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ADVANCED BIOREMEDIATION TECHNIQUES, AN APPROPRIATE METHOD FOR THE REMOVAL OF BIOLOGICAL EMERGING POLLUTANTS IN WASTEWATER

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ABSTRACT

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Abstract

The relevance of bioremediation techniques used for the elimination of pollutants of emerging concerns is one of the main issues in this research work. These pollutants when exposed to the environment, can results in environmental and health related issues. Therefore, they must be controlled by environmental bodies. In some water bodies such as oceans and seas, there exist several emerging pollutants which often lead to unbalanced atmospheric conditions, especially when exposed to non-target species. The negative effects caused by emerging pollutants due to their accumulation rate in the environment have risen a growing need for a sustainable remediation method such as bioremediation. Due to large volumes of natural water contamination as a result of industrialization, rapid urbanization, and inadequate sanitation. Due to these activities, it has resulted to serious negative effects on human health. Because of the high level of contamination, water treatment requires special consideration through the preservation of environmental factors (pH, temperature, and nutrient content), and biological parameters that guarantee their stability and growth in polluted water bodies.
Microbial bioremediation has the potential to tackle these issues through different mechanisms to ensure complete eradication of several contaminants by degradation. Different processes are used by bacterial, fungal, and algal remediations to reduce these contaminants. This thesis focuses mostly on the removal of hazardous pollutants such as heavy metals, pharmaceuticals and health care products, and agricultural products using different microorganisms.

Keywords:(bioremediation, emerging pollutants, wastewater treatment plants, removal technique)

CONCEPT DEFINITIONS

BEPs

Biological emerging pollutants

Eps

Emerging pollutants

HM

Heavy metals

WWTPs

Wastewater treatment plants

EPS

Extra polymeric substance

PPCPs

Pharmaceuticals and personal care products

ABSTRACT
CONCEPT DEFINITIONS
CONTENTS

FIGURES

TABLE

1 INTRODUCTION

The advancement in human activities such as agriculture and industrialization has resulted in an increase in the release of toxic chemical substances into the environment. By improper discharge of these chemicals into aquatic environment, they circulate around as emerging pollutants. As a result, water surfaces such as groundwater, effluents of wastewater treatment plants (WWTPs), have been polluted with large concentrations of emerging pollutants which, when consumed for agricultural purposes, often introduce toxic chemicals (EPs) into the food chain (Ahmad, Mofijur, Parisa, Islam, Kusumo & Inayat 2022, 2-4). Therefore, based on prevention, remediation, and detection, contaminated wastewater has emerged as a serious environmental issue that requires urgent attention. Wastewater treatment facilities are widely acknowledged as the primary defences against the discharge of new pollutants and diseases into the environment. While >95% of wastewater in developing nations does not receive adequate treatment, 80% of wastewater worldwide is discharged directly into the environment without treatment (UNESCO World Water Assessment Programme, 2019).

Emerging pollutants are toxic chemicals released into the environment due to human activities (anthropogenic). They can either be considered as organic (substances used for personal care, pharmaceutical compounds, endocrine disrupting chemicals), or inorganic such as heavy metals (arsenic, chromium, and fluoride) (Wanda et al., 2017, 1-2). The presence of these emerging pollutants in the water bodies is a major challenge for the environment due to their potentially harmful effects and wide distribution. Moreover, many of them are chemical or biological contaminants that are not controlled by national or international environmental authorities, especially in Mexico (Sanchez-Ocampo et al., 2022, 1-2). Therefore, biological processes can be used to degrade, convert, and eliminate dangerous pollutants from the environment in order to reduce the increasing levels and impacts of these contaminants.

However, bioremediation in the past ten years has been considered the best remediation technique for the removal of emerging pollutants in wastewater. In comparison to chemical treatment methods, bioremediation using algal has proven to be more effective in the removal of heavy metals (HMs) found in wastewater (Banu et al., 2020, 1-2). This method of treating wastewater also has its own limitations, such as its availability to other microorganisms is limited. Chemicals such as chlorinated compounds and radionuclides cannot undergo the process of biodegradation and the water solubility of the pollutants is low. Due to their trophic independence for nitrogen and carbon, microalgae and cyanobacteria constitute a potential new option for the bioremediation of wastewaters. But because they are lightdependent reactions, it is necessary to dilute the colourful effluents before treatment to prevent light obstruction (Sanchez-Amores et al., 2015, 58-70). Therefore, this thesis aims to emphasize on the relevance of addressing the present environmental issues that has been expressed in the Sustainable Development Goals as proposed by the United Nations, discussing the main Eps and their effects on wastewater and to explain justifiable reasons if advanced bioremediation has more tendency in the removal of emerging pollutants in comparison to chemical treatments which cannot remove large concentrations of pollutants like heavy metals.

2. EMERGING POLLUTANTS FOUND IN WASTEWATER

The effluents of wastewater have been found with pollutants which have caused a growing concern for the environment and human health when released into the environment. Therefore, a valuable and efficient waste management system can be provided if the main sources of Eps obtained from the main waste streams (domestic and industrial), can be identified. The widespread of these pollutants is mainly facilitated by municipal, industrial, and home wastewater when poorly disposed. They have an extended lifespan and have the tendency to resist due to their bio-accumulative and bioactive nature.

