Biostimulation and Bioaugmentation: An Alternative Strategy for Bioremediation of Ground Water Contaminated Mixed Landfill Leachate and Sea Water in Low Income ASEAN Countries

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Abstract

The occurrence of groundwater pollution in some parts of Association of Southeast Asian Nations (ASEAN) countries has been studied in the last two decades, and it has been found that the groundwater in these regions is in a critical state owing to contamination. Owing to financial constraints and a lack of available land, coastal areas and salt marshes, which generally have relatively little direct economic value, are often converted into waste disposal sites. Many landfills are not properly constructed. Consequently, leachate flows may contaminate the groundwater. Landfill leachate contains complex pollutants, which can lead to difficulties in groundwater remediation. In some cases, it was found that groundwater had been contaminated by a landfill leachate–seawater mix. Numerous

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studies have reported that the remediation of contaminated groundwater could be carried out on site in several ways, such as augmentation and biostimulation. Conceptually, both strategies could be applied and widely accepted as remediation technologies. A proper understanding of bioaugmentation and biostimulation protocols is key. Added nutrients and specific compounds such as osmolytes are required to protect microbes from osmotic stress. Some researchers have reported that various low-molecular-weight organic compounds such as amino acids, quaternary ammonium, and glycine betaine could function as osmoregulatory compounds. Screening of microbes for augmentation and monitoring the fate of a microbial community during such processes are very important and can be done using laboratory assays (microcosm study) and by targeting functional genes or other molecular microbial techniques.

Keywords
Bioaugmentation · Biostimulation bioremediation · Leachate · Osmolyte · Functional gene

Introduction

More than two billion people in low- and lower-middle-income Association of Southeast Asian Nations (ASEAN) countries depend on groundwater for daily needs such as drinking water supply, agriculture, and industry. Thus, groundwater is an essential source of freshwater for economic, social, and environmental benefits. However, because of environmental degradation, urbanization, rapid expansion, and exploitation may pose several problems, such as human health risks and clean water resource availability (Ha et al. 2014).

The occurrence of groundwater pollution in some parts of ASEAN countries has been studied over the last two decades, and groundwater in these regions has been found to be in a critical state owing to contamination (Shivakoti 1998; Hara 2006; Abbaspour 2011). In general, sources of groundwater contamination can be grouped into two main categories: (a) naturally occurring pollutants and (b) anthropogenic pollutants. Natural occurring pollutants refer to the alteration and deposits of elements, including salts, arsenic, fluoride, chromium, and cadmium, that exceed international or national standards for drinking water (USEPA 2006). For instance, it has been reported that groundwater in some parts of Bangladesh, Vietnam, Pakistan, Nepal, India, Vietnam, and Thailand contains arsenic and fluorine (Hara 2006; Islam et al. 2004; Jindal and Ratanamalaya 2003). Further, more than 35 million people in Bangladesh consumed drinking water from groundwater contaminated with arsenic (Islam et al. 2004). Arsenic occurs naturally in sedimentary and volcanic rocks and is often found in sulfide forms such as realgar (Selvin et al. 2002). In addition, arsenic is present in the crystalline structure of many sulfide minerals as a substitute for sulfur (Smedley and Kinniburgh 2002). On the other hand, seawater intrusion into groundwater is a source of natural occurring pollutants. Tole (1997) reported that coastal groundwater resources are in very critical danger of contamination by seawater. Rapid extraction of groundwater near shorelines will cause groundwater levels to drop and allows seawater to flow into groundwater. The rise of seawater levels and natural disasters like tsunamis may increase the occurrence of seawater intrusion (Kontar 2007). Anthropogenic pollutants refer to waste from human activities in agriculture, industry, and urban areas. Such waste (residual) contains hazardous compounds and may affect the quality of groundwater resources (Hossain et al. 2014).

Because of financial constraints and a lack of available land, coastal areas and salt marshes, which generally have relatively little direct economic value, are often converted to waste disposal sites (Hoornweg et al. 1999). In many Third World countries, landfills are been located in coastal areas, where they can become polluted (Khoury et al. 2000; Olobaniyi and Owoyemi 2006). For instance, groundwater surrounding the Keputih landfills in Surabaya-Indonesia was contaminated by leachate and affected by seawater intrusion (Rachmansyah 2001; Mangimbulude et al. 2016).

