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Green engineering principles and application: bioremediation

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Abstract

The adulteration of the environment by hazardous waste, such as heavy metals, nuclear wastes, hydrocarbons, pesticides and greenhouse gases is the major serious problem which need to be reduced. Common remediation technique such as physical, chemical and biological process are being applied. Chemical process can transform and change organic contaminant of interest which is not sufficient to clean the environment, while physical technique requires additional equipment. Thus, bioremediation exist as green approach to eliminate the hazardous waste in the environment. In this review, bioremediation is comprehensively presented. Remediation process types, challenges, limitations, mechanisms, and future suggestion has been elaborated to develop bioremediation technology for future prospect.

Keywords :

Bioremediation, in-situ remediation, ex-situ remediation, green approach

1 Introduction

Over the past couple of decades, environmental concerns have exponentially risen in coherence with increasing human activities such as unsustainable agricultural practices, urbanization and rapid industrialization (Azubuike et al., 2016; Mosa et al., 2016). Pollutants, such as heavy metals, nuclear wastes, hydrocarbons, pesticides and greenhouse gases are of major concerns due to their toxicity and potential hazard to both environment and human health (Dzionek et al., 2016). These pollutants infiltrate the environment in various ways, contaminating the environment.

Treating such pollutants require suitable cost-effective and eco-friendly processes which take into account the characteristics of the medium contaminated and apply the most effective method for remedial work. Generally, the treatment process is governed by three major processes (Lim et al., 2014; Li and Yang, 2018; Lin and Kiang, 2003): chemical processes, physical processes, and biological processes. Chemical processes consist of chemical compound which able to destroy or transform the organic contaminant of interest. One of the techniques is oxidation process, for instance the oxidation of the contaminant to carbon dioxide. The transformation techniques involve chemical reduction reactions and application of alkaline reagents to achieve successful chlorination. Physical treatment entails organic contaminants being transferred from one form of medium to another. It can be formed from soil to water or even soil to air.

* Corresponding Author. Email Address : hadibarata@curtin.edu.my https://doi.org/10.33086/etm.vli3.2504 Received from 24 November 2021; Received in revised from 29 November 2021; Accepted 29 November 2021; Available online 30 November 2021; The process requires a step for capturing the contaminant which produces concentrated contaminants for later recovery and further treatment or disposal via other treatment methods. One example of the process is the stabilization/ solidification technique which incorporates the immobilization of the metal and other inorganic contaminant (Fox, 1996). Lastly, biological processes refer to the usage of micro-organisms such of bacteria, fungi and plants to biodegrade of the contaminants. The process incurs the conversion of contaminants to smaller and safer compounds (Dhall et al., 2012). Current literature review has shown many cases whereby both physical and chemical treatment methods of contaminants can be expensive and has limited effectiveness on the contaminant (Das and Chandran, 2011). Thus, bioremediation technology is preferred to be used for pollutants elimination.

The aim of this report is to review current literature on the bioremediation technology, analysis should show the major trends, identifying and elaborating on newly developed solutions which have enabled past challenges to be overcome. Taking into consideration the theoretical aspects of the numerous methods employed by the technology, current limitations are to be identified whilst showcasing the future prospects of the bioremediation technology.

2 Remediation process types

2.1 History of bioremediation

Having been under consistent study since the 1940's, the application of the technology only became prominent in 1980's within the US when it successfully cleaned up oil spills on the shorelines. Studies have shown that numerous micro-organisms have the capabilities of degrading a range of pollutants, although their rate of degrading a pollutant depend on the micro-organism's environment (Dzionek et al., 2016; Dhall et al., 2012). Considering such capabilities, continuous experimentation with dirt jars and abandoned oil sumps, finally, the bioremediation process in the 1960's was developed and later successfully organized the first large-scale clean-up of an oil spill via the use of microbes (Ikhimiukor and Nneji, 2013). It enabled to restore the contaminated sites into their natural state through the application of micro-organisms. It degrades or transforms the toxic compound of interest into safer compounds (Mosa et al., 2016).

