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Biotechnological remedies for the estuarine environment polluted with heavy metals and persistent organic pollutants

Ganiyu Oladunjoye Oyetibo [a, b, *, 1], Keisuke Miyauchi [b], Yi Huang [b, c], Mei-Fang Chien [c], Matthew Olusoji Ilori [a], Olukayode Oladipo Amund [a], Ginro Endo [b]

[a] Department of Microbiology, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria
[b] Biotechnology Research Common, Faculty of Engineering, Tohoku Gakuin University, Tagajo, Miyagi, 985-8537, Japan
[c] Graduate School of Environmental Studies, Tohoku University, Sendai, Miyagi, 980-8579, Japan

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ABSTRACT

Estuaries in the areas prone to anthropogenic activities are exposed to multifarious pollutants. Toxic concentrations of heavy metals do exist with persistent organic compounds in such estuaries prolonging the recalcitrance and ecotoxicological consequences of the chemicals, which impact on the health of the brackish water and by extension, the oceans. The quest for high aesthetic quality of the estuarine environment is gaining attention from global campaign, which requires effective remedial strategies to replace the physical and chemical methods in use that are costly and often leave behind toxic residues in the environment. Contrary to physico-chemical remedial processes, bioremediation strategies are projected as a promising green technology to remove pollutants from the estuarine environment. The concept of bioremediation involves the use of competent biological elements such as microorganisms and plants, along with or without the biomolecules they produced, to ameliorate pollution. Therefore, this paper reviews the various bioremediation technologies that would be applicable to decommissioning estuarine environments polluted with toxic metals and persistent organic compounds.

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1. Introduction

An estuary is a partially enclosed body of water along coastlines where freshwater and saltwater meet and mix. It is a transition zone between ocean and continent that freely connects with the open sea, whereby the sea water is diluted with fresh water derived from land drainage. It is generally referred to as “nurseries of the sea” because it is a vital habitat to several marine species. It serves as buffering zone between terrestrial and marine ecosystems in urban and industrial areas by removing pollutants and filtering sediments through biochemical activities in the environment before it empties into the ocean, thereby, contributing to the health of the ocean. Anthropogenic activities frequently introduce elevated concentrations of heavy metals (HMs) and metalloids along with organic pollutants into estuary water in urbanized areas (Oyetibo et al., 2010, 2013a). Some specific examples of estuaries where HMs and organic pollutants have been reportedly co-exists due to anthropogenic activities include Lagos Lagoon in Nigeria (Oyetibo et al., 2015a; Obi et al., 2016); Victoria Harbor in Hong Kong (Zhang et al., 2008), Ancona Harbor of Adriatic Sea in Italy (Barbato et al., 2016), Gujarat coastline of the Arabian Sea in India (Patel et al., 2015), Santos-Sao Vicente estuary in Brazil (Pinto et al., 2015), and Tinto, Odiel and Piedras rivers' estuaries in Spain (Mesa et al., 2015a, 2015b) among others across the globe.

The toxicity of HMs to microbial community in polluted environments had been reported to inhibit biodegradation of organic pollutants in co-contaminated sites (Said and Lewis, 1991; Sandrin et al., 2000; Bamforth and Singleton, 2005), prolonging the recalcitrance of organic pollutants in the environments (Bamforth and Singleton, 2005). The continual outflow of effluents laden with HMs and organic pollutants alters the ecological status of the affected estuary with the evolution of (i) competent microorganisms that can simultaneously reduce and detoxify HMs while metabolising the organic pollutants, (ii) HM-resistant microorganisms that perform their metabolic activity in the presence of the

* Corresponding author. Department of Microbiology, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria.
E-mail addresses: goyetibo@unilag.edu.ng, goyetibo@mail.tohoku-gakuin.ac.jp (G.O. Oyetibo).
[1] Visiting Postdoctoral Research Scientist at Biotechnology Research Common, Faculty of Engineering, Tohoku Gakuin University, Tagajo, Miyagi, 985-8537, Japan.

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metals, and (iii) microorganisms, which are inactive in the presence of HMs in the affected estuarine environment, and only perform their metabolic activities once the metals have been reduced via extracellular processes (Nakatsu et al., 2005; Oyetibo et al., 2013a). The evolved traits in microorganisms, widely borne in transposons and plasmids (mercury resistance [*mer*] operon, for example, have been extensively described as transposon-borne: Huang et al., 1999, 2002; Endo et al., 2002; Narita et al., 2002, 2004; Matsui et al., 2005, 2016; Chen et al., 2008; Chien et al., 2008, 2010), are often reported as resistance/tolerance mechanisms that are exploited in environmental biotechnological decommissioning strategies of polluted estuarine environment (modelled in Fig. 1).

2. Toxic metals and organic pollutants of importance in estuarine environment

HMs are usually those elements with a molecular weight greater than 53, a density greater than 6 g cm^{-3}, and an atomic number greater than 20 (AMAP/UNEP, 2013). In the context of this definition, arsenic (As) and selenium (Se) are not metals but regarded as metalloids and are generally lumped with toxic metals whenever ecotoxicology of metals is discussed. They belong to the group of elements described geochemically as ‘trace elements’ that occur naturally in the earth's crust. All trace elements are toxic to living organisms at excessive concentrations, but some including iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), molybdenum (Mo) and so on, at low but critical concentrations are micronutrients used in the redox processes, regulation of osmotic pressure, and also enzyme components that are essential for the normal healthy growth and reproduction by living organisms (Perales-Vela et al., 2006). The classic metal(loid)s about which there is most concern in the estuarine environment are cadmium (Cd), cobalt (Co), chromium (Cr), mercury (Hg), nickel (Ni), lead (Pb), As, Cu, Mo, Se, and Zn (ATSDR, 2008; Oyetibo et al., 2010). While some of the metals provide metabolic functions in the estuarine ecosystem, others including Hg, Pb, Cr and Cd are with no known metabolic and physiological merit to life and, therefore, tagged “bad/toxic metals” (Todorova et al., 2007). Toxic metals do not only impact the estuarine ecological balance as notable environmental stressors, they also arouse or exacerbate environmental-induced diseases including cancer, chronic lung disease, diabetes and neurodegeneration (Hansen et al., 2006; Franco et al., 2009). In unrecognised or inappropriately treated cases, HM toxicity can result in significant deformation, morbidity and mortality as reported for ‘Minamata’ and ‘Itai-itai’ diseases caused by Hg and Cd poisoning,

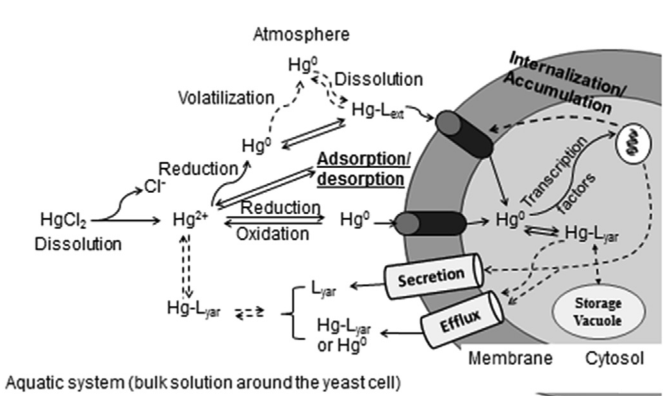

Fig. 1. Conceptual model of the biogeochemical activities of *Yarrowia* sp. on naturally-occurring and/or anthropogenically-derived mercury, as $HgCl_2$, in lagoon environment. L_{yar}, intracellular ligands; L_{ext}, extracellular ligands; Hg-L_{yar} and Hg-L_{ext} are complexation of Hg by ligands (Oyetibo et al., 2015b).