This chapter discusses the occurrence of groundwater pollution caused by landfill leachate–seawater mixtures in coastal areas in some parts of low- and lower-middle-income ASEAN countries and considers an alternative strategy for bioremediation.

Landfill Leachate–Contaminated Groundwater

Final waste disposal is a part of urban waste management, which is widely practiced around the world, especially in developing countries, because it is cheap and easy to do. However, in many cases, final disposal can be a threat to groundwater resources if not properly designed and managed. Visvanathan et al. (2005) reported that more than 90% of all landfills in South and Southeast Asia are nonengineered disposal facilities. This creates considerable health, safety, and environmental problems such as soil and aquifer pollution (UNEP 2004; Chofqi et al. 2004). Hence, in many cases groundwater-related problems in coastal areas of some parts of Southeast Asia are due to unregulated final waste disposal (landfill). This leads to landfills becoming contaminated with a dark-brown liquid called leachate. It is generated as a consequence of water contact with solid waste and due to decomposition processes of solid wastes in landfills. Leachate may percolate into the soil and eventually reach groundwater. Generally, leachate contains a variety of chemical substances (dissolved organic matter, inorganic compounds, heavy metals, and XOC compounds). To date, more than 1000 organic chemicals have been identified in groundwater contaminated by landfill leachate (Christensen et al. 2001). Those chemicals can be categorized into four groups: (a) aromatic hydrocarbons, (b) halogenated hydrocarbons, (c) phenols, and (d) pesticides. Table 1 shows the organic chemicals observed in groundwater. A detailed discussion of organic compounds in groundwater contaminated by landfill leachate can be found in Cozzarelli et al. (2000), Christensen et al. (1994a, 1994b, 2001), Bjerg et al. (2003), and Li et al. (2015).

A simple parameter often used to determine the presence of organic matter in contaminated groundwater is biochemical oxygen demand (BOD) or chemical oxygen demand (COD). By definition BOD is refers to the amount of oxygen required by microbes to break down the organic matter in a water of sample, while

Table 1 Observed selected organic pollutants in contaminated groundwater

Compounds
Aromatic hydrocarbon
Benzene
Toluene
Ethylbenzene
Xylene
Naphthalene
Halogenated hydrocarbon
Chlorobenzene
Tetrachloroethylene
Chloroethane
Chloroform
1,1-Dichloroethane
1,1,1-Trichloroethane
Trichloroethylene
Phenols
Phenol
Cresol
Chlorophenol
Penta chlorophenol
Nitrophenol
Pesticides
2-Hydroxybiphenyl
Benzamide
Furan
Atrazine

Source: Christensen et al. (2001) and Bjerg et al. (2003)

COD is refers to the amount of oxygen required by chemical (Potassium dicromate) to oxidize organic matter present in sample of water (Tchobanoglous and Burton 1991; APHA 1998). A BOD value of 1 mg/L indicates the presence of oxidizable contaminants or water status of high quality. On the other hand, high BOD values (5–10 mg/L) indicate the presence high amounts of organic contaminants or a water status of low quality (Kim 2005). The COD value also indicates the presence of organic contaminants. A COD value of groundwater greater than 7.5 mg/L is considered to indicate water of poor quality (Esa 1983).

Intrusion of Seawater into Contaminated Groundwater

A groundwater-related problem in coastal areas of some parts of ASEAN countries is the intrusion of seawater. Nevertheless, the main groundwater issue in coastal area basically has to do with landfill leachate and seawater intrusion simultaneously.