2.2 Bioremediation type

Bioremediation is generally broken down into three major types: microbial remediation, phytoremediation and mycoremediation. The microbial remediation primarily focuses on the microbe's enzymic activity for the conversion of contaminants to harmless substances (Ratnasari et al., 2021a). The interaction between microbes and the contaminant consequently initialized an immobilization and compartmentalization of the pollutant. Microbes used within the industry in classified into aerobes, anaerobes and facultative anaerobes (Ratnasari et al., 2021b). Primarily, the technique aids the decomposition of pesticides, hydrocarbons, and heavy metals. This bioremediation technique has developed into three groups which are bio stimulation, natural attenuation and bioaugmentation.

Phytoremediation is a type of remediation which involves the use of green plants since it is relatively cheap and regarded as a solar driven technique. The plants aid via in-place degradation and removal of contaminants in the medium. A common plant used for this process is the 'poplar tree', although high concentration levels of contaminant result on the death of the plant. Lastly, the mycoremediation is a type of bioremediation which employ fungi to break down contaminants. One common fungus is mushrooms, the technique relies on the enzyme to degrade the numerous substrates present in the contaminant. Alternatively, another process may commence via this type of remediation, whereby biosorption occurs. A process in which the pollutants are absorbed by the fungi (mushroom), into its mycelium which essentially makes the fungi not suitable for consumption (Kulshreshtha et al., 2014). Studies have shown the following fungi: Trichoderma and Aspergillus species to be very effective in removing a range of heavy metals (Ayad et al., 2018).

2.3 Bioremediation techniques

Since its development, bioremediation has grown to diverge into two major techniques, those being the in situ and the exsitu. The in-situ technique occurs within the sub-surface of the medium, those being soil or groundwater. Advantages which can arise from this technique of bioremediation are the high elimination rate of the contaminants, it is cost-effective, there is a lower impact risk level on the surrounding environment undergoing treatment and there is no need for extensive equipment (Azubuike et al., 2016). Furthermore, there is less human involvement to change the site's physiology which ultimately reduces the environmental impact as the site does not require excavation for removal and transportation. Although, one disadvantage is with regards to the interference of the existing environmental state through indirectly reducing/retaining the contaminated site from being treated. Thus, the need to be monitored closely on a consistent basis. The ex-situ technique is considered an alternate solution for the in-situ technique. This technique excavates and removes the contaminated medium (eg. soil) from the site and treats the medium with above-ground treatment methods involving the indigenous microbial population (Azubuike et al., 2016). The presence of oxygen is critical for this process to achieve exceptional bacterial growth, ideally producing water and oxygen as the contaminant is broken down further to simple non-toxic forms. Past applications of this technique have shown high successful rate on the field, but with regards to versatility it is limited on some soils and metals.

2.4 Classification of bioremediation

The bioremediation process can be classified into three categories: the biotransformation class, the biodegradation class and the mineralization class. Within the biotransformation class, are contaminant compounds which undergo alteration of their molecular structure to less harmful or non-harmful molecules. While in the biodegradation class, are the organic compounds which broken down simple inorganic and organic compounds or molecules. Lastly, is the mineralization class where the organic compound of interest has been completely biodegraded into the simplest inorganic molecules such as water and carbon dioxide.

3 An overview of the major challenges faced in this area of research and practice

3.1 Hydrocarbons

Fuel oils are primarily composed of n-alkanes and forms of hydrocarbons such as monoaromatics, aliphatic hydrocarbons and polynuclear aromatic hydrocarbons (Mohanty and Mukherji, 2007; Weber et al., 2016). There are vast abundant hydrocarbon degrading cultures present in natural existing ecosystems, the structural complexity of oil plays a key role in determining the extent of the oil's biodegradation (Das and Chandran, 2011; Kostka et al., 2011). Even under the most favorable environmental conditions, the degradation may not be completed as culture specificity also plays a critical role in determining substrate interaction and the degree of altering the oil bioavailability (Mohanty et al., 2013). Hydrocarbon contamination remains a major environmental issue till this day as a result of continuous activities from petrochemical industries. In 2003, a study showed that the natural crude oil seepage was estimated to be 600000 metric ton annually with an estimation uncertainty of 200000 metric ton annually. The estimation indicated that 47% of the seepage rates were natural occurring, the other 53% originated from anthropogenic sources such as extraction, processing and transportation (Kvenvolden and Cooper, 2003). One of the most iconic disastrous oil spills took place in Mexico in the year 2010 (Edwards et al., 2011). It leaked over 600 million liters of crude oil and damaged a range of marine ecosystems. It also influenced an immense decrease in both flora and fauna population within the region (Ainsworth et al., 2018).