respectively (AMAP/UNEP, 2013). The bioavailable toxic metal(loid) ion inside the cell interacts with cellular proteins producing their toxicity by forming complexes that make biological molecules lose their ability to function properly via induced alterations in redox homeostasis (Hansen et al., 2006; Franco et al., 2009), and result in malfunctioning or death of the affected cells (Franco et al., 2009). Children are more susceptible to the toxic effects of HMs and are more prone to accidental exposures (ATSDR, 2008), just as dozens of children were reportedly killed in Northern Nigeria due to Pb poisoning from illegal artisanal mining of gold.

Persistent organic pollutants (POPs) are extremely stable (being resistant to environmental degradation processes) toxic organic compounds released into the estuary through various anthropogenic activities, affecting the health of estuarine ecosystems and humans (Lasserre et al., 2009; Kanissery and Sims, 2011; Sanchez-Sanchez et al., 2013). POPs are also of global concerns and remained banned in many countries because they persist in the environment, have capability of long-range transport, bioaccumulate in human and animal tissues, and biomagnify in food chains (Tchounwon et al., 2003; Sanchez-Sanchez et al., 2013). In 2004, the Stockholm Convention committed more than 90 signatory countries to phasing out or eliminating large stocks or other sources of POPs (Jepson and Law, 2016). Yet, POPs still continue to threaten the health of the estuarine environment via lithospheric run-off pollution or accidental spillage as they are currently or were in the past used as pesticides, industrial chemicals or by-products. The 12 POPs, otherwise called “dirty dozen” POPs, that the United Nations Environment Program (UNEP) classified as the most hazardous to human health and the environment are presented in Table 1. POPs exposure through diet, environmental exposure, or accidents can cause death and illnesses including disruption of the endocrine, reproductive, and immune systems; neurobehavioral disorders; and cancers possibly including breast cancer (Goff et al., 2005; ATSDR, 2008).

3. Heavy metal and persistent organic pollutants remediation techniques

The primary management goal during the remediation of a contaminated environmental-component is to obtain closure, i.e., to achieve a set of conditions that are considered environmentally acceptable and which will ensure that no future action will be required at the site (Sebai et al., 2011; Sagarkar et al., 2013). Generally, remediation technologies are classified into four categories based on the process acting on the contaminants. These include removal, separation, and destruction as processes that reduce or remove the contaminants (Oyetibo et al., 2013b, 2014, 2015a, 2015b); and containment technologies that control the migration of a contaminant to sensitive receptors without reducing or removing the contaminant. The only approaches available for remediating heavy metal-polluted estuary are to remove the metals or to convert the metals into less bioavailable forms. In case of POP pollution, degradation or process leading to structural transformation to non-toxic compound is aimed (Sagarkar et al., 2013). The conventional procedures for removing pollutants (HM ions in particular) from aqueous system include chemical precipitation, ion exchange, reverse osmosis, ultrafiltration, electrodialysis, solvent extraction (Salt et al., 1998; Singh et al., 2003).

According to Khan et al. (2004), the various pitfalls of the common pollutant removing mechanisms include:

i) Very expensive with respect to cost of procuring chemicals and energy requirements in reverse osmosis, ion exchange
ii) Formation of metal hydroxides that clog membranes in electrodialysis

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Table 1
The most hazardous POPs as classified by UNEP and their ecotoxicological properties.

Name	Toxicity	Effects	Persistence (in half-lives)
Pesticides			
Aldrin	LD_{50} of 39 mg kg^{-1}	Highly toxic to aquatic animals. It damages foetus of pregnant women.	5 years
Chlordane	LD_{50} of 39 mg kg^{-1}; estimated LD_{50} of 25–50 mg kg^{-1} of body weight in humans	Highly toxic to crustaceans, fish, and other aquatic animals. It is linked to liver, kidney, and blood disorders. It damages endocrine, cardiovascular, and reproductive systems.	1-3 years
Dichloro diphenyl trichloro ethane (DDT)	LD_{50} of 113–800 mg kg^{-1}	High levels may be linked to tremors, imparting the kidney, liver, and immune and nervous systems. Low levels may cause nausea, diarrhoea, and irritations of eye, nose and throat.	2-15 years
Dieldrin	LD_{50} of 49 mg kg^{-1}	Highly toxic to aquatic animals. It causes foetus damage to pregnant women	5 years
Endrin	LD_{50} of 43.4 mg kg^{-1}	Highly toxic to aquatic animals. It affects central nervous system and liver, causes convulsions.	12-15 years
Heptachlor	LD_{50} of 30–162 mg kg^{-1}	Impedes reproductive potentials in humans.	Up to 2 years
Hexachloro benzene (HCB)	LD_{50} of 19–245 mg kg^{-1}	Probably carcinogenic. Liver, kidney, or thyroid cancer may originate from chronic ingestion	2.7–22.9 years
Mirex	LD_{50} of 740 mg kg^{-1}	Probable human carcinogen; increases risk of miscarriage.	Up to 10 years
Toxaphene	LD_{50} of 80–293 mg kg^{-1}	Probable human carcinogen; damage to liver, lung, kidney, and nervous system; death from large doses	100 days to 12 years
Industrial chemicals or by-products			
Polychlorinated biphenyls (PCBs)	LD_{50} of 1010–4250 mg kg^{-1} day^{-1}	Probable human carcinogen; acne, rashes, other skin conditions; irritated lungs and nose.	0.91–7.25 years
Dioxins and Furans	LD_{50} of 22 μg kg^{-1}	Reasonably suspected to cause cancer; chloracne, red skin rashes; excessive body hair; changes in blood and urine that signal liver damage	Over 20 years

iii) Generation of large amount of sludge containing toxic compounds during ultrafiltration and chemical precipitation
iv) Partial removal of certain ions in ion exchange resin

4. Bioremediation of polluted estuaries

Environmental bioremediation technology is a biological process that attempt to eliminate, reduce, isolate, or stabilise a contaminant or a group of contaminants (Kanissery and Sims, 2011). Metals, unlike organic pollutants, cannot be degraded into a harmless form such as carbon dioxide and water (Lima et al., 2009; Morgante et al., 2010). A diverse group of bacteria, fungi and algae are key players in pollutant remedial processes. Microbial degradation depends not only on the presence of microbes with the appropriate degradative enzymes, but also on a wide range of environmental parameters (Sagarkar et al., 2013).

Degradation of POPs occurs via:

i) Microbial degradation or biodegradation,
ii) Chemical degradation, and
iii) Photodegradation.