2.1 Pharmaceuticals and personal care products

The emergence of pharmaceutical compounds was to treat illness through biological reactions in animals and humans. However, when inadequately released, they can cause harm to non-target species (Sarkar et al., 2017, p2.), when they infiltrate the environmental matrices**.** The production of such pharmaceuticals into their final form involves the generation and release of toxic waste materials such as liquid and vapour solvent, detergent, and biological waste (Shallini et al., 2010, 2265–2270).

One of the major local sources that have been reported is the manufacturing plants which was reported by a research group to have found significant concentration of active pharmaceutical products on different samples collected from the areas of a pharmaceutical plant located in South India (Lübbert et al., 2017, 479-481). Therefore, through inadequate disposal of expired pharmaceutical in common garbage can cause such effluent to end up in WWTPs which must be subjected to several treatment such as filtration, sedimentation, and chlorination. These treatment methods help to eliminate conventional pollutants, but they cannot completely remove active pharmaceutical compounds (Sim et al., 2010). Therefore, residues of pharmaceutical products have been detected in wastewater and living organisms.

2.2 Agricultural compounds

The utilization of agricultural chemicals such as fertilizers and pesticides intended to control pests and diseases in crops (Sarkar et al., 2021, 2), even though excessive use of these agrochemicals often infiltrates the environment resulting to waste water pollution. They should be regulated accurately, otherwise they can leach from the irrigation site into groundwater and surface water (Jayasiri et al., 2022, 2-3). The uptake movement of these agrochemicals from the soil to plants depends on several factors such as the physiochemical properties of the pesticides (pH and moisture content), and the physiological properties of the plants as well (Hwang et al., 2017, 2). This can create unbalance in the ecosystem caused by the residues of the pesticides which can negatively cause harm to non-targeted species.

2.3 Biological contaminants (virus, enteric bacteria, mycoplasma, protozoa)

Through the discharge of wastewater effluents into surface and groundwater, biological contaminants reach the environment which is considered the major pathway (Abd-Elmaksoud et al., 2021, 4-5). Some pathogenic organisms are resistant to disinfection process making traditional biological method not reliable on the WWTPs in the removal of pathogens (G´eba et al., 2021). This results to the presence of biological contamination in aquatic bodies leading to two main effects: health hazard to living forms of aquatic environments and serious biohazards to human health due to unsafe seafood products consumption that are contaminated with pathogenic microorganisms (Longo et al., 2010, 1182–1187).

The bacteria Escherichia coli which is a Gram-negative bacterium is frequently used as an indicator microorganism for the monitoring of pathogens in wastewater samples (Wang et al., 2021, 291.). Its presence is primarily associated with faecal pollution and suggests potential contamination by other enteric bacteria like Salmonella spp. and Yersinia spp. Due to its incapacity to develop in water and its simple correlation with unique pollution, Enterococcus faecalis is also frequently employed as an indicator of pathogenic wastewater contamination (Ryu et al., 2007, 283–290).

2.4 Heavy metals

Heavy metals such as, lead (Pb), cadmium (Cd), zinc (Zn), and Chromium (Cr) etc, are emerging pollutants that have become more prevalent, especially in urban sewage development, are often found in different concentrations of wastewater (Singh et al., 2017, 183-199). Their accumulation into wastewater leads to biomagnification (Ali et al., 2019, 6730305), and respiratory disorder. The usage of algae helps to facilitate the removal of heavy metals through two stages. Firstly, it absorbs the metals over the cell surface and the ions are being transported slowly into the cells where they are further detoxified (El-Sheekh et al., 2015). These metals have a high tendency of biodegradation and contain toxic substances.

2.4.1 Mechanisms of algae action on heavy metals

Toxic heavy metals tend to affect the growth, survival, and enzymatic actions of algae, which have evolved the implementation of three mechanisms aimed at eliminating the effects of metal toxicity. The three mechanisms include bio adsorption, bioaccumulation, and extracellular and intracellular bioremediation. The presence of many functional groups attached to the cell wall surfaces carrying a negative charge enables the algae to act as binding site and the metal ions containing a positive charge are adsorbed by the functional groups which enable some of them to bind only to a specific site of the metal particles while others bind to numerous metal ions sites. This defense action helps to prevent direct entry of heavy metal ions into the algal cells (Bilal et al., 2021).

Furthermore, algal plants also help to generate additional systems in their bodies for the capture, elimination, and release of heavy metal ions in wastewater. This serves as an additional resistance mechanism outside of the cell. In algae cells, metal chelating proteins are being synthesized in the form of phytochelatins (Howe & Merchant, 1992).PCsynthase grows at the positive sites of the metal in the cell which result in numerous phytochelatins combined with the metal ions which facilitate the transfer of metal ions from the cytoplasm to the vesicles which aid removal of toxic metals from wastewater. Through the application of phytoremediation, heavy metals in wastewater can be absorbed due to special morphology and high growth rate of some plant species. These plant species also resist heavy metal contamination through chelation and compartmentalization in vacuoles. They also help eliminate heavy metals in wastewater through adsorption and intracellular accumulation (Rezania et al., 2016, 587-599).