3.2 Pesticides

Pesticides are being globally used in agricultural practices to control harmful organisms, preventing spoilage of both crops and livestock, ultimately prevent yield reduction and pest infestation (Ghorab and Khalil, 2016). These pesticides are most beneficial in agricultural production, studies have shown that in low concentrations amounts some residues persist in the soils, water, air and in the food supply (Nicolopoulou-Stamati et al., 2016). A moderate pesticide residue into an ecosystem can significantly affect the equilibrium of the existing food web and can result in severe health risks such as raspatory problems for human health (Nicolopoulou-Stamati et al., 2016; Mamane et al., 2015).

3.3 Radioactive waste and heavy metals

Industrialization and wars have also lead to significantly higher concentration levels of heavy metals, radioactive wastes and greenhouse gases on a global scale (Lawrence et al., 2015). Uranium as a naturally occurring element exists in low abundance and therefore would be generally pose very low risk to health or danger to its surrounding environment (Ma and Zhai, 2012). Increasing anthropogenic activities such as uranium mining, nuclear fueled powerplants for electric generation purposes, uranium enrichment for nuclear weaponry and the combustion of fossil fuel has significantly increased uranium concentrations in the environment (Bearman, 1979). One study carried out in 2004 showed that in the U.S alone had an estimate 2 million acres of land accounted for uranium contamination in 30 states. When present in groundwater, Uranyl, U(VI) has been documented to be the most dominant species of the element. Characteristic properties of the element include high solubility and mobility within subsurface environments (Ma and Zhai, 2012). Heavy metals such as cadmium, copper, lead and zinc are common non-degradable toxic contaminants compared to organic compound which can be degraded via physical, chemical or biological means. Hence, metals compounds can only be transformed from one organic complex to anther or oxidized in to a different state (Ayangbenro and Babalola, 2017).

3.4 Textile Production: Dyes

The exponential growth and advancement of textile industries over the years has surely aided in the lack of reinforcement for proper treatment facilities to be developed, this has resulted in toxic and carcinogenic pollutants such as dyes, volatile organic compounds and heavy metals being discharged into the environment (Vikrant et al., 2018). Shabbir, et. al states the annual estimated mass of synthetic dyes discharged annually into aquatic environments ranges from 30 to 150 Kilotons. Having a surplus of dye concentration present within a water body can pose significant negative impacts on the ecosystem. Thus, it is crucial that new cost-effective methods are development to treat, recover and safeguard our present ecosystems and environment (Shabbir et al., 2017).

4 A review of the approaches and 4.2 studies used in the past to overcome The these challenges

4.1 Hydrocarbons

One solution towards enabling a more favorable condition for biodegradation of hydrocarbons to occur via microbial degradation included the surfactant enhanced bioremediation (SEB) approach which aimed to overcome the oil's bioavailability constraints encountered during the biotransformation process of Nonaqueous Phase Liquid Pollutants (Mohanty et al., 2013; Churchill et al., 1995). Although, the success rate is highly dependent on the choice of surfactant type and on the dosage, amount used on the hydrocarbon. Application of surfactant improves the uptake capacity of constituents through solubilization and emulsion whilst potentially modifying the cell surface of the microbe being used (Mohanty et al., 2013). Application of surfactants allows the enhancement of constituent uptake via emulsification and solubilization mechanisms whilst potentially changing characteristics of the microbe cell surface (Sheng, 2017).

Emulsification is the process of reducing interfacial tension between the oil and the aqueous phase, providing a route for mass transfer to occur from oil phase to the liquid phase (Mohanty and Mukherji, 2007). Surfactants are have three mechanisms to enhance bioavailability, these include pseudo solubilization, emulsification and facilitated transport (Mohanty et al., 2013). During solubilization, partitioning within the surfactant's micelle occurs which enables the hydrocarbon to become more soluble. Surfactant monomers will form a 'regular' micelle whereby monomer hydrophilic tails face inwards, hydrocarbons will naturally partition within the hydrophilic core.