Table 2 Categories of groundwater contaminated due to seawater intrusion

SR value	Category
(<0.5)	Good quality
(0.5–1.3)	Slightly contaminated
(1.3–2.8)	Moderately contaminated
(2.8–6.6)	Injuriously contaminated
(6.6–15.5)	Highly contaminated

As mentioned, landfill leachate–contaminated groundwater is indicated by the presence of organic compounds and contamination by seawater intrusion is indicated by elevated levels of concentration of several major ions such as Cl^-, Na^+, and SO_4^{2-} (Ekhmaj et al. 2014; El Moujabber et al. 2006). A commonly used parameter to determine the occurrence of seawater intrusion is the Simpson ratio (SR) as described by Todd (1959). This ratio can be calculated using the following equation:

$$\mathrm{SR} = \left(\mathrm{Cl}^-\right)/\left(\mathrm{HCO_3}^- \pm \mathrm{CO_3}^{2-}\right). \tag{1}$$

Todd (1959) suggested, based on Eq. 1., that contamination of water due to seawater intrusion can be classified into five categories (Table 2).

Lee and Song (2007) reported that, besides the Simpson ratio, another ratio that includes HCO_3/Cl, Na/Ca, Ca/Cl, Mg/Cl, and Ca/SO_4 would be useful to determine seawater intrusion, which they demonstrated when they studied the implications of seawater intrusion on groundwater chemistry in a western coastal aquifer of Buan, Korea.

The occurrence of seawater intrusion into groundwater contaminated by landfill leachate using SR values remains critical. It is hard to distinguish between present saline water from seawater and contaminated groundwater-landfill leachate. However, this chapter does not discuss which is the proper method to use to determine the occurrence of seawater intrusion. The important thing to consider is that seawater intrusion may influence temporal hydrochemistry processes and may result in elevated saline groundwater.

Groundwater Management Strategies

Landfills produce leachate over long periods, even 30 years postclosure (Kjeldsen et al. 2002).Therefore, organic compounds from landfill leachate are persistent in groundwater, while groundwater in coastal areas is vulnerable to becoming mixed with seawater owing to intrusion. Groundwater contaminated by a landfill leachate–seawater mixture undergoes more complex hydrochemical processes. This condition creates difficulties for remediation.

The critical issue of groundwater contaminated by landfill leachate–seawater mixture in coastal areas of ASEAN countries has received serious attention from governments in the past decade. This could explain the implementation of several strategic policies and regulations in connection with groundwater resources so as to protect groundwater and even remediate contaminated groundwater (WEPA 2012). Groundwater quality management therefore involves the maintenance of the fitness for use of water resources on a sustained basis by achieving a balance between socioeconomic development and environmental protection (Abbaspour 2011).

A basic question in this connection concerns the proper technology to use in low-income ASEAN countries. This and other questions will be discussed in the following sections.

Bioremediation as a Technology for Contaminated Groundwater Remediation

Cleaning up contaminated groundwater is part of groundwater management and policy in order to provide sustainable clean water for human activities. The National Research Council (2000) has reviewed engineered systems like a conventional pump and treatment system for groundwater restoration at 77 sites and concluded that engineered systems show promise but remain unproven for the wide range of contaminants and geologic settings of concern. Pumps and treatment systems for groundwater restoration have been used in the USA and Europe. This method is resource intensive and expensive. In the context of low- and lower-middle-income countries with limited skilled human resources and financial constraints, low-cost, effective technology is required.

Nowadays, bioremediation is used widely as a technology and strategy for environmental remediation. By definition, bioremediation is the use of living organisms, primarily microorganisms (microbes), to degrade environmental contaminants into less toxic forms (Mary Kenza 2011). According to EPA (2013), bioremediation is an engineered technology that modifies environmental conditions (physical, chemical, biochemical, microbiological) to encourage microorganisms to destroy or detoxify organic and inorganic contaminants in the environment. Many studies have reported on the use of bioremediation at a number of sites worldwide, including Europe and the USA, with varying degrees of success (Sims et al. 1992; EPA 2013; Alvares and Illman 2005). Some researchers assert that bioremediation is effective at restoring polluted environments in an eco-friendly way and at very low cost (Thompson et al. 2005; M'rassi et al. 2015; Stroo 2010; Azubuike et al. 2016). Referring to the foregoing definitions, it is clear that microorganisms are key players in all steps of bioremediation. A better understanding of how microorganisms function is required for a proper implementation of protocols.

