IOP Conference Series: Earth and Environmental Science

PAPER • **OPEN ACCESS**

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To cite this article: Aliya Naz *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **796** 012039

Conference:

Community based Research and Innovations in Civil Engineering, 2021

Title:

Applications of Microbes in Bioremediation of Point Source Pollutants from Wastewater

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Abstract

Water pollution is the major concern of this era. Industrial developments, agriculture patterns, construction processes and unsustainable mining have contributed to increased level of pollutants in most of the fresh water bodies across the globe. This review categorizes different sources of water pollution and focuses on remediation of wastewater through microbial applications. The point source pollutants can be remediated using 'at source' microbial bioreactor installations**.** This work concludes that hybrid biofilm reactors and membrane bioreactors can be utilize for the removal of micro-pollutants. However, anaerobic bioreactors are more efficient in treating wastewater having high concentration of organic matter.

Keywords: *Microbes; wastewater; drinking water; heavy metals; petroleum*

1. Introduction:

Pollution can be defined as presence of toxic substances with negative ecological, health and other environmental impacts due to anthropogenic or geogenic interferences. Water pollutants such as heavy metals, pesticides, micro-plastics are the major concern in recent era. These pollutants are gradually entering into food chain and bio concentrating in the living tissues with potential of getting bio-magnified in higher trophic levels. Toxicants present in the water eventually affect the human health through direct exposure and via biomagnifications process **[1-4].** Water pollutants can be classified into inorganic, organic or microbial agents. Heavy metals are the major contributor of the inorganic pollution load in any water system. Exposure of toxic metals, pesticides and microplastics through different sources can cause acute and chronic toxicity in human **[5-8].** The acute toxicity includes dizziness, irritation, ulcer, vomiting, and diarrhoea **[9].** While the chronic toxicity can cause even more prominent and serious illness related to endocrine, neurological, immunological, developmental and reproductive changes which cause cancer, affect mental growth, lower the IQ in children, poor gross and developmental coordination disorder **[10-12].** Thus, clean water and recycling of polluted water is prime necessity nowadays. In this era of development, urbanization and unsustainable industrialization, toxic substances are spewed into the water bodies polluting both surface as well as groundwater sources **[13].** Entry of these pollutant into the water bodies directly impact the aquatic fauna by interfering with their physiological, anatomical or embryogenesis processes producing health abnormalities like birth defects, stunted growth, ion imbalances, endocrine signal disruptions in insects and fishes. In present day and age the pollutants in major fresh water bodies have reached to alarming levels which warrants immediate remediation interventions. There are many technologies available to remove pollutants from water sources but microbial remediation methods is gaining popularity due to the advocacy by the scientific fraternity because of their remediation efficacy, and cost effectiveness **[14,15].**

Various microbes are able to utilize wide range of pollutants in both aerobic and anaerobic conditions depending on their cellular metabolic and physiological pathways **[16].** Microbes bioremediation can be either enzyme linked biotransformation or adsorption through cell surface. Few microbes produced responsible enzymes through various metabolic pathways which certainly participated in degradation of pollutants **[17,18].** Thus microbes can be used in pollutant removal technologies which give satisfactory results in terms of pollution degradation and their removal from contaminated water **[16].**

Here, in this review, different biological system has been elaborated in details to understand the utility of microbe based technology to treat different organic and inorganic pollutants from contaminated water.

2. Water pollution

Contaminated water with various pollutants is the major concern of environmentalist in the present scenario. Polluted water not only destroys human health but it also can affect the whole ecosystem. There are two main division between sources of water pollution i.e. point and nonpoint sources. If the source of pollution can be identified to a specific location in a geographical extent it is referred to as point source of pollution **[19].** In case of water pollution and water resource management, any industrial effluent discharge, and municipal sewage can be an example of point source pollution. Point sources pollution can be managed ‘at the source’ by installing water treatment facilities before the waste water is let out into the nearby water bodies such as river stream or sea.