Lastly, facilitated transport mechanism refers to other hydrocarbon mass transfer processes via oil interaction with surfactant micelles of single surfactant monomers (Mohanty et al., 2013). While considering this solution, it is critical to note that many toxic intermediates have been seen to accumulate during the surfactant degradation process which can cause endocrine disruption (Mezzanotte et al., 2003; Chen et al., 2005).







Figure 2 Illustration of reverse monomer-micelle equilibrium (Rangel-Yagui et al., 2004)

4.2 Radioactive waste and heavy metals

The most successful well documented bioremediation strategies for uranium treatment have been immobilization, whereby bioreduction and bioprecipitation techniques are applied. Through natural evolution, a range of bacterial species have been found to be able to reduce Uranium (VI) to Uranium (IV). Studies have shown these species are dominated by sulphate reducing bacteria and Iron (III)- reducing bacteria (Ma and Zhai, 2012). Alternative methods such biosorption can also be implemented to immobilize the uranium in the environment. This is achieved via use of negatively charged microbes and other present molecules such as carboxylic and sulphate groups. Multiple studies in the biosorption have proved successful and shown promising potential microbe for the method. One promising fungi-based bio sorbent is the Saccharomyces cerevisiae, this bio sorbent has shown high resistance towards toxic uranyl ions whilst surviving low pH external environments in comparison to other microbes (Zheng et al., 2017). It is easily attained, environmentally safe, low cost and the model is ideal for genetic manipulation. Past studies have indicated correlation between uranyl ions, phosphate ions and hydrogen ions, actively present in the precipitation of chernikovite when the Saccharomyces cerevisiae were used as bio sorbent, during the desorbing of uranium phosphates were released whilst dramatically reducing toxicity levels from the uranium sample. Analysis showed how uranium phosphate precipitates from can

be transform to crystalline state-tetragonal chernikovite which is more stable form of uranium (Zheng et al., 2017). The *Saccharomyces cerevisiae* species has also been used in the past to treat a range of heavy metal; the species proved successful. Although, more research is required for further development as many mechanisms on a micro-scale is still unknown (Massoud et al., 2019).

4.3 Textile production: dyes

Recent development of both microbiology and biotechnological advancement has enabled bioremediation to be an alternative method over the prior conventional chemical and physical treatment methods. Over the years, many physicochemical treatment options have been developed to remediate dve pollution from facilities. Such methods include adsorption, oxidation remediation, membrane separation, ion-exchange and coagulation (Pan et al., 2017; Huang et al., 2014; Rondon et al., 2015; Robinson et al., 2001). While these methods have had many applications in the past, they have not been very economical nor energy efficient and resulting in high operational cost whilst generating a vast amount of unwanted wastes such as sludge which require more capital, energy and manpower to treat and dispose (Vikrant et al., 2018). Furthermore, each of the previous technologies encountered major constraints as the technology became saturated. Oxidation remediation, although was simplistic and yielded good results, the process generated large amounts of sludge which were difficult to dispose. Other drawbacks included the high energy cost and the formation of carcinogenic by-products (Robinson et al., 2001). Adsorption method has also experienced enhancements over the decade through the use of porous sorbents which can be activated for the sorption process. This is achieved via using materials such as activated carbon, peat or wood (Ratnasari et al., 2022a). Although, the method is limited as it cannot degrade contaminants, the retention time is very long, it is not very costeffective and the fact that unwanted side reactions can occur during the process makes the method not favored for commercialization (Vikrant et al., 2018). The membrane separation process has had advantages whereby there is ease in operation and installation of the equipment, less maintenance required and has showcased a smaller footprint that the other physicochemical methods (Rondon et al., 2015). Despite the advantages, the methods require high capital investment, the later disposal of the concentrated residue and the constant clogging of the membrane being used (Rondon et al., 2015). The next method is ion-exchange, a technology whereby rich-wastewater dyes are passed over an ion-exchange resin till the resin is fully saturated. This method is very high cost and very inefficient when the treating a non-rich wastewater along with the capability to only treat specific. Lastly, the coagulation method removes dyes from wastewater via the use of ferrous sulphate and ferric chloride. While this method has shown to be very effective with direct dyes, the method has also shown ineffectiveness against acidic dyes and has a high processing cost (Vikrant et al., 2018)