Principles of Bioremediation

Microbes (archaea and bacteria) are unicellular microscopic organisms, as varied and diverse as the kinds of environments on Earth (Capelle 1993). In nature, microbial populations interact with other species' populations as a microbial

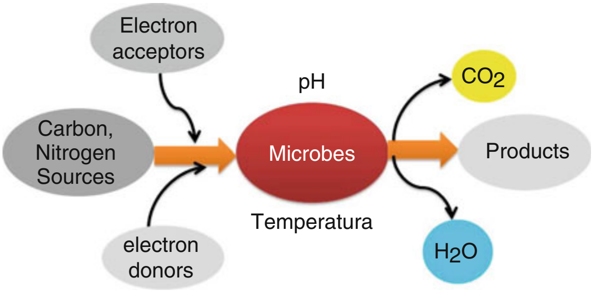

Fig. 1 Illustration of nutrient requirement of microbes. Microbes require carbon, nitrogen, and energy and are supported by favorable conditions such as pH and temperature for their growth and metabolism. Microbes are able to use various organic compounds including groundwater contaminants as carbon and energy sources and can grow in varied environmental conditions

Table 3 Types of microbes based on energy, carbon, and electron sources

Nutrient	Source	Type of microbe
Energy	Light	Phototroph
	Chemicals	Chemotroph
Carbon	Organic compound	Heterotroph
	Inorganic compound	Autotroph
Electron donors	Organic compound	Organotroph
	Inorganic compound	Litotroph

Table 4 Groups of microbes based on nutritional requirement

Energy source	Electron donor	Carbon source	Name
Sunlight	Organic	Organic	Photoorganoheterotroph
		Inorganic	Photolorganoautotroph
	Inorganic	Organic	Photolitoheterotroph
		Inorganic	Photolitoautotroph
Chemical compounds	Organic	Organic	Chemoorganoheterotroph
		Inorganic	Chemoorganoautotroph
	Inorganic	Organic	Chemolitohetertroph
		Inorganic	Chemolitoautotroph

community. The diversity and abundance of microbes in a microbial community are affected by several resources (carbon, energy, and nitrogen), electron donor/acceptor, and environmental conditions (e.g., temperature, pH) that prevail in their habitat (Brock 2012). In other words, all microbes require sufficient resources and suitable conditions for their growth and metabolism, as illustrated in Fig. 1. Generally, microbes can be classified based on their nutritional requirements (Tables 3 and 4). The types of microbes used for remediation are well known based on the nutrients present at contaminated sites.

The literature describes in detail the fate of organic compounds in nature, which depends on the availability of electron acceptors (Baun et al. 2003; Christensen et al. 2001; Cozzarelli 2001). Thus, the presence of electron acceptors in contaminated groundwater indicates the potential of microbial transformation. The state and fate of contaminants in all environments are highly dependent on the redox or valence state of the environment. The redox potential of the environment will control the direction of chemical balance and whether the contaminant is reduced or oxidized (Baker and Herson 1990).

Microbial degradation of organic compounds in contaminated groundwater occurs under different redox zones. When microbial degradation involves the use of oxygen as electron acceptor, it is called an aerobic process, and when it uses other electron acceptors instead of oxygen (such as nitrate, manganese, iron III, and sulfate), it is called an anaerobic process. If all electron acceptors are present, oxygen will be used first, followed by nitrate, manganese, iron, and sulfate. Finally, methanogenesis and fermentation reactions dominate when the most favorable electron acceptors are depleted (Christensen et al. 2001; Bjerg et al. 2003). Sequence redox zones is illustrated in Fig. 2. Christensen et al. (2001) and Bjerg et al. (2003) explain in detailed that in aquifers with continuous leachate (contaminant source) release, a methanogenic zone is close to the source. Within this zone and down gradient of it, sulfate reduction may take place. Iron reduction takes place further down the gradient where conditions become less sulfate reducing. Manganese and nitrate reduction zones have been observed to sometimes overlap with the iron reduction zone. Aerobic conditions may exist on the outskirts of a reduced plume if a pristine aquifer is oxidized and contains significant amounts of dissolved oxygen (>1 mg/L). A similar illustration of redox zones was also reported by Lovely (2003), that is, there are distinct zones in which different degradation processes predominate. At the source of contamination, such as the leachate from landfill, methane production often predominates. In this zone, microbes convert organic contaminants into simpler molecules, such as acetate and hydrogen. In other zones, organic contaminants are oxidized to carbon dioxide with the reduction of sulfate, iron (III), nitrate, or oxygen. Generally, the degradation of organic contaminants takes place in different redox zones, but chlorinated contaminants, which are not easily oxidized, undergo reductive dechlorination in methanogenic, sulfate-reduction, and iron (III)-reduction zones (Lovely 2003).