Non- point sources are pollution locations dispersed over a wide area or region and cannot be traced back to a single location. This is possible in case of agricultural run- offs, washing activity along river banks, urban runoff etc. This sort of pollution is hard to tackle because of dispersed sources and ‘at source’ mitigation measures seldom works. Awareness campaigns, policy level interventions can tackle these issues to certain extent.

3. General standard limits for wastewater

To tackle with point water pollution, different statutory bodies of different countries have given guidelines to treat wastewater before the discharging in natural water bodies. For an example, In India, general standard limits for various parameters to discharge in surface water bodies, public sewers, land of irrigation and marine or coastal areas **[20].** According to this, pH should be under 5.5 to 9.0, Biochemical oxygen demand (BOD) should not exceed 30mg/L, concentration limits (mg/L) for toxic heavy metals like Arsenic, Mercury, Lead, Cadmium, Chromium (VI), total Chromium, Copper should be under 0.2, 0.01, 0.1, 2.0, 0.1, 2.0, and 3.0 respectively.

Now, the challenge is to treat water at point source so that it cannot contaminate into natural ecosystem and impact human health. The major water pollutants are carcinogenic heavy metals, pesticides, micro-plastic (Table1). There are various chemical and biological methods are available to treat polluted water such as adsorption, chemical precipitation, ion exchange, membrane filtration, biological hybrid reactor, activated sludge process, trickling filter. The biological methods are basically based on microbes proven effective and sustainable to remove contaminants at limited extent **[21].** As chemical treatment process produced huge waste the biological treatment process is proved more beneficial.

Table 1: List of major pollutants in the various water bodies of globe

Sl. No.	Place	Time of sampling	Major pollutants	Reference
1.	West Bengal, India	2012-2014	Insecticides such as malathion, chlorpyrifos, and lindane and Herbicides -alachlor and atrazine	**[68]**
2.	Tekeze Dam, Tigray, Ethiopia	-	DDT, DDE, Lindane, Endosulfan	**[69]**
3.	Kali River, Western Uttar Pradesh (India)	-	Organophosphorus-Parathion, Organochlorine- α- BHC, γ-BHC, Endrin, Endosulfon	**[6]**
4.	Chandigarh, India	February to June 2019	Endosulphan (ES) and hexachlorocyclohexane (HCH)),	**[8]**
5.	Wuxi, Jiangsu Province (Eastern China)	February 2018	The influent municipal wastewater treatment plant consisted of polyethylene terephthalate (PET, 47%), polystyrene (PS, 20%), polyethylene (PE, 18%) and polypropylene (PP, 15%). MP morphotypes dominated in fragments (65%) and fibers (21%), which mainly were PET, with limited films (12%) and foams (2%).	**[70]**
6.	Baluchistan province, Iran	-	Heavy metals (Cd, Cd, Pb)	**[71]**
7.	Sukinda Chromite mine, Odisha, India	2013	Cr, Cd, Pb, Co	**[3,4]**
8.	Indian Sunderbans	2014	Cr, Cd	**[5]**

4. Role of Microbes in bioremediation of water

There are a number of microbial strains showed different mechanisms in order to reduce, adsorb or remove pollutants from aquatic media **[15, 22].** Early bioreactor tests were reported by Alexander Mueller in 1865 **[23].** Various bioreactors have been operating under aerobic or anaerobic conditions. Over the years the advanced biological bioreactors have been designed and field tested such as trickling filter, rotated biological bioreactors, and suspended sludge bioreactor, aerobic and anaerobic bioreactors. Nowadays, membrane filter and biofilm filter is more popular for remediation of pollutants from water because of their high contaminant removal efficiency. A list of few microbes with their efficiencies in order to remove major pollutants like pesticides, micro-plastic and heavy metals are given in the Table 2.