Within the past decade, biological technique awareness has risen significantly, this has been as a result of the many advantages the techniques has shown over those of the conventional techniques which incorporated both chemical and physical means (Ghosh et al., 2017). The technology has proven to be environmentally friendly, cost-effective, simple and ensures safe operation. For bioremediation of dyes, techniques such as bio stimulation, bioventing, bio sparging, bioaugmentation, leaching and sorption has been widely used as they can be carried out both in-situ and ex-situ (Vikrant et al., 2018). Three main types of biodegradations can be achieved with dyes, those being biodegradation via bacterial strains, biodegradation via fungal strains and lastly biodegradation via algae strains (Vikrant et al., 2018; Ratnasari et al., 2022b). Degradation of dyes via bacteria usually incurs the cleavage of azo linkages along with numerous enzymes such as the azoreductase present. Another study showed the reactive black 5 dye was degraded up to 93% for the concentration of 500 mg/L within 120 hours under aeration. Although, studies have shown that while some intermediates are also further degraded by oxygenase and hydrolase enzymes released by the bacteria. While this occurrence may benefit the process, other studies have shown more complex azo dyes to be more resilient as they are non-permeable in origin (Vikrant et al., 2018).

Biodegradation by fungal strains in application has been quite effective in the past decade, fungi have degraded and successfully mineralized multiple textile dyes via their extracellular ligninolytic enzyme system. The availability of diverse metabolic capacity and exceptional morphology allows fungi to be a robust efficient process (Rahimnejad et al., 2015). One promising fungus was the 'Penicillium Oxalicum strain SAR-3' which degraded azo dyes acid red 183, direct blue 15 and direct red 75. The general mechanism initially starts once through the adsorption and onto hyphae, the fungi then proceed to breaking the dye chemical bond by the extracellular enzymes inside of the hyphae. It is important to note that the result and efficiency of the process is critically dependent on external factor such as pH, biomass dosage and contact time (Mahmoud et al., 2017).

Lastly, the biodegradation of dye via algae strains have been effectively employed for a variation of textile dyes(Vikrant et al., 2018). Routinely exercising the anoxic method (both aeration and anaerobic). An exemplary algae strain *'phormidium autumnale* UTEX1580' showcased complete degradation of the dye indigo (Dellamatrice et al., 2017). When considering the biodegradation process of textile dyes via bacterial strain means, the rate of substrate diffusion in the cell can be limiting compared to fungal strains which does not experience such issues. Between the various fungal strains, white-rot fungus has proven to be highly effective in the biodegradation process (Vikrant et al., 2018).

Ligninolytic enzymes which secrets from the white-rot fungus can bind to numerous compounds which recalcitrant, complex compounds such as textile dyes and does not require preconditioning of white-root prior to application (Kaushik and Malik, 2009). Several challenges and limitations may be experienced when considering biodegradation and mineralization of textile dyes, although this can eventually be overcome with genetically engineered micro-organisms in future application as the methods and biological techniques developed has overwhelmingly proven to be more sustainable and cost-efficient compared to conventional methods of dye treatment.

4.4 Heavy metals

Previous methods used for the removal of metal ions from aqueous solution included physical, biological and chemical technologies. Although, previous studies have shown electrochemical treatment and chemical precipitation to be quite ineffective when ion metal concentration within the aqueous sample was between ranges of 1 to 100 mg/L (Wang and Chen, 2009). Furthermore, these two treatment techniques resulted in large volumes of sludge being produced which recommended harsher processing treatment. Treatments such as carbon adsorption process, ion exchange and membrane technologies have also shown to be very expensive upon application for treating large amount of water/wastewater even when the medium contains low concentration heavy metal (Wang and Chen, 2009).

Ex-situ remediation techniques (physical and chemical) such as landfill, excavation, electro-reclamation and acid leaching temporary aided in disposal and treatment of the contaminated area. These conventional methods have continuously proved to result in high-costs, vast physical destruction of soil fertility and overall low efficiency (Banik et al., 2014). Thus, newly bio-remedial techniques such as microbial remediation was developed (See Figure 3). Through microbial remediation, micro-organisms detoxify the targeted heavy metals through extracellular chemical precipitation and valence transformations. Few potential heavy metals have been detoxified via enzymic reduction processes carried out by microbes (Figure 4).



Figure 3 Bacterial degradation of heavy metal contaminants (Banik et al., 2014).