Generally, microbes gain energy for growth through substrate breakdown. However, in some cases, under mixed substrates, some microbe communities are able to degrade a certain substrate partly or completely, but not in support of growth. In such conditions this is called cometabolism. According to Dalton and Stirling (1982), cometabolism is the transformation of a nongrowth substrate in the obligatory presence of a growth substrate or another transformable compound. The term “nongrowth substrate” describes compounds that are unable to support cell replication as opposed to an increase in biomass. This definition was devised primarily as a result of nongrowth substrate metabolism studies with methane-utilizing bacteria. Janke and Fritsche (1985) reviewed the significance of microbial cometabolism, and they explained that microbial cometabolism of xenobiotics in natural ecosystems occurs at slow rates and will not increase in number or biomass. However, they

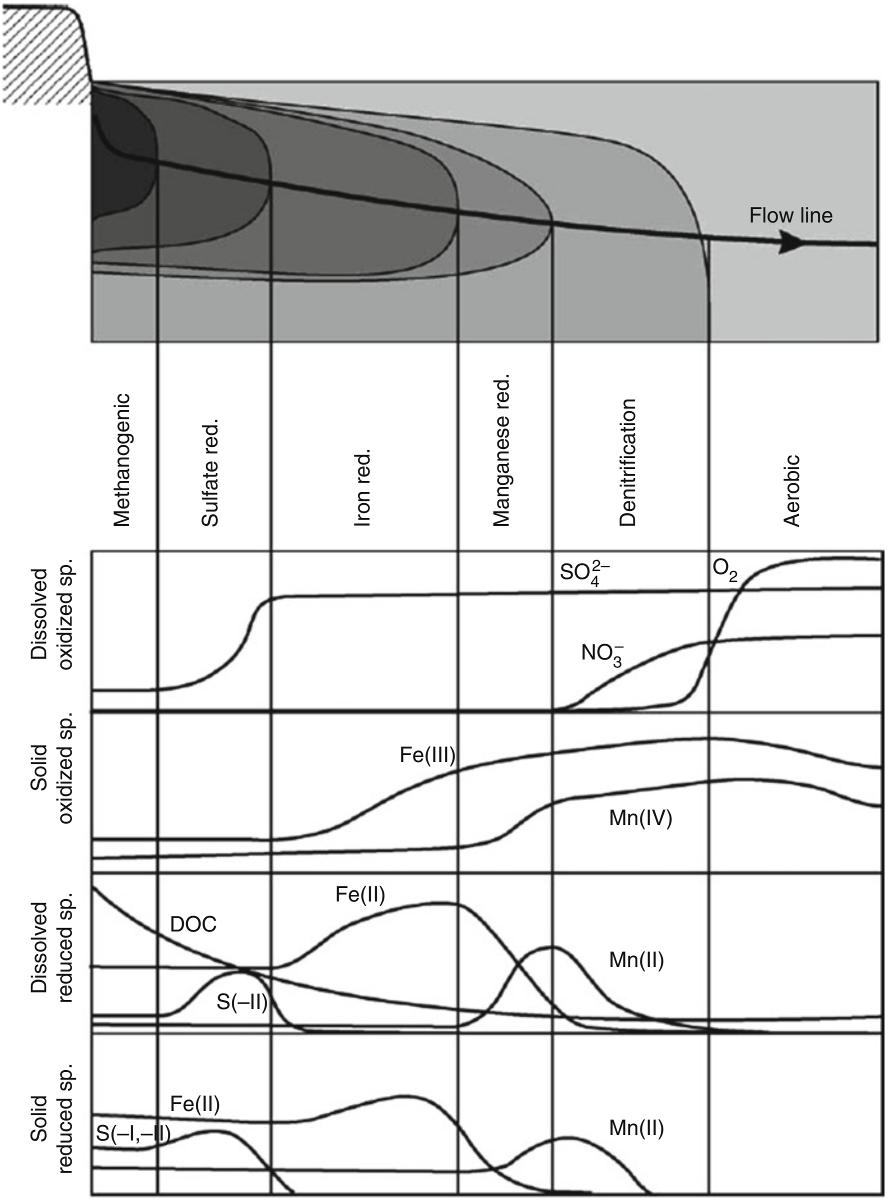

Fig. 2 Schematic redox zonation in an originally aerobic aquifer down-gradient from landfill, and distribution of redox species along a streamline (axes not to scale) (Source: Christensen et al. 2001; Bjerg et al. 2003)

concluded that under high concentrations of biomass and appropriate substrate mixtures, cometabolism of synthetic chemicals may be a useful technique of considerable practical importance to accumulate biochemical products at high yields. In addition, the cometabolic capabilities of wild-type microorganisms may serve as a tool for the construction of microbial strains with a new degradative potential for recalcitrant xenobiotic compounds. Since the microbes do not rely on pollutants for growth, the cometabolic degradation of environmental pollutants has the potential to achieve biodegradation goals.

The presence of microbes with the appropriate metabolic capabilities is the most important requirement in bioremediation. Thus, analysis and selected desired microbes as a biological engine for remediation may be recommended as parts of a bioremediation procedure. The microbes may be indigenous to a contaminated area or they may be brought in from other habitats into the contaminated sites.

AN understanding of the correlation between microbes and their nutrients in nature is an important step toward developing an innovative, strategic approach to bioremediation technology. It should be noted that, individually, microbes cannot mineralize most hazardous compounds completely. Complete mineralization results in a sequential degradation by a consortium of microbes and involves synergism and cometabolism actions. Natural communities of microorganisms in various habitats have an amazing physiological versatility; they are able to metabolize and often mineralize an enormous number of organic molecules (Singh et al. 2014).