Microbial degradation occurs when microorganisms in contaminated estuary use POPs as a source of carbon, cell building material, electrons, and energy; or mineralise the organic pollutants along with other sources of nutrients and energy. Cells catalyse the oxidation of organic chemicals (electron donors), causing the transfer of electrons from organic chemicals to some electron acceptors, which can be O_2 in aerobic oxidation, or NO_3^-, Mn_2, Fe_2, SO_4^{2-} (with decreasing efficiency) in anaerobic oxidation (Wang and Xie, 2012). The great versatility of microorganisms in estuarine ecosystem offers simpler, inexpensive strategies involving secretion of extracellular enzymes as catalysts, to bring about extensive modification in the structure and toxicological properties of pollutants or potential pollutants (Wang et al., 2013; Sagarkar et al., 2014).

Biodegradation can be categorised into three types that have importance in an ecosystem setting:

i) Primary biodegradation, which involves degradation to the minimum extent necessary to change the identity of the compound.
ii) Ultimate biodegradation, which entails degradation to water, CO_2, and inorganic compounds if elements other than C, H, and O are present (mineralisation)
iii) Acceptable biodegradation, which is degradation to the minimum extent necessary to remove some undesirable property of the compound, such as toxicity (biotransformation).

Although the exact mechanism for microbe adaptation to POPs is not entirely understood, microorganisms may acquire genetic material to encode the biochemical mechanisms necessary to deal with potential substrates. Consortia of microorganisms have been reportedly involved in the pesticide degradation phenomenon (Vryzas et al., 2012; Sagarkar et al., 2014). A pesticide in contaminated estuary has to move to the microbial colonies and cross the microbial cell membrane into the cell to metabolize. Some microbes produce extracellular enzymes, which are exported from the cell to predigest POPs that are poorly transformed (Sebai et al., 2011; Sagarkar et al., 2014). Once inside an organism, a POP can metabolize via internal enzyme systems. Depending on the specific pollutant, the biological degradation process may be very fast due to the presence of enzymes, or otherwise very slow for other compounds (Zhang et al., 2011; Sagarkar et al., 2014). The ability of microorganisms to degrade or modify compounds depends on their capacity to produce requisite enzymes and ideal environmental conditions for the reactions to occur. In addition, sufficient biomass and communication between the pollutants and the enzymes (intracellular or extracellular) are indispensable (Sebai et al., 2011; Zhang et al., 2011).

However, there are cases of partial (incomplete) degradation of POPs, which often leave behind intermediates that may also be toxic and lead to bioaccumulation (Sebai et al., 2011). It is therefore important to monitor the disappearance of the pollutants along with the intermediates of the degradation pathway. Sagarkar et al. (2013) used molecular tools in analysis of microbial community based on quantifications of genes involved in POP degradation pathway to provide information on catabolic potential and

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diversity involved in bioremediation of POPs. Thus, specific target genes can be monitored, quantified and correlated to degradation analysis which would help in predicting the outcome of any bioremediation strategy (Sebai et al., 2011; Zhang et al., 2011; Sagarkar et al., 2013; Wang et al., 2013).

5. Selection of technologies exploiting biodegradation

Bioremediation can be the major type of remediation occurring in a particular technology or it can be a consequence of another technology or an integral part of that technology. Some elements necessary to be considered to access remediation technologies are as follows:

i) Applicability (target contaminants)
ii) Minimum achievable concentration
iii) Clean-up time required
iv) Reliability and maintenance
v) The quality of the decontaminated matrix
vi) Residuals produced (post treatment needed for by-products)
vii) Site data, with respect to prevailing conditions
viii) Overall cost
ix) Public acceptability
x) Safety
xi) Development status
xii) Environmental impacts
xiii) Potential biological pathways to degrade/sequester a contaminant
xiv) Performance dependency on site characteristics

Selecting the most appropriate strategy to remediate an estuary can generally be guided by considering three basic principles:

i) Biochemistry, the amenability of the pollutant to biological transformation to less toxic products;
ii) Bioavailability, the accessibility of the contaminant to microorganisms
iii) Bioactivity, the opportunity for optimization of biological activity.

Some specific criteria for selecting technologies used in the treatment of POPs and metal(loid)s in estuarine matrixes are summarised in Table 2.

The main groups of the technologies employed in biodegradation, as part or all of their remedial strategies, include enhanced (engineered)-, and natural-bioremediation. Natural attenuation also known as intrinsic bioremediation describes 'hands-off' processes that rely entirely on natural processes with no human intervention to reduce/remove contaminant concentrations in the natural environment. These processes may include dilution, dispersion, volatilization, biodegradation, adsorption, and chemical reactions. Natural attenuation has been employed at sites where the potential for contaminant migration is low, or where other remedial measures are impractical. This category includes passive technologies. It relies on the capacity of microorganisms or other organisms in the system to metabolize, remove, reduce (Sebai et al., 2011), or inactivate the pollutant at the polluted site (Oyetibo et al., 2014). The normal microorganisms on the site degrade the toxic compounds without additions, modifications, or interference (Obayori et al., 2008; Vryzas et al., 2012). Natural attenuation is, or should be, a component of all remedial solutions (Ilori et al., 2007; Sagarkar et al., 2014), since only a few (if any) remediation technologies can achieve final site-specific remediation objectives like natural attenuation. Therefore, it is important to understand the basics of biochemical reactions, physical attenuation mechanisms, the regulatory basis for the technology, how natural attenuation should be applied, its advantages and disadvantages, and the evaluation process. The benefits of natural attenuation are:

i) Minimal disturbance of the site, i.e., the site is simply left to be remediated by natural processes.
ii) The operational costs are low to non-existent since it involves no human intervention. The costs associated with natural attenuation are typically related to monitoring to make sure the process is working.

The main limitation of natural attenuation is that it is slower than any other remediation. In addition, the most appropriate plants and microorganisms may not be present and/or natural environmental conditions may not be optimal to facilitate natural remediation of the contamination (Sagarkar et al., 2014). Health risks from the contaminated site may, therefore, exist for a period of time that is not acceptable from either a social or business standpoint. In that case, engineered bioremediation is applied. In the engineered bioremediation, operators take an active role in promoting or carrying out the bioremediation process. This can be either *in situ* or *ex situ*. Some processes originally designed to carry out remediation through chemical or physical means are now known to, at least partially, involve bioremediation processes. It is presently thought that the microbial activities in the volume being treated are enhanced by this process (Hsieh et al., 2009). The Installation Restoration Program Site 40 of Naval Weapons Station Seal Beach, California, for example, involved biostimulation via sodium lactate injection regimen followed by bioaugmentation with a mixed community that included *Dehalococcoides* strains to achieve efficient degradation of chlorinated solvents in the polluted system (Rahm et al., 2006).

6. Bioremediation processes

A number of bioremediation strategies, as illustrated in Fig. 2, have been developed to mitigate estuaries polluted with HMs and POPs (Mesa et al., 2015a, 2015b; de Quadros et al., 2016; Li et al., 2016). Typical examples of these estuaries were Spanish Tinto, Odiel and Piedras rivers that have been reportedly decommissioned of their contaminants using phytoremediation strategies (Mesa et al., 2015a, 2015b). The biotechnological processes projected for possible restoration of polluted estuaries include the use of whole cells and/or transcriptomes of bacteria, fungi, and plants.