From Figure 3, mechanism A shows the process of bacterial absorption of the heavy metals, via intracellular accumulation, cell surface absorption and extracellular precipitation. Mechanism B shows bacterial remediation of heavy metals via siderophore formation, this process is carried out with the formation of membrane protein-mediated metal transport and siderophore-metal complexes. Lastly, mechanism C showcases bacterial remediation through biosurfactant production. Sorption and desorption of surfactants which help in precipitation of the heavy metals (Banik et al., 2014).

Metals	Microorganisms	Source	MRL/Uptake Efficiency	Removal (%)
Cd	Bacillus strain H9	Metal-contaminated soil	275 µg/ml	36 (48h)
	Aspergillusterreus	Industrially polluted sediments	: 122 ppm/g	70 (13 days)
Cr	Pseudomonas aeruginosa	Wastewater	0.08 mg/ml	46 (2 days)
	Aspergillusniger	Acquired	5.1-6.6 mg/g	21- 36 (7 days)
Pb	Pseudomonas aeruginosa PU21 (Rip64)		0.5 mg/ml (110 mg/g)	80 (2 days)
	Aspergillusniger	Acquired	5.3-34.4 mg/g	13-88 (7 days)
Cu	Thiobacillus ferrooxidans	Adopted	0.02 mg/ml (700 mg/g)	25 (15 min)
	Schizosaccharomyces pombe	Acquired	0.6-1.3 mg/g	11 - 25 (4 days)
Ni	Pseudomonas spp.	Local isolate	74.9 mg/g	98 (4 days)
	Candida spp.	Sewage	10.3-46.8 mg/g	29- 57 (5-15 day:
Abbrevia	tions: MRL: Maximum Resistance Level			

Figure 4 List of heavy metal accumulating micro-organism with respective removal efficiency.

Considering the biosorption method, one of the biggest challenges was to select a type of biomass from a vast selection of readily available inexpensive biomaterials. Naturally, biomaterials have the capability to bind heavy metals, but upon considering a biomaterial for a full-scale biosorption process only those of highbinding heavy metal capacity are most suitable (Wang and Chen, 2009). Applying such a method aims to remove the contaminant metals from the sample solution via the use of living or dead microbial biomass (Ayangbenro and Babalola, 2017).

5 A summary of the status of the field

The bioremediation technology is still within its infancy stage but has shown to be very promising for remediating a range of contaminants across multiple media such as water, soil and subsurface materials. Being both cost-effective on field applications

and having the potential to achieve complete mineralization, the technology plays a key role in becoming an alternative to previously used physical and chemical conventional remediations. Since its initial development, the technology has branched out significantly in regard to technique specification for treatment of certain contaminants. Although, the technology still faces many limitations in various areas due to current available technology and limited research which prevents further advancement of the technology's versatility, efficiency and ease of feasibility altogether (Das and Chandran, 2011).

6 Limitations - Overview

While bioremediation has shown many successes in application towards treatment of contaminated sites, some concerns do arise which makes the technology less favorable. This incurs the various disadvantages associated with the technology and the need for more research which ultimately imposes many limitations:

6.1 Incomplete transformation

The idea of a complete transformation into harmless substances is merely a theoretical probability, thus there is uncertainty on the extent to which a successful degradation process can occur (Das and Chandran, 2011). Factors such as the nature of the contaminant play a significant role in the contaminant's breakdown. One example would be the resulting toxicity of the daughter compound being higher than that of the parent compound, thus it showcased that transformation into safe compound are not most certain. Another case would be the incomplete transformation of natural oil due to the oil's bioavailability (Mohanty et al., 2013).

6.2 Biological processes are highly specific

Micro-organisms are very condition specific when carrying out a process, whether in relation to growth conditions or degradation activity the environmental factors play a significant role in determining the success of the activity (Das and Chandran, 2011). Upon conditioning the site of interest, the environmental conditions and the level of nutrients fed are most crucial in monitoring the process. In reality, conditions from one contaminated site to another may vary due to a range of complex existing ecosystems and may be difficult in maintaining consistency. Furthermore, even upon ideal conditions the organisms may favor metabolizing the introduced nutrients over interaction with the contaminant of interest. Moreover, the addition of nutrients may have been primarily to initiate population growth for the introduced organisms. The similar nutrient may also promote growth for unwanted of other organisms whilst indirectly impacting the introduced organism's efficiency towards degradation of the pollutants.