Overall, the essential point of bioremediation is how to optimize all environmental conditions and sufficient nutrients (including electron acceptors) to support the biological function of microbes in breaking down contaminants.

On-Site Bioremediation

Successful bioremediation technology in practice is strongly correlated with the user's (practitioner's) understanding of the principles of bioremediation. In the context of low- and lower-middle-income ASEAN countries with financial constraints and a lack of available land, it would be wise to use an appropriate technology and strategy. Among existing bioremediation strategies, on-site bioremediation is the right choice. On-site bioremediation refers to a bioremediation process that takes place directly on the site of contamination. Many studies have shown that on-site bioremediation is economical because it does not involve the removal of contaminated groundwater to the surface (Mary Kenza 2001; Baker and Herson 1990), so it significantly reduces operating costs and exposure risk for personnel.

Innovations aimed at enhancing bioremediation processes could be made with reference to the principles of bioremediation. Currently, bioaugmentation and biostimulation have been applied to improve the rates of contaminant biodegradation in contaminated sites.

Bioaugmentation

The rate of biodegradation of contaminants in groundwater depends on the concentration of the contaminants and the amount of microbes present. The concentrations of contaminants and microbes often change over time. When the amount of microbes is low, the addition of microbial cultures to the contaminated sites is required to enhance biodegradation rates; this is called bioaugmentation. The issue is what type of microbes to add. The microbes that are used should have the capability to grow and degrade the existing contaminants. Commonly, indigenous or microbe communities from other environments are used. Generally, microbes identified as active members of microbial consortiums include *Acinethobacter, Actinobacter, Acaligenes, Arthrobacter, Bacillins, Berijerinckia, Flavobacterium, Methylosinus, Mycrobacterium, Mycococcus, Nitrosomonas, Nocardia, Penicillium, Phanerochaete, Pseudomonas, Rhizoctomia, Serratio, Trametes*, and *Xanthofacter* (Singh et al. 2014). Adebusoye et al. (2007) have found nine microbial strains that degraded petroleum hydrocarbons in a polluted tropical stream in Lagos, Nigeria. Those strains are *Pseudomonas fluorescens, P. aeruginosa, Bacillus subtilis, Bacillus spp., Alcaligenes* sp., *Acinetobacter lwoffii, Flavobacterium spp., Micrococcus roseus,* and *Corynebacterium* spp. Wenderoth et al. (2003) demonstrated in microcosm experiments the effectiveness of adding aerobic chlorobenzene-degrading bacteria (*Pseudomonas putida* GJ31, *Pseudomonas aeruginosa* RHO1, *Pseudomonas putida* F1DCC) to groundwater contaminated with chlorobenzene, which stimulated chlorobenzene depletion.