2.4.2 Challenges and opportunities involved in the removal of heavy metals using algal bioremediation techniques.

Even though there are numerous benefits in algal bioremediation towards removing heavy metals from wastewater, there are also numerous challenges associated with the system. A greater concentration of emerging pollutants such as heavy metals inhibits the activity of microbial growth. Based on de Wilt et al. (2016), the investigation of heavy metals in wastewater were reported to be recalcitrant to photolysis and this can be handled by increasing the biomass concentration. During phycoremediation of heavy metals, biomass productivity decreases, resulting in a decline in lipid production, nanoparticles, and other valuable products. To overcome these challenges, other advanced biometric systems should be implemented (Leong et al., 2020). Therefore, to ensure maximum remediation of heavy metals, strains should be selected to accumulate maximum biproducts such as lipids and pigments.

Another challenge faced nowadays is that most bio remediators are grown in the waste streams which makes the process cost- effective. From observations, wastewater contains excess nutrients which affects algal growth rate. Therefore, it is necessary to analyze the wastewater and balance the nutrients level. To overcome this challenge, other methods such as chlorination, acidification and membrane filtration should be implemented (Wu et al., 2022,126930). Furthermore, one of the major issues faced by scientists is to extract heavy metals from the bio remediators since they have poor desorption efficiency (Pratush et al., 2018). This problem can be handled by utilizing a better technique where modification of biomass is carried out with the aid of extracellular polymeric substances, alginate, and other chemicals (Naveed et al., 2019).

2.5 Endocrine disrupting chemicals

Endocrine disrupting chemicals such as phthalates, triclosan, and bisphenols are often found in many commercial products used in daily life such as pharmaceutical drugs and personal care products. Furthermore, some industries and food production companies act as sources of EDCs which include toxic metals, pesticides, brominated flame retardants, and plasticizers (Gonzalez-González et al., 2022). EDCs have a higher bioaccumulation and toxicity rate and have been detected in low concentrations as ng/L in the environment where they can be bioaccumulated until they reach certain toxic concentrations for living forms of life. Such chemicals can alter natural hormonal functions in both humans and animals, which caused adverse effects even at low concentration (Sornalingam et al., 2018, 4-5).

2 FACTORS AFFECTING ALGAL BIOREMEDIATION FOR EPS REMOVAL

The rising prevalence and harmful consequences of EPs in the environment, combined with the difficulty of WWTPs to remove them, have prompted a significant need for effective remediation strategies that are practicable, affordable, and sustainable. This can be achieved by using algae mechanisms that are controlled by numerous physio-chemical factors such as pH, temperature, concentration of the emerging pollutants, redox, duration and intensity of light exposure and hydraulic retention time (Gondi et al., 2022).

3.1 Temperature and pH

The wastewater generated from industries usually has a high temperature and disposing them directly can result to thermal pollution especially in aquatic environments (Chen et al., 2020,122806). Microalgae can be cultivated in a wide range of temperatures even though they have specific strain optimal temperatures. Most microalgal strains such as Spirulina plantesis are usually cultivated in optimal temperatures ranging from 15°C to 35°C (Nwoba et al., 2020, 7-8). Once the optimal value is achieved, the productivity of the biomass decreases with an increased temperature. Therefore, at temperature range of 30-40°C, the microalgae species that have the ability to grow in wastewater are known to be an important microorganism for algal-bioremediation of emerging pollutants (Cheah et al., 2015).

The mechanism of algae bioremediation can be affected by the pH level. The different functional groups found on the adsorbent surface are influenced by the ionization states (Beevi et al., 2014, 216-221). The rate of reaction can be lowered by any change in the optimal pH of the biological process. This can be possible because, at lower pH levels, the surface of the algae becomes positively charged, which tends to lower the molecular adsorption. At pH greater than the isoelectric point, the surfaces of the algae become negatively charged, resulting to an increased in adsorption (Chen et al., 2011). For example, pH value greater than 9 tends to have a negative impact on algal growth. This is because it lowers the capacity of carbon dioxide absorption resulting to lack of RuBisCO maintenance (Sutherland et al., 2015, 222–229).

3.2 Concentration of emerging pollutants

The concentrations of most emerging pollutants in water systems are generally low, making their analysis difficult which requires improvements (Borea et al., 2018, 89-95). However, because of these difficulties, spectroscopic methods have been implemented to measure the concentration depending on the type of emerging pollutant present. In wastewater, the concentration of emerging pollutants ranges from 0.007 to 56.63 g/L (Alturki et al., 2015, 201-206).

3.3 Dose of adsorbents and particle size

Permeability increases when the pollutant particle size is smaller which helps to promote absorption into the cell wall based on the toxicity of the pollutant involved. Nano compounds tend to be adsorbed more readily due to the presence of more binding sites on their surfaces (Hayat et al., 2015, 149-153). Different factors responsible for algal- bioremediation includes surface area and the functional group present. An increase in hydraulic retention time increases the absorption between the pollutants and the adsorbent (Hlongwane et al., 2019; Sarkar et al, 2021, 808-833).

3.4 Advantages of advanced bioremediation (algal) for the removal of emerging pollutants over other chemical treatments techniques.