6.3 Introduction of non – native organisms

While the introduction of non-native organisms may have had good intentions towards treatment of the contaminated site, issues arise as to whether the organisms themselves do not pose threat to other native organisms within the existing ecosystem. As knowledge towards the long-term effects of the biodegradation process are still in need of further study.

6.4 Technology still at its infancy

Bioremediation as a method for treatment of pollutant is still rather new, whilst it has successfully been applied onto a range of projects. Several research are yet to be done for further comprehension to set forth a reasonable theoretical basis to allow improvement of existing technology and further development of newer methodologies to achieve higher efficiency and increasing the range of application. Thus, currently there is difficulty onto extrapolating a benchmark and pilot case-studies towards large field operations (Kostka et al., 2011). One challenge encountered by the newly developed biosorption method was when considering the method's application range (Tsezos, 1999).

6.5 Not all contaminants are biodegradable

The initial concentration and toxicity of the contaminants are the drivers towards the extent to which remediation can take place, whilst consideration is also taken upon the properties of the soil or medium in which the contaminant has polluted. One situation where bioremediation was not successful were sites with consisted of high-level metal concentrations such as mercury, others include organics. They have been known to be toxic to the microbes. Studies have shown that some compounds may not be degradable at all, such as the high molecular weight polycyclic aromatic hydrocarbons (Das and Chandran, 2011; Atlas and Bragg, 2009).

7 Future Suggestion

7.1 Deficit of knowledge of microbial processes

The substantially lack of knowledge on the fundamentals of science which are involved in the bioremediation process often restrains process in the field of bioremediation. These include the disciplines of both chemistry and biology along with hydrology (inclusive of transportation process). To this date, little is known in relation to the interaction between a newly introduced micro-organisms with various hydrological environments. Furthermore, comprehension of the biodegradation and biotransformation routes are still uncertain. Research in the chemical activity/interaction between the on-site contaminants, remedial organisms and on-site micro-organisms are needed. Different contaminated sites are composed of various complex systems, ranging from diverse organisms to the difference in the types of contaminants. Thus, it is mandatory for further study on these aspects to allow advancement of the bioremediation process.

7.2 Lack of Integrated Research

Bioremediation is itself a multidisciplinary approach that incorporates the use of biological systems to degrade or transform pollutants to safer substances. This showcases the need for researches of various background to integrate known knowledges. In achieving a successful and highly efficient technology, the involvement of engineers, geologists, microbiologists, biochemists are of minimum requirements. In achieving this, the process is bound to reach its potential optimization limit. This can also be achieved through introducing formal education programs which integrate both practices and principles for the next generations.

7.3 Lack of Revenue

Many funds from both the private sector and the government are without question being invested towards biotechnology-based companies, although in comparison few of the revenue are really allocated towards bioremediation improvement. Thus, lack in revenue can also act as an obstacle towards contributing to the technology's research and development and improving the overall efficiency.

7.4 Inadequate tools and Infrastructure

Materials and infrastructure are necessities for experimental finding. The accuracy in experimental results is most critical as it confirms the degree of success for the treatment when introduced to the contaminated site. Considering the technology is fairly new, insufficiency of advanced tools designed specifically for the technology would ultimately be an obstacle towards the development and research capabilities of the bioremediation process.

7.5 Technological advancement

Accumulation of heavy metals is increasing at an exponential rate around the globe, enhancing and revolutionizing the existing microbial remediation to fasten the process, making them recyclable and enabling control of the microbes still remains a major challenge. Another challenge incurs the ability to supply materials which allow stimulation of the micro-organisms to further enhance remediation treatment efficiency and versatility

8 Conclusion

This paper was undertaken to understand bioremediation of toxic pollutants in waters comprehensively and describe the limitation of the bioremediation which had been exposed. Bioremediation to remove several pollutants, such as hydrocarbon, heavy metals, dyes, and radioactive waste have been projected. More than a few major challenges of bioremediation for these pollutants have been prescribed. To cover the challenges and limitations, numerous of future suggestion has been presented.

Declaration of competing interest

The authors declare no known competing interests that could have influenced the work reported in this paper.

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