6.1. Bacteria-based bioremediation

Bacteria-based bioremediation requires that competent bacterial strains function in the presence of the target contaminant, as well as other contaminants. The bacteria used for bioremediation are selected to target a specific form and oxidation state of toxic pollutants, such as reduction of soluble divalent and hexavalent HMs and/or degradation of specific POPs. The recovery of nutrient cycling mediated by soil microbes is often of major importance in HM—polluted sites (Oyetibo et al., 2010).

Bacterial approaches to pollutants' degradation involve:

i) Use of POPs as growth substrates (Vryzas et al., 2012; Oyetibo et al., 2013a, 2015b; Sagarkar et al., 2014). The efficiency of bacterial degradation thus relies on a positive feedback loop between pollutant degradation and the formation of more bacteria. However, unlike chemical catalysts, organisms require minimum substrate fluxes to persist. Below a crucial 'per cell' flux of maintenance energy, the biocatalyst concentration and its catabolic capacity decrease (Oyetibo et al., 2013a). This occurs when

Table 2
The technology selection criteria for some POPs and metal(loid)s in the estuarine environment.

Types of technology	Selection considerations	Advantages	Disadvantages
In situ			
Oxidative biodegradation	Release of contaminant from sediment-sorbed phase to aqueous phase; enzymatic biodegradation of contaminant in sediment-sorbed phase; site conditions; availability of active biopolymers;	The most cost-efficient; noninvasive; relatively passive; natural attenuation processes.	Environmental constrains; extended treatment time; monitoring difficulties
Biosorption	Redox potentials of indigenous organisms; increase microbial populations to facilitate wide surface area available for adsorption; wide pH (2–8) to remove various metal(loid)s; productions of exopolymers as contaminant ligands;	High contaminant retention capacity; low cost	Contaminant precipitates may remobilize with change in sediment pH; desorption do easily occur under high acidic condition.
Biostimulation: biosparging, bioventing etc.	Site condition with respect to physico-chemical components; ecological stoichiometry via supplies (rate and ratio) of nutrients in relation nutritional demands of the cell's innate physiology; biodiversity of indigenous microorganisms; ecophysiological status of pollutant-degrading microorganisms that provides the theoretical framework for optimizing nutrient formation.	Equipment is readily available and easy to install; short treatment times; easy to combine with other technologies.	High concentration of contaminants may be toxic to organisms; cannot always reach low cleanup limits.
Bioaugmentation Biodetoxification	Biodegradative abilities of autochthonous microorganisms; presence of factors that cause antibiosis; biodegradability of the POPs; chemical solubility; distribution of pollutants; exposure history and adaptive status of microbial degraders	The most cost-efficient; noninvasive; relatively passive; natural attenuation processes	Environmental constrains; extended treatment time; monitoring difficulties; there may be long lag phase.
Phytoextraction Rhizofiltration	Same as phytoremediation	Same as phytoremediation	Same as phytoremediation
Ex situ			
Landfarming Composting Biopiles	Same as biostimulation above in addition to phytoremediation below sometimes	Cost-efficient; low cost	Space requirements; extended treatment time; need to control abiotic loss; mass transfer problem; bioavailability limitations.
Slurry reactors Aqueous reactors	In addition to bioaugmentation considerations, toxicity of amendments and toxic concentrations of contaminants need be considered.	Rapid degradation kinetics; consistency of the process; optimized environmental parameters; enhances mass transfer; effective use of inoculants and biopolymers.	Matrices (water and sediments) require excavation; relatively high cost capital; relatively high operating cost
Phytoremediation	Climatic and geological conditions of the treatment site	Relatively low cost; easily implemented and maintained; several mechanisms for removal; no need for disposal sites; aesthetically pleasing; environmentally friendly.	Contaminants accumulated may be released again during litter fall; longer than other technologies; possible accumulation in fuel woods; may increase solubility of contaminants

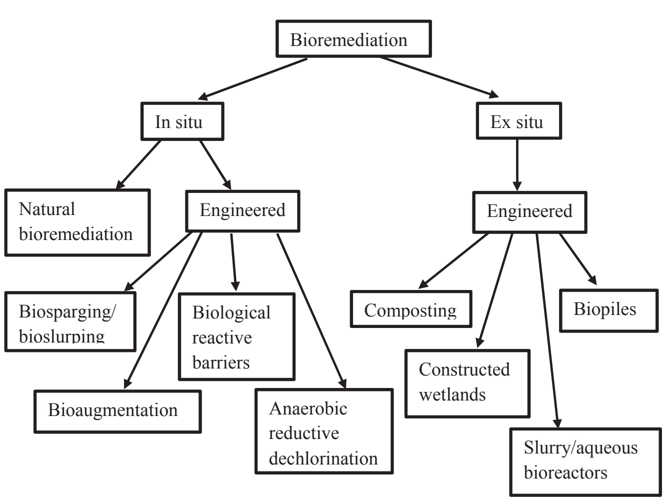

Fig. 2. Classification of bioremediation processes.

pollutant concentrations are very low (some trace chemicals in estuary), or when pollutants are poorly bioavailable (high-molecular-mass compounds), or contain very little energy (highly oxidized chemicals, such as chlorinated or nitrated compounds). Under such conditions, an organism persists only if it succeeds in reducing its maintenance requirements – usually coupled with a loss in activity (for example, entering dormancy or undergoing sporulation) – or in using other substrates along with the pollutant (Ilori et al., 2007).

ii) Use of specific biochemical pathways. Degradation pathways for new pollutants are mostly modifications or extensions of existing pathways. Alternatively, they may be newly assembled but based on existing enzymes gained, for example, by genetic transfer between species. However, degradative pathways will only evolve and radiate when there is a selective benefit for their encoding bacterium. Specific pathways are thus unlikely to exist for environmental chemicals that always occur at concentrations below those required for multiplication or for chemicals containing rare or novel structural elements (Banitz et al., 2011).