In wastewater treatment, the major techniques utilised include reverse osmosis, flocculation, ultraviolet disinfection, ion exchange and electro-coagulation (Teh et al., 2016). However, these approaches are economically non- friendly and require extensive energy input and labour intensity (Sankaran et al., 2020). These chemical treatment methods cannot eliminate large concentrations of emerging pollutants found in wastewater which therefore requires a special technique that can overcome such barriers (Chiu et al., 2015, 179-189). Furthermore, bioremediation using algae demands no chemical dosage and has the ability to achieve efficient P and N elimination through photosynthetic mechanisms and CO_2 sequestration. This occurs through the release of oxygen into wastewater which result into an increase in dissolved oxygen concentrations (Ummalyma & Sukumaran, 2014, 295-301). Based on previous analysis, the production of energy (diesel and biofuel) from organisms such as microalgae is commercially capable if the process is carried out using bioremediation which solely relies on the cost of energy input (Gouvale, 2011, 1-69). Important nutrients from wastewater can be recovered during microalgae culturing which prevents deterioration of fresh water through eutrophication, whereas in chemical treatment processes, atmospheric nitrogen is stripped out by nitrogen, carbon as carbon dioxide, and precipitation of phosphorus (Nagarajan et al., 2020, 122817). Removal of emerging pollutants using different mechanisms as shown in table 1, significantly remove one or more pollutants but are limited to eliminate most of the contaminants effectively from wastewater. This helps to provide a good quality technique suitable for the removal of a particular emerging pollutant.

Table 1: Summary comparison between different removal techniques of emerging pollutants in wastewater (Touliabah et al., 2022).

Treatment techniques	**Advantages**	**Disadvantages**	**process**
Phycoremediation	-Eco-friendly and low cost -High nutrient removal	-Difficult and expensive to harvest algae -poor and limited effluent quality	-Not applicable for certain contaminants -Reduced pH tolerance
Fungi and bacteria	-Cost effective -Minimal energy needs	Produces some unwanted microorganism that produces gases and bad odours	Sludge production
Chemical precipitation	-High level pollution adaptation -simple process	-High pH sensitivity -Low solubility of metal sulphides	-Sludge production -Chemical consumption
Electrical oxidation	-Improve biodegradability -No chemical requirements or high temperature	-Formation of toxic intermediate metabolite -High energy consumption and sludge generation	-Selective and low reaction rates

4 BIOLOGICAL REMEDIATION TECHNIQUES FOR EPS REMOVAL

The process of removing contaminants from wastewater using bioremediation depends on whether the right organism, the needed environment and environmental factors are present for optimal removal of these pollutants. To degrade emerging pollutants, the organisms suitable are bacteria, fungi, algae, and plants. When compared to traditional methods like land filling, bioremediation has several benefits. For example, it is a natural process that is typically accepted as a waste treatment method and leaves behind harmless residues. Additionally, the process can be carried out on site because of the low rate of site disruption.

However, some compounds such as heavy metals, radionuclides, and chlorinated compounds do not undergo complete degradation. Also, biological processes require the presence of metabolites that are capable of microbial growth. The use of many different organisms in bioremediation processes helps to improve the efficiency and enable a higher microbial diversity (Ławniczak et al., 2020, p856). Re-mediation using biological microorganisms (Microbial remediation), plants (phytoremediation), and combine techniques is presented in Figure 1. Bioremediation method can be classified mainly as in-situ and ex-situ. Through the process of in-situ remediation, the contaminated material is treated in place while ex situ remediation is a physical method which removes contaminated materials from wastewater using bioreactors during chemical, physical, and thermal processes to be treated elsewhere As a result of the growing environmental threat of Eps and WWTPs' incapacity to remove them from the environment, researchers are looking for more effective remediation methods that are affordable, practical, sustainable, and of significance (Koning et al., 2000, 304).

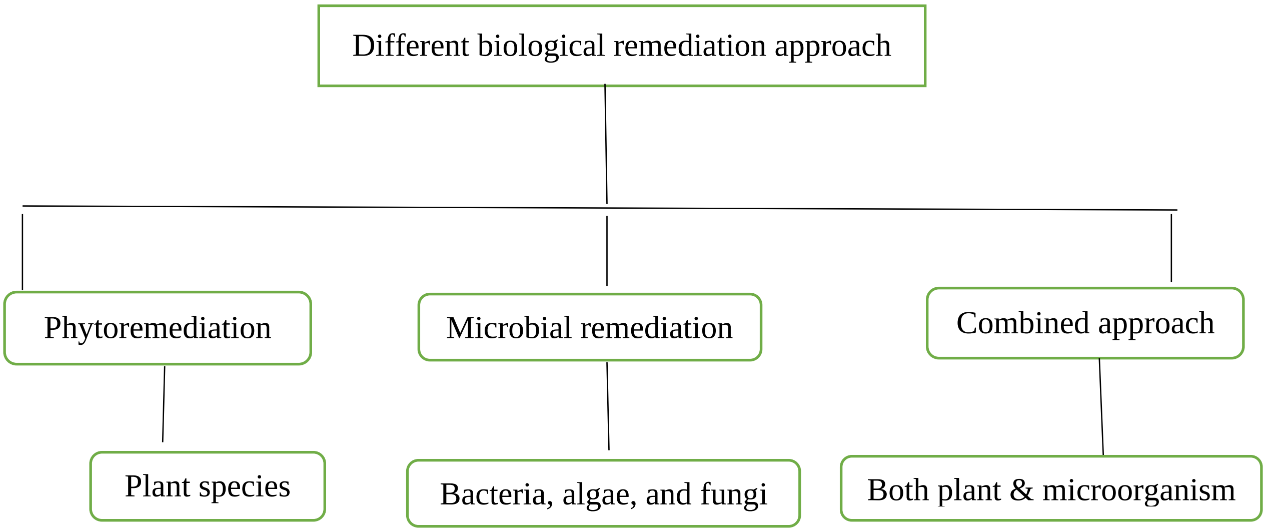

FIGURE 1. Different biological remediation technologies for eliminating emerging pollutants. (Ullah et al., 2015, 28-40).