Table 2: Usage of microorganism for water treatment

Sl. No.	Pollutant	Microorganism	Remarks	Reference
1.	Micro plastics (polyethylene, polystyrene, polyethylene terephthalate)	Bacillus strains *B. cereus* and *B. gottheilii*	The weight loss percentage by *B. cereus* were recorded 1.6%, 6.6%, and 7.4% for polyethylene (PE), polyethylene terephthalate (PET), and polystyrene (PS), respectively. *B. gottheilii* recorded weight loss percentages of 6.2%, 3.0%, 3.6%, and 5.8% for PE, PET, PP, and PS, respectively.	**[7]**
2.	Pesticides (aldrin, atrazine, captan and diflubenzuron)	*Bacillus* sp. and *Exiguobacterium aurantiacum*	Growth and pesticide removal effiencies of microbial strains were analysed at 50 lg/ml of aldrin, atrazine, captan and diflubenzuron.	**[72]**
3.	Organic matter	*Trichococcus* sp.and *Simplicispira* sp.	About 96% organic matter removal were achieved from wastewater in a full-scale moving bed biofilm reactor	**[73]**
4.	Total nitrogen	*Trichococcus* sp *and Polaromonas*	About 62-75% total nitrogen removal were achieved from wastewater	**[73]**
5.	Total phosphorus	*Candidatus_Accumulibacter, Acinetobacter* sp, *Candidatus_Competibacter*	An anaerobic/oxic/anoxic mode was adopted to achieve phosphorus removal along with denitrification.	**[74]**
6.	Cd and Pb	*Bifidobacterium longum*,	54.7 mg Cd/g and 175.7 mg Pb/g dry biomas	**[75]**
7.	Cd	*Microbacterium oxydans* CM3 and *Rhodococcus* sp. AM1	58 and 39% of 400 mg Pb/L	**[76]**
8.	Cd	*B. cereus* S5	70.16 mgCd/g (dry weight)	**[77]**
9.	Cr	A co-operative endeavour by nitrifying bacteria *Nitrosomonas*	100% of Cr(VI) was observed at pH 4.0, with initial Cr(VI) concentration of 5 mg/L	**[78]**
10.	Pb	Phosphate solubilizing bacteria (*Bacillus* sp.)	95% removal of Pb+2 at 110 mgPb/L with NH_4Cl broth	**[79]**

11.	Hg	Dead Biomass of *Lysinibacillus sphaericus*	Removal of over 95% of Hg in solution of 28.4 µg Hg per mg of bacteria	**[80]**

5. Bioreactor systems

5.1. Trickling filter

A number of studies have been proven that trickling filter one of the appropriate method to remove pollutants from wastewater. Trickling filter is based on attached growth on a support material **[23, 24].** Trickling filter is a unit in which wastewater is passed through zoolgia i.e. a mixture of microbes and fungi. Recent studies suggested the uses of trickling filter to remove biological nutrient (Nitrogen, phosphorous) and organic compound removal [**25-27**].

5.2. Activated sludge process

A unit which consist microbial composition (sludge) in aeration chamber used to treat wastewater known as activated sludge process. A dense microbial flocs circulated in the chamber responsible for biodegradation of organic matter present in the wastewater. Few organic matters are getting oxidised in presence of oxygen which produce CO_2 and water, and few utilized by the bacteria for synthesis of new microbial cells **[28].** The first activated sludge processes introduced by Ardern and Lockett in 1914 **[23].** Initially, this process was used to treat sewage, tannery effluents and wastewater which generally contains huge organic matter, but now a day's Activated Sludge Process is used for the removal of microplastic and different pharmaceuticals like fluoroquinolones, tetracycline and macrolide from wastewater **[29-33].**

5.3. Anaerobic reactors

Anaerobic bioreactors have been used since the 1880s to treat wastewater especially the water with very high organic solid concentrations **[23,34].** Anaerobic reactor consist anaerobic bacteria which utilized organic matter present in wastewater for their growth. In 1978, the upflow anaerobic sludge blanket reactor was introduced for the first time by Lettinga et al., 1980 **[35].** These bioreactors are widely used to treat pharmaceutical wastewater, slaughter house wastewater, citrus industry effluents, potato starch processing wastewater and coffee processing wastewater [**36-41**].