In cases of groundwater contaminated by landfill leachate–seawater mixtures, microbial communities with specific capabilities for degrading contaminants and for growing in high-osmotic-pressure or high-saline environments must be used. Halophilic microbes are very important in the bioremediation of organic contaminants in coastal groundwater contaminated with seawater. For instance, Karajić et al. (2010) reported that halotolerant microbes are able to decrease organic compounds in saline wastewater treatment. Moreover, in a recent study, Bonete et al.(2015) reported that *haloarchaea* (salt-loving organisms) that can grow in media with high salt concentrations in a range of 12–30% salt (2–5 M NaCl) are good biological agents for bioremediation in water treatment processes and in saline and hypersaline environments contaminated with toxic compounds such as nitrate, nitrite, ammonia, chlorine compounds such as perchlorate and chlorate, hydrocarbons, and heavy metals. New advances in the understanding of haloarchaea metabolism, biochemistry, and molecular biology suggest that general biochemical pathways related to nitrogen (nitrogen cycle), metals (iron, mercury), hydrocarbons, or phenols can be used in bioremediation.

Determining Potential Microbes

Laboratory assays of potential microbes from site samples are an important step in the evaluation of the efficacy of a process. The assay, which measures microbial activity in a microcosm, should be done as soon as possible after taking site samples. However, determination of the potential for contaminant degradation requires long incubation times (in itself a disadvantage) and thus might be affected by post-sampling changes (Röling and van Verseveld 2002). In some cases, differences in results measuring microbial potential obtained by laboratory assays (microcosms) and in the field were found. For instance, Smith et al. (2005) showed in laboratory assays that the bacterial strain PM1 rapidly and completely biodegraded MTBE in groundwater sediments. The bacterial culture was injected in an in situ field study at Port Hueneme Naval Construction Battalion Center in Oxnard, California. Six months after treatment began, MTBE concentrations in monitoring wells down-gradient from the injection bed decreased substantially in the shallow zone of the groundwater.

In recent years, advances in technology in molecular microbial and analytical chemistry have been developed together, making it possible to identify in situ microbial population structures, and even individual cells, responsible for triggering specific processes (Lovely 2003; Thompson et al. 2005). Molecular techniques, such as denaturing/temperature gradient gel electrophoresis (D/TGGE), terminal restriction fragment length polymorphism (tRFLP), and polymerase chain reaction (PCR), are better approaches to obtain a more comprehensive assessment of the composition and structure of microbial communities in contaminated sites (Watanebe and Baker 2000; Röling and van Verseveld 2003; Lovely 2003).

An important question is how to analyze the functions (physiological features) of microbial populations detected by molecular ecological methods. Several molecular methods for analyzing the in situ functions of microbial populations have been developed. For instance, metabolically active members of microbial consortia can be identified by quantifying rRNA molecules of different species, since the ribosome content of microbe cells is linearly related to growth rate (Watanabe and Baker 2000). Specific information on the potential for bioremediation of a certain contaminant can be obtained by assessing the functional genes that are responsible for its degradation (Brockman 1995; Stapleton et al. 1998).

Specific functional targeting genes (catabolic gene) can be amplified by PCR from environmental DNA samples and sequenced to analyze the composition and diversity of catabolic populations. For instance, Staat et al. (2011) applied targeting functional genes encoding specific enzymes, benzylsuccinate synthase a-subunit (bssA) and 6-oxocyclohex-1-ene-1-carbonyl CoA hydrolase (bamA), to determine the presence of mono-aromatic-degrading bacteria in groundwater contaminated by landfill leachate. DNA extracts from contaminated sites were amplified by PCR using specific primers for *bamA and bssA* genes. The positive results from the PCR products indicated the presence of those microbes at the contaminated sites. The same approaches can be used for other targeting functional genes. Recently, functional genes encoding osmolyte synthesis (*Mpgsmt* and *Mpsdmt* genes) have been amplified by PCR and can be used as probes for characterizing the presence of osmotolerant microbes in sites (Lai and Lai 2011).