4.1 Microbial bioremediation of emerging pollutants (bacteria)

Microbial treatments of pollutants either by the usage of fungi, algae, bacteria, or a combination have been used for the decontamination of Eps from wastewater as shown in Figure. 2. There exist numerous bacteria strains that are part of the symbiotic consortium which enable the removal of undesired Eps. Such bacteria populations can be found in wastewater. As described by Bhatt et al. (2022, 134344), there was an evaluation of bioremediation capacity of Serratia marcescens strain WW1 that were isolated from a WWTP. Antibiotics such as tetracycline are utilized during the strain growth phase as a source of carbon and nitrogen. Thus, the strain of the bacterial was able to degrade 89.5% of the tetracycline after 48 hours of treatment, demonstrating its ability to metabolize antibiotics from the environment.

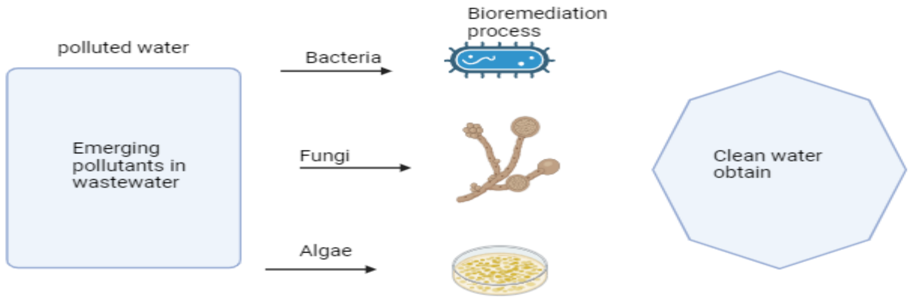

FIGURE 2. Removal of emerging pollutants using different microorganism (Bhatt et al., 2022)

4.2 Myco-remediation

The usage of fungi as a potential in bioremediation have the capacity to degrade petroleum hydrocarbons consisting of aromatics, alkanes and nitrogen-sulphur-oxygen containing compounds (Adenipekun & Lawal et al., 2012, 62-68). They can target and metabolize a variety of molecule classes, including inorganic and organic contaminants, due to their intra and extracellular enzymatic machinery and their capacity to excrete acids (Dashtban et al., 2010, 36-50). They have been recently attracting more attention as a suitable agent in the treatment of wastewater (Gonz-Gonzalez, 2022). PPCPs found in wastewater are transported through hospitals and municipal wastewater treatment plants, which have been identified to be the primary source for the increase in anti-microbial resistance genes (Frascaroli et al., 2021,1-6). The intake of contaminants into wastewater has changed the chemical, physical and biological properties which makes my-coremediation on site a complex system. Application of fungus in-situ myco-remediation is advantageous since it causes less disturbance to dirty wastewater, especially in the treatment of wastewater contaminated with hydrocarbon and dyes. Furthermore, by stirring in bioreactors, on-site treatment with fungus helps to reduce the amount of shear pressures that fungi are exposed to. However, ex-situ my-coremediation allows for better monitoring and environmental conditions, as well as the growth and performance of the fungi, which gives the process more flexibility (Harms et al., 2011, 12-13).

Ex-situ myco-bioremediation can be improved by using variety of fungi and other microorganisms, and/or higher species, or by combining it with other physicochemical remediation techniques. Fungi that live in symbiosis with plants (maize) can speed up the breakdown of a variety of chemicals, including the herbicide Atrazine, which can degrade wide range of compounds (Huang et al., 2007). According to Bilal et al. (2019), the method of using microbial consortia, fungal cultures, and their enzymes appears promising for eliminating PPCPs in traditional wastewater treatment plants.

4.3 Phycoremediation (Using algae)

Organism such as microalgae has played an important role in the treatment of sewage and their ability to regulate energy production and carbon dioxide emissions have created a greater exploitation (Li et al., 2019). Therefore, a greater negative carbon emission can be obtained if these microorganisms are properly used, thereby resulting to effective control of carbon dioxide which is a major greenhouse gas. In comparison to traditional wastewater treatment methods, the usage of microalgae can eliminate organic pollutants, heavy metals, nutrients, and pathogens found in wastewater. Through rhizofiltration, algae can absorb, concentrate, and precipitate metals in their biomass from toxic wastewater (Yadav et al., 2011). Heavy metals such as Ni, Cu, Zn & Pb are being extracted from wastewater through rhizofiltration. Using algae as a means of treating wastewater helps to form a large amount of biomass and has a greater tendency of absorbing and eliminating heavy metals (Baghour et al., 2002).

Some of the characteristics which enable algae suitable for wastewater treatment is their high surface area to volume ratios, heavy metals resistance, amenability to genetic modification and their capability to grow both autotrophically and heterotrophically. Therefore, since algae require nutrients like phosphorus and nitrogen to convert sunlight into useable biomass, phytoremediation is considered a relatively safe method (Chekroun & Baghour, 2013).