6.2. Fungi-based bioremediation

Bioremediation by fungi has not been an unfettered success story, as it tends to disregard the ecological demands of fungi and often uses ecologically displaced organisms in competition with bacteria more suited to the polluted environment. As such, the potential use for fungi in bioremediation of polluted estuary has not received the attention merited by the extensive metabolic

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capabilities of these organisms. In environmental biotechnology, fungal degradation is considered for pollutant classes that are inefficiently degraded by bacteria. For example, bacteria might be disadvantaged if substrates contain rare structural elements, have a low bioavailability, contain little energy or occur permanently at minute concentrations. The problem of concentration applies to a range of structurally quite different chemicals that combine low environmental concentrations with worryingly high biological activities, traits that are attributable to their design as human and veterinary drugs. However, there are other characteristics that could make the use of fungi more attractive irrespective of whether the bacterial option is available. These include:

i) Long-range transport, whereby essential factors like nutrients, water, the pollutants itself etc., are translocated within the fungi hyphae or between different parts of their mycelium for the transformation or detoxification of environmental chemicals to occur (Allen et al., 2003). Resource translocation includes the recycling of hyphal biomass located in substrate depleted regions for the benefit of exploration for food in other regions. Fungi have also been found to stimulate POPs degradation by bacteria in soil environments in which the active movement of bacteria to pollutant reservoirs is limited by physical barriers such as air-filled pores or dense aggregates. Owing to their wedge-shaped, hydrophobic tips, growing fungal hyphae penetrate air-water interfaces and soil aggregates. The surfaces of the resulting mycelia have been found to be surrounded by water films, representing continuous pathways for motile bacteria. Random and chemotactic swimming of bacteria along fungal mycelia have been found to facilitate bacterial degradation of heterogeneously distributed chemicals (Wick et al., 2007; Banitz et al., 2011).

ii) Catabolism of POPs.

iii) Remediation of metal(loid)s. Fungi interact with metal(loid)s in various ways, including:
   a) Metal(loid) mobilization resulting from the production and excretion of organic acids (for example, citrate and oxalate), which increase metal(loid) solubility through acidification of the mycosphere and provision of metal-complexing structures (Gadd, 1999);
   b) Extra-hyphal immobilization occurs through the formation of secondary minerals (Sayer et al., 1999; Oyetibo et al., 2016), biosorption to cell wall constituents such as chitin and chitosan (Volesky and Holan, 1995; Gadd, 2009; Oyetibo et al., 2015a), complexation by metal-sorbing glycoproteins excreted by arbuscular mycorrhizal fungi (Gonzalez-Chavez et al., 2004);
   c) Intracellular metal immobilization involves storage in vacuoles and complexation by cytoplasmic metallothioneins (Mehra and Winge, 1991; Oyetibo et al., submitted but undergoing peer-review);
   d) Metal transformations such as reactions involving organometals like methylations (Barkay and Wagner-Dobler, 2005), and redox reactions frequently result in metal volatilization (Oyetibo et al., 2015b);
   e) Streams of cytoplasmic vesicles and vacuoles along fungal hyphae may translocate metals to other parts of the mycelium and to the plant symbionts of the fungi (Gray, 1998; Darrah et al., 2006).

6.3. Phytoremediation

Certain plants located in contaminated areas can facilitate uptake and/or degradation of pollutants in a process termed phytoremediation. It is an innovative and progressive technology comprising several different techniques that utilise vegetation, its related enzymes, and other complex processes to clean-up polluted systems. Collectively, these processes are able to isolate, destroy, transport, and remove organic and inorganic pollutants from contaminated media. Mechanisms of phytoremediation that take place outside, inside, or through plant systems, which are adoptable in decommissioning contaminated estuaries are illustrated in Fig. 3. Rhizodegradation and phytodegradation are most effective with organic contaminants, while phytoextraction is best when HMs and POPs co-exists as contaminants (Huang et al., 2015). Phytoremediation involves numerous biological, chemical and physical processes such as adsorption, accumulation, translocation and transformation, which can drastically affect the surrounding environment (Payne et al., 2013; Huang et al., 2015). Phytoremediation mechanisms generally include i) phytodegradation (phytotransformation), ii) phytoextraction (phytoaccumulation), iii) phytostabilization, iv) rhizodegradation, v) rhizofiltration, vi) phytovolatilization, and vii) hydraulic control.

6.4. Biological exopolymer-based bioremediation

Biological exopolymer, generally referred to as extracellular polymeric substances (EPS), is a complex biopolymer that mainly composed of polysaccharide, protein, humic substances, uronic acid, nucleic acid and lipids (Guibaud et al., 2005; Comte et al., 2008; Oyetibo et al., 2016). EPS are distinguished as homopolymeric when their chemical structures are only sugars, or heteropolymeric when they are made up of a number of monosaccharides and non-carbohydrate substituents with various linkage types. While the homopolymeric EPS are neutral, majority of the heteropolymeric EPS are polyanionic due to the presence of either uronic acids or ketal-linked pyruvate, or hydrophobic amino acids, or inorganic residues, like phosphate, may equally confer polyanionic status. Hydrophobic and electrostatic interactions, via negatively charged and hydrophobic amino acids, link proteins to the EPS

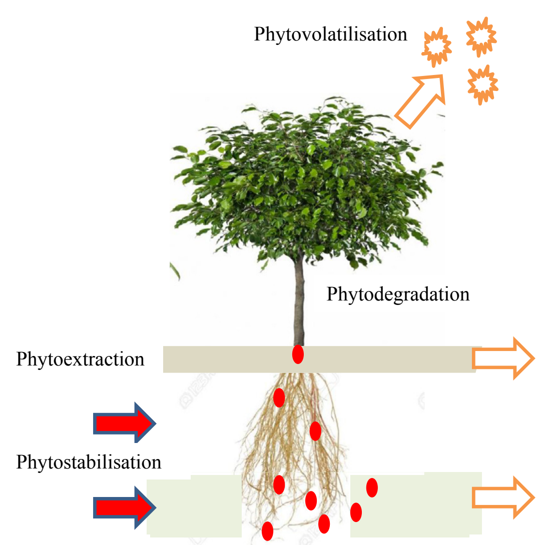

Fig. 3. Hypothetical strategies to phytoremediate pollutants in estuarine sediments after excavation of polluted sediments to large expanse of treatment site. The pollutants (red circles) can be stabilized in excavated sediment matrix, sequestered into the plant, degraded or volatilized due to enzymatic modifications to originate innocuous forms of the contaminants (yellow explosions). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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matrix (Ras et al., 2008). The production of EPS by microorganisms is intimately linked to adherence processes that facilitate cellular adhesion to surfaces, formation of cell-to-cell aggregation in form of flocs, biofilm, sludges and biogranules that are useful in the biosorption and bio-sequestration of metal(loid) ions (Guibaud et al., 2005, 2008; Comte et al., 2008; Panwichian et al., 2011; Wang and Xie, 2012; Oyetibo et al., 2016), and mineralisation of POPs (Jia et al., 2011; Bao et al., 2016). EPS produced by many microorganisms are innately for cellular protection from hostile environments (Jia et al., 2011; Miajlovic and Smith, 2014), but adopted in biotechnology on basis of their polyanionic nature for various bioremediation strategies of milieus polluted with HMs and POPs (Wilen et al., 2003; Lamelas et al., 2006; Comte et al., 2008; Jia et al., 2011; Bao et al., 2016; Oyetibo et al., 2016). The use of isolated biopolymers in bioremediation phenomenon appears to be more economical, effective and safe alternative to chemical methods. As a non-living bioremediation agent, EPS is preferred for its easy availability in the treatment process and avoidance of pathogenicity issues of the organisms concerned (Gavrilescu, 2004).

