3.5 h. The bioremediation of polluted surface water by using biofilm on filamentous bamboo is feasible and effective. As a result, the mean COD removal rate reached 66.1% in a batch hybrid reactor, and glucose can be used to substantially increase the COD removal. Under continuous flow conditions, the removal rates of COD, NH$_4^+$-N, turbidity, and bacteria were 11.2-74.3%, 2.2-56.1%, 20-100%, and more than 86.6%, respectively. Therefore, Polluted surface water with refractory organic pollution, low transparency, and high nitrogen pollution can be remediated by using biofilms on filamentous bamboo. The filamentous bamboo is beneficial to forming a rich microbial community. It is recommended that filamentous bamboo be widely used for the bioremediation of polluted river water instead of conventional bio-carriers and phytoremediation techniques.

Biocord is a man-made bio-reactor substrate, developed and manufactured for water management using microbe activity to passively treat water in controlled flow or storage applications. Biocord can also be used to treat wastewater in oceans, rivers, lakes, marshes and manmade reed beds [45]. Research results illustrated that the biocord exhibited good filtration performance and effectively removed COD, NH$_4^+$-N and TN with 26%, 65%, and 50% respectively. The flow rate of 4 L/min for 120 min, resulted in the water being completely replaced once every 10 min. The bio-cord fibers also provided suitable conditions and support media for microbial growth.

Recirculating ration is an important to improve the treatment efficiency. Liehr and Rubin [46] compared peat filter and a recirculating sand filter (RSF) for onsite treatment. Both systems were able to meet secondary effluent standards for BOD and TSS. The RSF also was moderately effective at removing nitrogen (58%) while the non-recirculating peat filter was not (26%).

In addition, hydraulic loading rate, aspect ratio, granular medium size and water depth are determining factors in the performance of the biofilm-based systems [47]. However, these techniques have drawbacks, such as complex water and air distribution systems, backwash requirements, occasional biofilm sloughing and a high nitrite residue in the effluent [48,49].

Conclusions and Recommendations

In-situ bioremediation methods can overcome the shortcomings of chemical and physical methods. Bioremediation methods also have advantages such as low cost requirements, fewer environmental influences, and no secondary pollution. In addition, understanding in-situ treatment systems is essential for maintaining and controlling hydraulic conditions in open streams.

After comparing the latest surface water remediation technologies that can be applied in-situ (i.e., aquatic plants, aquatic animals, and microorganisms), the following conclusions are made:

- There is an increased focus on simulated (artificial) aquatic plants in order to overcome the disadvantages of resident plants,
- Aquatic animals can mitigate pollution in water bodies and promote nutrient regeneration,
- More studies in water quality security and toxicology should be conducted when using aquatic animals as in-situ treatment methods,
- Microorganism-based systems are promising in-situ treatment methods that are low-cost and efficient,
- These methods should be tested not only on rivers and lakes, but also on other polluted surface streams such as agricultural drains,
- Bioremediation processes should be optimized, taking flow conditions and nutrient availability into account,
- It is important to integrate primary bioremediation options into water quality models to ensure effective design and management, and
- Research efforts should focus on understanding the effects of bioremediation on local ecosystems and biodiversity.

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