4.4. Phytoremediation of emerging pollutants

An effective and non-destructive in-situ bioremediation technique for the removal of emerging pollutants is through phytoremediation by using plants to filter wastewater through biological processes. By using a variety of mechanisms such as degradation, transformation, accumulation, and stabilization, phytoremediation removes EPs from the environment and wastewater (Chen et al., 2021; Kanwar et al., 2020). Through this procedure, hazardous Eps can be removed from the environmental matrices (Chen et al., 2020, 8). Based on the type of pollutants and environmental conditions involved, the plant species must be carefully chosen which is considered as the primary factor related to the effectiveness of phytoremediation. Duck weed plant species have been used in the treatment of wastewater due to their low cost. For efficient removal of agrochemicals such as N and P, it has been estimated, that these plants species have the tendency to accumulate 9.1t/ha/year of total N and 0.8t/ha/year of total P in their biomass (Zhou et al., 2018, 57-63). After 3 days of incubation, it was observed that the duckweed *Lemna turionefera* found in municipal wastewater had a lower concentration of total N and P than those present in the effluent from a local wastewater treatment plant. Based on these same studies, it was observed that after 15 days of culturing and growth, four duckweed plant species were able to remove 93% of total N and P found in local municipal wastewater making the final concentration of N to be 1mg/L which is lower than the national standard for wastewater treatment (15mg/L, China Standard GB 18918-2002).

Another factor which helps in the breakdown of contaminants by phytoremediation is the growth stage of the chosen plant species. For example, plant species such as Alternanthera species, can effectively eliminate Acetaminophen and methylparaben from wastewater samples. These pollutants seen in plants slowed down the rate of plant growth and there is a significant change in the removal of pollutants: acetaminophen and methylparaben removal rates were 88.6% and 66.4%, respectively, compared to unplanted controls' removal rates of 29.7% and 21.9%, respectively (Mohammed et al., 2021, p7).

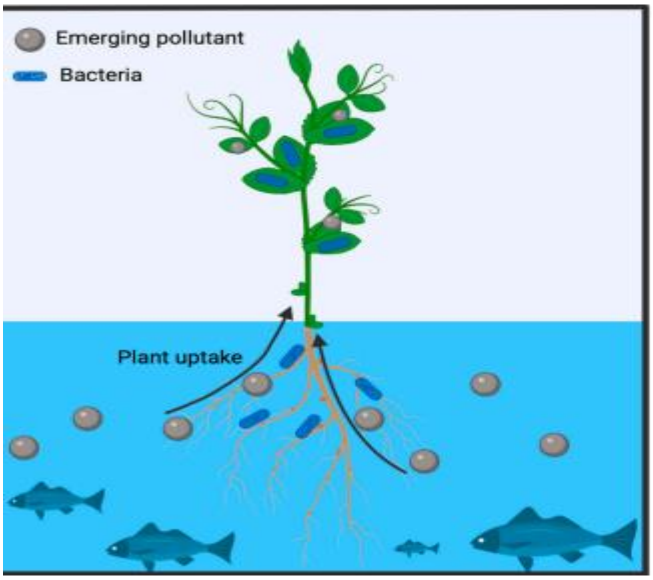

FIGURE 3. Emerging pollutants removal by enhanced degradation using the combined technique (Chen et al., 2021).

4.5 Combined technique using both microbes and plants.

Both microbial and phytoremediation have demonstrated their potential to eliminate Eps even though their long processing time is a disadvantage (Supreeth, 2021, p8). Thus, the combination of both plants and microorganisms has emerged as an alternative to accumulate and transform Eps to a greater extent. Microalgae have a wide usage in the biological treatment of wastewater, thus through proper evaluation of environmental issues, combined technique can yield considerable economic benefits with the production of value-added products. The process of using both plants and microorganisms in the degradation of pollutants functions differently. Plants degrade pollutants by exudation of nutrients used by rhizosphere bacteria to their metabolism and growth (Glick, 2014; Supreeth, 2021). On the other hand, plant resistance is increased by microorganisms to the toxicity of some pollutants. Symbiotic association between Arbuscular mycorrhizal fungi (AMF) and plants helps to increase nutrient uptake and resist emerging pollutants in wastewater and different environmental factors (Chen et al., 2021).

4.6 Current challenges associated with advanced bioremediation.

Although bioremediation techniques have made considerable relevance in the removal of emerging pollutants, there are still several obstacles to be cleared before their removal rates and industrial application may be increased. The difficulties can be divided into three categories: operational, biological, and economic. Any microbial remediation solution must have high operational functionality and cost efficiency to be successfully employed, which might be challenging in some instances. Additionally, some bioremediation procedures can only remove Eps partially; as a result, pre- or post-treatments are necessary, which could increase overall energy consumption and costs (Ahmed et al., 2022). Additional research is also needed to address biological problems. In this context, investigations using algae-based approaches showed that microalgae cannot grow well in wastewater when there are significant levels of total phosphorous, chemical oxygen demand, and total nitrogen contamination (Gupta et al., 2019, p9).

Regarding the operational difficulties, the optimum performance of biological therapies can be directly correlated with several environmental factors, including temperature, light intensity, pH, oxygen, carbon dioxide concentrations, and temperature. For instance, the ideal temperature for the growth of microalgae lies between 20° and 30° C. However, they should be tested in a natural environment considering the presence of co-existing pollutants/microorganisms and different conditions of temperature, sunlight, and pH values (Wu et al., 2022).