7. Types of bioremediation techniques applicable in the estuarine environment

Considering the dynamic nature of estuarine water, *in situ* techniques may scarcely be applicable during bioremediation campaign of polluted estuarine system. Excavation of the sediments and water to a large expanse of land (many hectares) will be necessary to actualise effective decommissioning of the pollutants and afterwards, the treated media would be released back to the estuary. A case study is the ‘Minamata Bay Pollution Prevention Project’ in Japan that saw the deepest part of Minamata Bay (approx.. 580,000 m^2), where about 780,000 m^2 of Hg-rich sedimentary sludge was dredged with a cutter-less pump ship and poured into a reclaimed land area.

The treatment processes for modified *in situ* remediation methods applicable to estuarine environment include:

i) Biodegradation, involving delivery of oxygen to the sediment and may require the addition of other nutrients and/or co-metabolites that are circulated through the contaminated zone to provide mixing and intimate contact between the oxygen, nutrients, contaminant, and microorganisms. Generally, this technique includes conditions such as the infiltration of water-containing nutrients and oxygen or other electron acceptors for groundwater treatment. Enhancement of atrazine degradation by indigenous bacteria has been demonstrated by applying appropriate and limiting nutrient amendments to soils (Qui et al., 2009). A single inoculation with the *Pseudomonas* sp. ADP was found not sufficient for clean-up of highly polluted medium (100 mg/kg) (Newcombe and Crowley, 1999). Repeated inoculations of the strain ADP combined with citrate bio-stimulation were necessary for rapid dissipation of atrazine (Lima et al., 2009). Silva et al. (2004) also reported that inoculations of strain ADP together with citrate or succinate bio-stimulation markedly increase ADP cell survival and atrazine mineralisation in matrix contaminated with high concentrations of atrazine.

ii) Bioaugmentation involves the addition of microorganisms indigenously or exogenously to the contaminated sites (Krutz et al., 2010; Sagarkar et al., 2013). It has been proposed as an effective and low-cost bioremediation technology for complete removal of POPs (Wang and Xie, 2012; Wang et al., 2013; Sagarkar et al., 2013, 2014), and to minimise their dispersion to non-agricultural environments (Morgante et al., 2010). Two factors limit the use of added microbial cultures in a land treatment unit: a) Nonindigenous cultures rarely compete well enough with an indigenous population to develop and sustain useful population levels, and b) Most systems with long-term exposure to biodegradable pollutants have indigenous microorganisms that are effective degraders if the sediment treatment unit is well managed. Although poor survival of the inoculated microorganisms may hinder the success of bioaugmentation (Silva et al., 2004), this strategy has been adjudged in some quarters as the fastest process for the removal of pollutants from polluted sites. For example, 92% atrazine was reportedly removed in the first week of bio-augmenting a contaminated site as compared to 52% in the bio-stimulation process and 16% under natural attenuation (Sagarkar et al., 2013, 2014). Also, bioaugmentation of solid matrix with *Bacillus* spp has been reported to reduce potentially bioavailable Cd up to a factor of 14 (Jezequel et al., 2005).

iii) Precipitation involves the use of whole microbial cells and/or biomolecules to precipitate, adsorb to surfaces (biosorption) or accumulate in organic matter, in tiny pores of solid matrices, or onto functional groups of microbial cells leading to a decline in pollutant bioavailability (Darrah et al., 2006; Oyetibo et al., 2014, 2015a, 2015b, 2016). Such accumulation often occurs in inhospitable, toxic environments that lack the nutrients, water or appropriate electron acceptors that would be needed to support the growth of microorganisms capable of remediation. During biofilm colonisation on a surface or interface, bacteria are encased in an extracellular polymeric substance (EPS) or “slime”, which allows them not only to share nutrients, but also to precipitate pollutants and remain protected from environmental stresses as illustrated in Fig. 4 (Oyetibo et al., 2016).

iv) Land farming is a simple technique in which contaminated sediment is excavated and spread over a prepared bed and periodically tilled until pollutants are degraded. It also involves mulching (Kiikkila et al., 2001), which has been found to immobilize toxic metals. The goal is to stimulate indigenous bio-degradative microorganisms and facilitate their aerobic degradation of contaminants (de Quadros et al., 2016). In general, the practice is limited to the treatment of superficial 10–35 cm of sediment. Since land farming has the potential to reduce monitoring and maintenance costs, as well as clean-up liabilities, it has received much attention as a disposal alternative. In most cases, land farming involves applying various phytoremediation strategies by cultivating hyper-resistant plants on the excavated sediments, with or without microbial association, to remove POPs and HMs from the sediments. With *Pteris vittata*, Huang et al. (2015) demonstrated arsenate removal from aqueous system with 80% of the arsenate accumulated in the fern fronds, resulting at decline in arsenate concentration from 1000 μg/l to undetectable level (<0.1 μg/l). Also, plant roots colonised by microorganisms as planktonic cells or biofilms have been reportedly enhanced absorption and microbial degradation of contaminants in the rhizosphere (Passatore et al., 2014; Huang et al., 2015; de Quadros et al., 2016; Sriprapat and Thiravetyan, 2016). Hyperaccumulator-endophytes was reported to improve phyto-stabilisation efficiency of a system because they were able to detoxify pollutants, alter toxicant solubility and facilitate plant growth upon reducing root to shoot translocation of the toxicants (Ma et al., 2016a, 2016b; Wang et al., 2016). Bioaugmentation of *Spartina maritima* (cordgrass) with selected bacterial consortium was demonstrated to enhance plant adaptation and HMs rhizo-

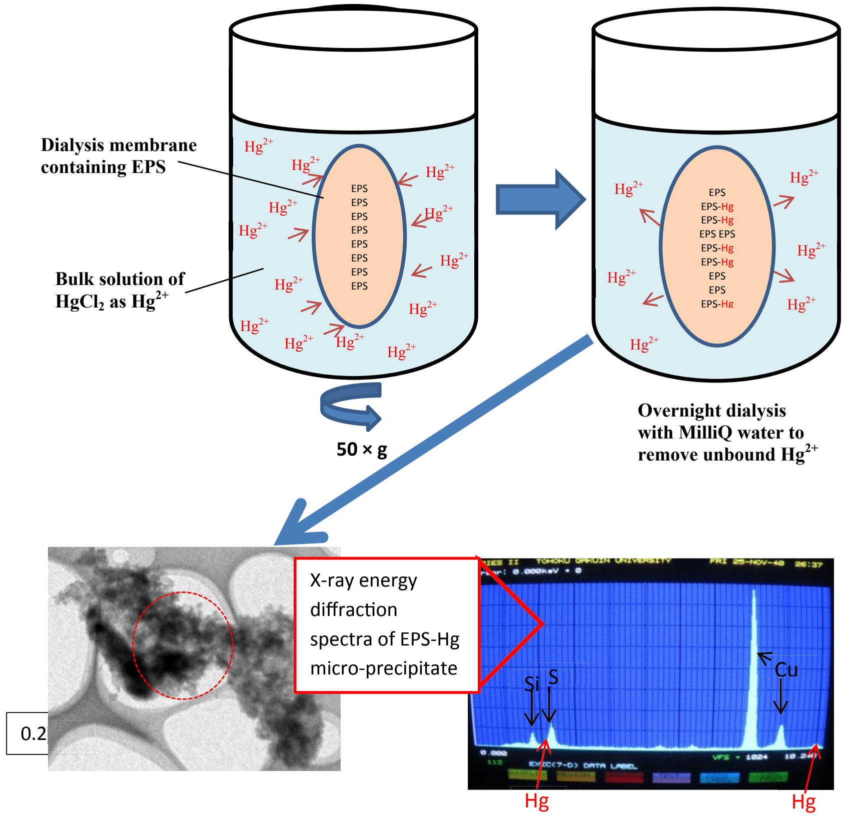

Fig. 4. Extracellular precipitation of pollutants as demonstrated with micro-precipitation of mercuric ions by EPS produced by *Yarrowia* spp. whereby nanoparticles of Hg as HgS were presumed from Hg-EPS complexation (Oyetibo et al., 2016).

accumulation during marsh restoration program (Mesa et al., 2015a, 2015b).