5.4. Aerobic Batch and continuous suspended bioreactor

This bioreactor may consist single strain of microbes and mixed microbial culture. In the bioreactor development completely mixed stirred tank reactors were started in the late 1950s **[42].** These systems used to treat wastewater contain pharmaceutical wastewater or water containing pesticides. Antibiotics like ofloxacin, norfloxacin and ciprofloxacin has been removed with aerobic granular sludge **[43, 44].** González et al. 2012 used aerobic bioreactor to treat Degradation and detoxification of the herbicide 2, 4 dichlorophenoxyacetic acid (2, 4-D) from contaminated water with single culture of *Delftia sp.* Strain **[44].** Continuous-flow aerobic granulation in plug-flow bioreactors were used to remove COD and NH_3 from contaminated wastewater by Sun et al. 2019 **[45].**

5.5. Batch and continuous packed bed bioreactor (PBBR)

The first bed bioreactors are started in the 1970s to treat wastewater **[46].** Now a number of wastewaters pollutants have been treated with packed bed and fludized bed bioreactor systems. For eg. malathion biodegradation in batch and continuous packed bed bioreactor (PBBR) **[47].** Bioremediation of Congo red dye has been carried out by researchers, they used *Brevibacillus parabrevis* strain to biodegraded red dye **[48].** Single strain microbes used to remove benzene in both batch and continuous packed bed bioreactor by researchers, and strain of *Bacillus sp.* were found suitable to remove moderate amount of benzene from contaminated wastewater **[49-50].** A study by Sonwani et al. 2020 also supports the utility of packed bed bioreactor for the removal of pollutants from contaminated water on the basis of its kinetic performance [**51**].

5.6. Airlift bioreactors

These advance bioreactors is designed especially for ex situ bioremediation of wastewater. Airlift bioreactors consists indigenous microbes, which found capable for ex-situ remediation of water [**52].** Khongkhaem et al. 2016 used airlift bioreactor to degrade phenolic compounds from palm oil mill wastewater with help of silica immobilized microbe **[53].** Researchers further used airlift bioreactor for toluene biodegradation, petroleum and hydrocarbon bioremediation from polluted wastewater **[54,55].** The utility of air lift bioreactor is not only limited to degrading pollutants but it is also used for carbon sequestration through the consortia based primary production **[56].**

5.7. Membrane bioreactor

Membrane bioreactors basically meant to treat micropollutants from water **[57].** Various organic and inorganic micro-pollutants can be degraded under membrane and integrated membrane bioreactor systems **[58].** Extractive membrane bioreactor (EMBR) used for phenol-laden saline wastewater and a significant concentration of phenol removal has been observed in wastewater containing 2.5g/L of Phenol, whereas high phenol content in wastewater showed adverse effect on the microbes growing in EMBR **[59].** The limitation of membrane bioreactor is higher capital and operating cost in comparison to general traditional bioreactors.

5.8. Biofilm remediation system

Biofilm bioreactor immobilizes the microbes and maintains biomass in the reactor **[60].** These reactors have now proven a better option to remove organic and inorganic content from wastewater **[61,62].** A lab set up of biofilm reactor performed the removal of ammonia nitrogen and permanganate reported by 84.41–94.21% and 69.66–76.60%, respectively **[62].** The biofilm bioreactor is required regular cleaning to restrict the growth of other microbes to maintain the efficacy of target microbe.

6. Disposal of sludge

Microbial bioremediation of municipal and industrial wastewater is getting attention, but the only limitation is the huge sludge generation through this process. Researchers have discovered many sustainable techniques for the sludge reuses and disposal. Most used sludge disposal technique is landfill after treatment and pyrolysis. Recent works shed lighted on the reuse of sludge for land application or to improve the degraded soil and energy recovery **[63-64].** However, reduction of sludge volume is required before the reuse or disposal. Anaerobic digestion can be the best method for reduction of sludge volume.