Biological remediation techniques, such as algae- and fungal-based treatments, have been successfully applied in the degradation of EPs under laboratory conditions. Additionally, wastewater effluents are often a complex combination of pollutants; as a result, it is necessary to consider the impact of other pollutants that coexist to assess whether or not the concept is applicable. Finally, the development of innovative and sustainable technologies has been prompted by the conventional WWTPs' failure to effectively remove biological emerging pollutants and other EPs. However, preventive strategies through public policies and regulations in industrial activity, the use of personal products, and disposal practices should also be considered (Géba et al., 2021).

5 MECHANISMS OF ALGAL BIOREMEDIATION FOR THE REMOVAL OF EMERGING POLLUTANTS.

Removing emerging pollutants from wastewater involves various mechanical reactions. These mechanisms include the passage of the Eps through the cell wall of the algal and binding it to the intracellular proteins contained in living cell, the process of bioaccumulation that occurs in the non-living cells, adsorption of the emerging pollutants into the cell wall of the algal or during biosorption, breakdown of complex Eps into tiny less toxic compounds through the process of biodegradation, and the exposure of Eps into direct UV light during the process of photodegradation as shown in Figure 4. The secretion of reactive oxygen species (ROS), from cell wall organelles such as mitochondria, chloroplast and peroxidases are induced when different microalgae species are attached with emerging pollutants (Kurade et al., 2016, 26-34). Anti oxidative enzymes such as superoxide dismutase, Catalase and Corbate peroxidase are responsible for the removal of ROS species. This helps to minimise cell destruction of microalgae (Zhang et al., 2011, 337-347). Therefore, the biodegradation of Eps is a sequential step involving enzymes metabolisms. Firstly, cytochrome P450 helps in the activation process through oxidation, reduction, and hydroxylation reactions leading to the formation of biodegradable compounds, the transfer of the enzymes from the cytosol to the activated in order to form conjugate and lastly, the shifting of the conjugate to the vacuoles using transporters (Hansda et al., 2016).

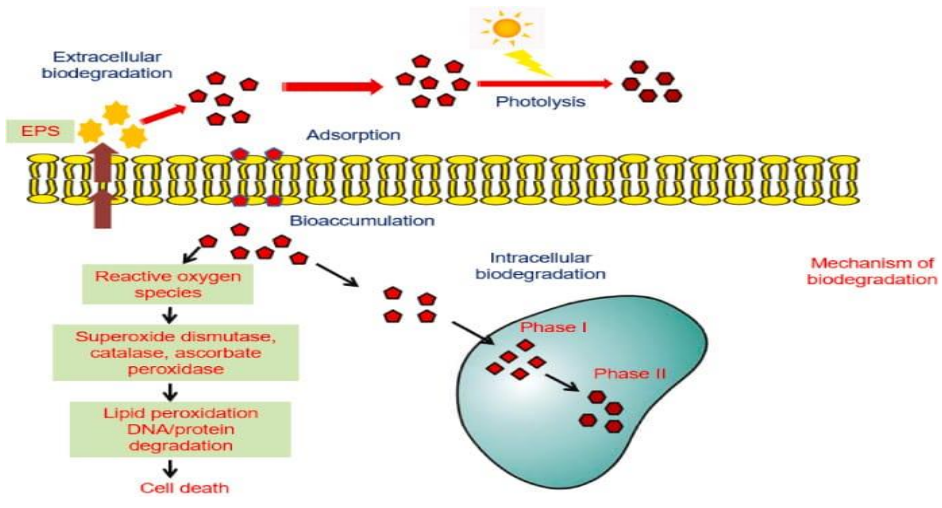

Figure 4: Algae mechanisms of emerging pollutants removal from wastewater (Rajesh Banu et al., 2020)

5.1 Biosorption

This technique entails the attachment of emerging pollutants on the microorganism surfaces especially in the removal of heavy metals from wastewater below the concentration limit as established by regulatory bodies (El-Naggar et al., 2019). The algae cell has different functional group such as polysaccharide, lipid and proteins which acts as adsorption site. The process takes place in two ways: physisorption and chemisorption. During physisorption, the sorbent surface containing the binding site interact with solute molecules while during chemisorption, there is formation of chemical bonds. Algae has the tendency to release exopolysaccharide which make it a suitable surface-active agent in the removal of emerging pollutants especially, heavy metals. These metals get adsorbed into the cell wall surface by the process of ion exchange (Lo et al., 2014, 182-190). For example, an investigation was conducted where Chlorella vulgaris was utilised for the biosorption of mercury found in wastewater which yielded a greater biosorption capacity showed by the algal cells (Kumar et al., 2020).

5.2 Bioaccumulation

The cell cytoplasm has an external environment where there is a buildup of contaminants during bioaccumulation. The contaminants found in the cell are effectively absorbed by microalgae which utilises them for growth purposes (Rezania et al., 2016). During bio-adsorption and bioaccumulation, the Eps are absorbed by microalgae for their maintenance and cell growth. The process of bioaccumulation begins with bio-adsorption which is considered as the initial stage where Eps such as pharmaceuticals (triclosan and sulfamethoxazole) have been bioaccumulated inside microalgal cell leading to the formation of excess ROS and cell death (Bai and Acharya, 2019, 534-540).