The treatment processes for *ex situ* remediation methods include:

i) Slurry phase biological treatment usually consists of a series of large tanks or bioreactor vessels in which water, nutrients, and other additives are mixed with excavated sediments to produce aqueous slurry under controlled environmental conditions (detail reviewed by Robles-Gonzalez et al., 2008). Combination of bioaugmentation and biostimulation in slurry phase bioremediation protocol has been reported to significantly mitigate pollutants than the conventional slurry bioreactors (Mansur et al., 2016). Some of the basic bioreactors useful for this purpose are shown in Fig. 5 (particularly Fig. 5a, b, and f).

ii) Composting is a technique that involves combining contaminated sediment with non-hazardous organic amendments such as manure or agricultural wastes (Vangronsveld et al., 1996). The presence of these organic materials supports the development of a rich microbial population and an elevated temperature characteristic of composting. Composting can be performed using windrows, aerated static piles, or specially designed composting vessels. It is proposed to serve as remedy to treat the sludge generated from bioreactors provided factors such as nutrients, pH, moisture, aeration and temperature within the compost pile prevail to promote degradation of POPs (Prakash et al., 2015), as well as lessening the bioavailability of metal(loid)s (Barker and Bryson, 2002; Megharaj et al., 2011; Prakash et al., 2015). The contained systems typically allow treatment to be completed in less time than the windrow or aerated pile by providing better control of composting conditions. Rapid treatment time is offset by the high initial cost of the composting reactor.

iii) Biopiles are a hybrid of land farming and composting. It is designed to provide optimum temperature, moisture content, aeration, and nutrient conditions to promote rapid biodegradation. In most cases, degradation is achieved by indigenous microorganisms. Essentially, engineered cells are constructed as aerated composted piles. They are a refined version of land farming typically used for treatment of surface contamination with organic compounds, which tends to control physical losses of the contaminants by leaching and volatilization.

iv) Bioreactors are biochemical-processing systems designed to degrade contaminants in pumped estuarine water or excavated sediments using microorganisms. Bioreactor treatment may be performed using microorganisms growing in suspension in the fluid (Fig. 6) or attached on a solid growth support medium as a batch or continuous system (Chen et al., 1998; Costa and Tavares, 2016; Li et al., 2016; Zhang et al., 2016). In suspended growth systems, such as fluidized beds or sequencing batch reactors, contaminated estuarine water is circulated in an aeration basin where a microbial population aerobically degrades organic matter and produces carbon dioxide, water, and biomass. Because of its high performance and controllability, bioreactor system for estuarine bioremediation are not enough economical but more reliable than composting and biopile systems. Schematic of all basic bioreactors variously adopted in decommissioning polluted water and sediments of estuarine system are shown

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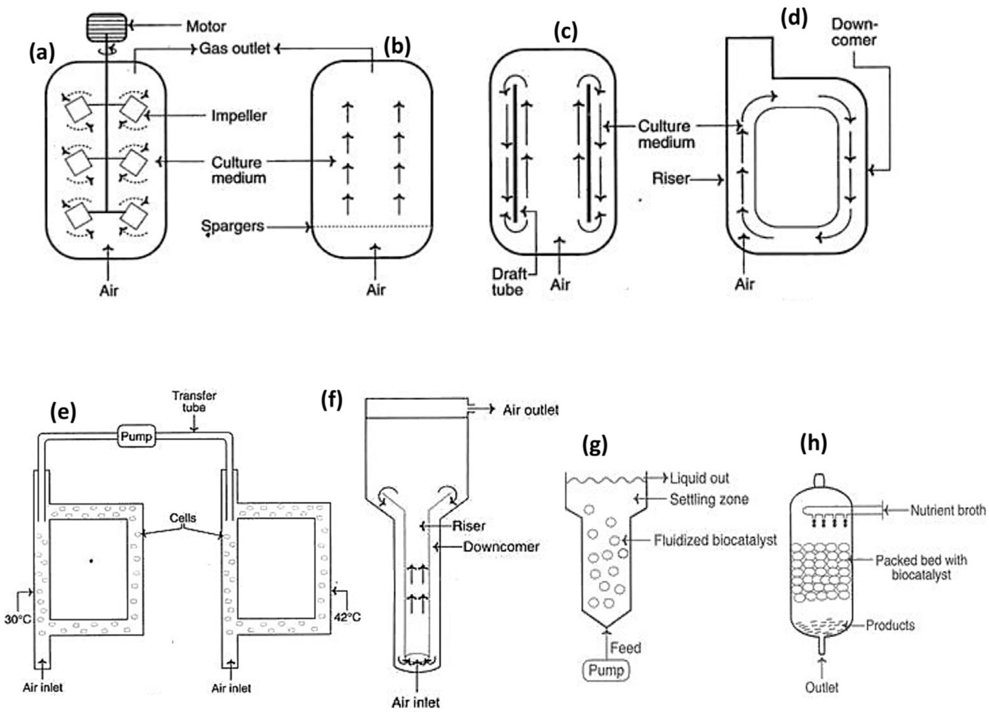

Fig. 5. Sketches of some types of bioreactors that is adoptable for *ad situ* and *ex situ* bioremediation techniques of estuarine water and sediments. These include (a) Continuous stirred tank bioreactor; (b) Bubble column bioreactor; (c) Internal-loop airlift bioreactor; (d) External-loop airlift bioreactor; (e) Two-stage airlift bioreactor; (f) Tower bioreactor; (g) Fluidized bed bioreactor; (h) Packed bed bioreactor.