6.1. Anaerobic digestion

Anaerobic digestion is a sustainable treatment option for the degrading of organic matter from wastewater sludge. The organic matter gets decomposed into biogas i.e. methane and carbon dioxide, with a trace amount of NH_3, and H_2S **[65].** The biogas released from this process can be used as a source of energy. During the time of high price and limited fossil fuel supplies in developing countries like India, energy generation through anaerobic digestion may prove beneficial. The utilization of stabilized sludge as an organic fertilizer adds on the potential and utility of anaerobic digestion of wastewater. Figure 1 shows the anaerobic digestion of wastewater. There are basically four phases of organic matter digestion through anaerobic digester, these are hydrolysis, acidogenesis, acetogenesis and methanogenesis. Anaerobic digestion reactors have commonly been operated at mesophilic (30–40 °C) and thermophilic (50–60 °C) temperatures.

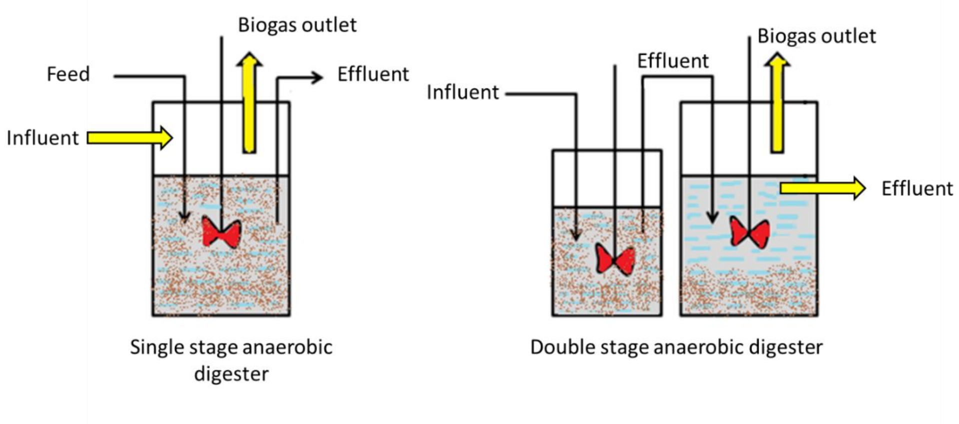

Figure 1: Single stage anaerobic digester and double stage anaerobic digester

The continuously stirred tank reactor is one of the foremost anaerobic digestion reactor used in various industries can be divided into two stages:

- Single Stage
- Two-Stage

In a single-stage digester, all four phases of anaerobic digestion take place in a common environment. A single-stage anaerobic digester is not ideal for all members of the consortia, and one of the possible reasons could be that the bioavailability of the enclosed essential nutrients is not sufficient to maintain enzymatic processing by microbes **[66].** However, in a two-stage anaerobic digester, the methanogenesis phase is typically separated from the other three stages of anaerobic digestion, which is reported to provide a higher efficiency and energy recovery, as well as greater biogas production compared to traditional single stage anaerobic digestion **[67].** Because of better efficiency, now days most of anaerobic digestion reactors are being constructed as two-stage configurations.\

7. Conclusion

This review concludes that microbial remediation systems can be utilized for removal of wide range of pollutants such as heavy metals, phenols, herbicide, pesticides, micro plastics - polyethylene and polystyrene from waste water. The batch bioreactors can be modernized to remediate broad spectrum of pollutants with continuous and hybrid reactor systems. The microbial bioreactors are comparatively better than other traditional methods as they do not required large space to install, are easy to use, and require modest maintenance. Commercialization of microbe base bioremediation process would be effective as well as economically feasible. This work concludes that hybrid biofilm reactors and membrane bioreactors can be utilize for the removal of micro-pollutants. However, anaerobic bioreactors are more efficient in treating wastewater having high concentration of organic matter.

Future research can be focused on fine tuning and scaling up of these lab scale microbial remediation systems for more cost effectiveness and applicability for wider industrial use. Thus the 'laboratory to industry' transition is mandatory to make microbial remediation system popular, sustainable and cost effective.

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