5.3 Bio uptake of emerging pollutants

Bio uptake of Eps takes place only in living microalgae cells where the pollutant enters the algal cell and binds to the intracellular proteins (Mulla et al., 2019, 41-55). During bio uptake, hazardous substances are transported from the ambient medium to the cells where nutrients can be cultured in larger quantities (Karya et al., 2013, 244-250). A reliable remedy has not been provided during bio-uptake because the process only helps in transforming Eps from one form to another which result to the inability of using algal biomass to produce biofuels. Therefore, there is a need for further studies regarding post treatment process and reduction of Eps toxicity using algal cell (Chen et al., 2017).

5.4 Biodegradation

During the process of biodegradation, there is metabolic breakdown of Eps using enzymes as catalyst (Varjani, 2017). Such enzymatic reactions include hydroxylation, glycosylation dehydrogenation and hydrolysis. This method occurs in three steps: addition of a hydroxyl group which converts the lipophilic molecules into hydrophilic molecules, formation of a conjugate bond with glutathione through the formation of compounds containing electrophilic group. This helps to protect the cells form oxidative damages and thirdly, enzymes such as carboxylase, dehydrogenase are used. The quality of the biodegradability of certain compounds depends on the complexity of the structure. For example, linear and unsaturated structures containing electron-donating group have a higher biodegradation rate as compared to cyclic compounds (Xiong et al., 2016, 183-190). The rate of Eps biodegradation depends on the C: N: P in wastewater and the optimum ratio of biodegradability is 100:18:2. Co-metabolism and metabolic biodegradation are the two processes that are responsible for biodegradation. When EPs are degraded by co-metabolism, enzymes are used to catalyse the process, but when EPs are degraded metabolically, algae use EP as a source of carbon (Norvill et al., 2016, 291-309).

5.5 Photodegradation

Photodegradation occurs in two ways: photooxidative degradation or photolysis. During photodegradation, the oxidants such as hydroxyl radicals reacts with the Eps present while photolysis takes place when UV light absorbs contaminants which results in conformation change (Abo et al., 2016, 27-35). Factors such as intensity and light wavelength affects the process of photodegradation through the addition of dissolved organic molecules (DOM) which are classes of compounds responsible for the enhancement of photodegradation. Based on the mode, photodegradation can either be direct or indirect. Indirect process deals with the absence of microalgae present in the wastewater while in direct photodegradation, there is removal of sterols (Reymann et al., 2020). For example, triclosan and ciprofloxacin have been eliminated from *Nannochloris sp* using photodegradation. This process is specifically to promote light exposure using turbulent mixers and algal scrubbers to remove contaminants. However, this technique is highly selective which makes some Eps to be resistant to photodegradation (Nguyen et al., 2021).

5.6 Hydrolysis

This method depends solely on the structure of the pollutant present. For example, pharmaceuticals such as sulphonamide are resistant to this process (Gavrilescu et al., 2015, Norvill et al., 2016). An increase in algal pond temperature result in an increase in the rate of chemical breakdown using water. This is because there are variations in the pH level of algal pond. Therefore, emerging pollutants that hydrolyses at higher pH level experience substantial daylight degradation. Contrarily, at neutral pH pollutant hydrolysis deteriorate during the night. However, Certain drugs such as fluroquinolones and sulphonamides, are resistant to hydrolysis (Zhang et al., 2020).

5 CONCLUSIONS

The utilization of traditional approach in the treatment of wastewater from steel companies is often difficult. This is due to high iron concentrations present in the residues. The removal of Eps from water bodies is of great concern since Eps causes serious health hazards to humans and the environment. Therefore, the source and different pathways in which emerging pollutants infiltrate the environment as well as their effects on humans and the environment were described in this thesis report. However, advanced bioremediation techniques were discussed since their advantageous features such as sustainability, cost-efficiency, and eco-friendliness make them suitable alternatives for decontamination of pathogens. Therefore, the most recent advancements in the removal of emerging pollutants through bioremediation techniques is a better alternative to conventional methods, thereby creating different avenues for implementation and optimization at a large scale. For a variety of wastewater, algal bioremediation offers an effective treatment thereby reducing the rate of carbon-dioxide emission and biomass product obtained. The utilization of harvested biomass for energy and biofertilizers should determine the best harvesting technique based on the algae strains being employed. Microalgae effectively remove organic and inorganic contaminants through the primary mechanisms of bio-adsorption, bioaccumulation, photodegradation, and biodegradation. To achieve the desired level of bioremediation effectiveness, bio-adsorption, a prominent interaction among others, can be deliberately improved by changing abiotic variables. Before harvesting, bio-adsorption can be further improved by controlling pH, temperature, and ionic species to promote interactions.

Results from the research work showed that wastewater treatment plants could perform better than private institution due to numerous treatment steps which helps to facilitate the removal of complicated developing contaminants and pathogens. The numerous treatment processes may provide different microenvironments that allow incorporating diverse mitigation mechanisms (such as biosorption and degradation). The parameters that affect the removal of emerging contaminants from wastewater in these systems (such as system operation settings, ambient conditions, and wastewater matrix) have been further identified and discussed. Therefore, a conclusion can be drawn that algae usage in biomonitoring and restoration of aquatic systems encourages bioremediation, even though some EPs are still resistant for algae recovery. In comparison to other microorganisms and conventional approaches, the bioremediation of different organic contaminants by microalgae and cyanobacteria is a sustainable and environmentally acceptable green technology for treating polluted wastewater.

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