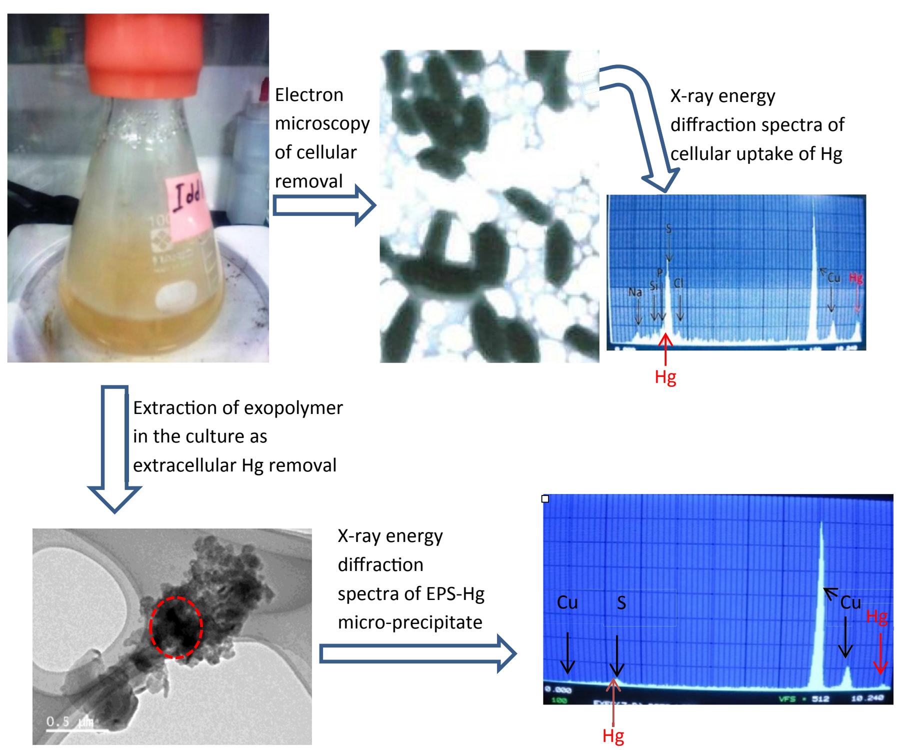

Fig. 6. Microbial removal of toxic metals from estuarine water using bioreactor. This is based on bioaccumulation and adsorption of bioavailable mercuric ions in liquid medium (manuscript submitted).

in Fig. 5, and the synopsis of their operational mechanism are displayed in Table 3 (Kantarci et al., 2005; Grace et al., 2008; Costa and Tavares, 2016; Denis et al., 2016; Xie et al., 2016; Zhang et al., 2016). The biomass is settled out in a clarifier, then either recycled back to the aeration basin or disposed of as sludge.

Table 3
General operational designs of bioreactors and their applications.

Type	Mode of operation	Applications	Reference
Stirred tank bioreactor	Air is added to the culture medium under pressure through sparger, which along with impellers enables better gas distribution system throughout the vessel. The bubbles generated by sparger are broken down to smaller ones by impellers and dispersed in the medium, enabling creation of a uniform and homogeneous environment throughout the bioreactor.	Water and sediments	Zhang et al., 2016
Bubble column bioreactor	Air is introduced at the base of the column through perforated pipes or plates, or metal micro-porous spargers	Water and sediments	Kantarci et al., 2005
Airlift bioreactor	The medium in the vessel is divided into two interconnected zones referred to a riser, where the air is pumped; and the other zone that receives no gas called the down comer. There are Internal-loop type, which has a single container with a central draft tube that creates interior liquid circulation channels; External loop type that possesses an external loop so that the liquid circulates through separate independent channels; Two-stage type involving growing cells from one bioreactor at a temperature and pumped afterwards into another bioreactor maintained at different temperature; and Tower type based on hydrostatic pressure generated at the bottom of the reactor to increase oxygen solubility in the medium	Water	Denis et al., 2016
Fluidized bed bioreactor	This involves allowing polluted estuarine water to flow through fluid suspended competent biocatalysts such as immobilized biomolecules, immobilized cells, and microbial flocs.	Water	Grace et al., 2008
Packed bed bioreactor	This comprises a bed of solid particles, with biocatalysts on or within the matrix of solids, packed in a column. The solids used may be porous or non-porous gels, and they may be compressible or rigid in nature. The flow of the fluid can be upward or downward.	Water	Costa and Tavares, 2016; Xie et al., 2016

Recent advancement has precluded the clarification stage in bioreactor system with the incorporation of a membrane process like microfiltration or ultrafiltration with a suspended growth bioreactor (Waheed et al., 2016). This is collectively called membrane bioreactor. Moreover, in overcoming the major set-back with use of stirred tank and bubble column bioreactors, the use of magnetotactic bacteria have been proposed recently, whereby magnetotatic biocatalysts would be successfully recovered from the bioreactors after bioremediation via an external magnetic field (Tanaka et al., 2016). In attached growth systems, such as up-flow fixed film bioreactors, rotating biological contactors (RBCs), and trickling filters, microorganisms are grown as a biofilm on a solid growth support matrix and water contaminants are degraded as they diffuse into the biofilm (Costa and Tavares, 2016; Li et al., 2016). Production of signal molecules like *N*-acyl homoserine lactones and dominance of quorum sensing strains in fluidized- and packed-bed bioreactors may be a limitation leading to microbial overload, or membrane biofouling and retardation of filtration performance in membrane bioreactor (Waheed et al., 2016).

Support media include solids that have a large surface area for bacterial attachment. The support matrixes extensively used in bioreactor design can be an adsorptive medium, such as activated carbon and silica gel (Xie et al., 2016) that can adsorb contaminants and slowly releases them to the microorganisms for degradation; plastic or ceramic packing, polyurethane foam (Li et al., 2016); and even clays (vermiculite), sands and gravel (Costa and Tavares, 2016). An efficient mitigation of matrix containing toxic POPs and HMs using attached growth systems have been reported (Costa and Tavares, 2016; Li et al., 2016; Xie et al., 2016). The microbial population in the reactor may be derived from natural selection, enrichment, the contaminated media, or an inoculum of organisms with a specific contaminant-degrading potential (Robles-Gonzalez et al., 2008; Costa and Tavares, 2016; Denis et al., 2016; Li et al., 2016; Mansur et al., 2016; Xie et al., 2016; Zhang et al., 2016).

Combinations of biostimulation strategies like efficient inflow oxygen and nutrients, application of intermittent operational mode, and the gradual cut of external carbon source do reportedly enhancing performance of bioreactors (Li et al., 2016). Further, a valuable tool in the design and scale-up of bioreactors for bioremediation of estuarine water and sediments is the mode by which air is added to the bioreactors as reported for airlift bioreactors (Denis et al., 2016).

8. Conclusions and future perspectives

Estuarine environment is vital to ocean health, serving as buffering zone where potential hazards of the ocean are eliminated. Due to its proximity to anthropogenic activities, toxic metals and POPs often contaminate the environment beyond levels the system could cope with and thereby provide a channel to ocean pollution. Bioremediation provides green technique for cleaning up estuarine pollution by enhancing the same bioprocesses that occur naturally in the ecosystem. This involves the use of bacteria, fungi and plants along with their metabolites in various strategies *in situ* or *ex situ* upon excavation. As such, this technology uses relatively low cost, low-technology techniques that generally have a high public acceptance. Research is needed to develop and engineer bioremediation technologies harnessing competent cells, nanomaterials, omics, biopolymers and proteins that are appropriated for estuaries contaminated with complex mixtures of POPs and metal(loid)s that are not evenly dispersed in the environment. New frontiers into evaluating the performance of bioremediation in estuarine milieus are proposed for future endeavours since bioremediation success is currently difficult to be evaluated, being often highly specific and there are no acceptable endpoints for the biotechnology.

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