The most important question in bioaugmentation is this: How do we monitor the fate of introduced microbes and their interactions within indigenous communities? To monitor the fate of introduced strains, fluorescence in situ hybridization (FISH) is used. To detect the structure and dynamics of indigenous communities during degradation experiments, single-strand conformation polymorphism (SSCP) analysis of 16S rDNA has been used (Schwieger and Tebbe 1998). Detailed explanations of molecular microbial techniques for bioremediation have been reviewed by Widada et al. (2002), Röling and van Verseveld (2002), Wenderoth et al. (2003), and Lovely (2003).

The study of microcosms (culture-dependent method) and molecular microbial techniques (culture-independent method) are complementary to each other and are still used to assess the microbial potential in environments.

Biostimulation

Biodegradation of contaminants in soil/groundwater can be affected by certain factors, including nutrient pH, temperature, electron acceptors, growth supplements, and contaminant concentrations. Biostimulation is defined as optimizing all environmental conditions such as by addition nutrients, electron acceptors, and essential growth factors and by controlling the pH/temperature to stimulates microbial activities (Margesin and Schinner 2001; Perfumo et al. 2007; Adams et al. 2015). Biostimulation is dependent on the indigenous organisms and thus requires that they be present and that the environment be capable of being altered in a way that will have the desired bioremediation effect (Hazen 2010). Studies have shown that nutrients (nitrogen, phosphate, or carbon in the form of molasses) are needed for microbial cell growth (Hazen 2010; Adam et al. 2015). Generally, the levels of nutrients (nitrogen and phosphate) are proportional to the presence of carbon (contaminants). The general theoretically calculated ratio of C:N:P for the biodegradation of polycyclic aromatic hydrocarbon (PAH) compounds is 100:10:1 (expressed in mol) (Ley et al. 2005). Litchfield (1993) suggested that the C:N:P ratio in practice is 100:10:2. Oxygen is often added to contaminated sites to stimulate aerobic degrading bacteria. In some cases, the degradation of specific organic compounds in groundwater occurs under aerobic conditions. In such conditions, dissolved oxygen will decrease, so the introduction of oxygen is required. The literature contains reports showing that aerobic conditions are indicated by dissolved oxygen concentrations exceeding 2 mg/L. In practice, on-site air sparging of water can supply 8 mg/L dissolved oxygen, sparging with pure oxygen can deliver 40 mg/L, while the application of hydrogen peroxide can provide more than 100 mg/L oxygen. Therefore, while air sparging is the simplest and most common oxygen delivery technique, the use of oxygen or hydrogen peroxide may speed the bioremediation process and decrease the pumping required. However, in some cases the increased cost and potential explosion hazard associated with a pure oxygen supply may limit the applicability of direct oxygen use (NRC 1993).

Biodegradation of organic contaminants takes place under anaerobic conditions. In such conditions, the availability of electron acceptors instead of oxygen should be considered. Nitrate/nitrite, manganese, iron (III), and sulfate are electron acceptors under anaerobic conditions.

In the case of coastal groundwater contaminated by landfill leachate–seawater mixtures, sulfate is abundant. This suggests that the occurrence of organic

Table 5 Types of osmolytes

Group	Types
Mono-di-oligo-polysaccharides	Glucose, fructose, sucrose, raffinose and fructans
Polyol (sugar alcohol)	Sorbitol, manitol, glycerol,inositol and methylated inositol
Amino acid	Methyl-proline,prolin betaine, ß-alanine betaine, choline O-sulphate
Tertiary sulfonium	Dimethylsulfoniopropionate (DMSP)

biodegradation should take place under sulfate-reduction conditions. A studied reported by Mangimbulude et al. (2016) showed that high sulfate concentrations were observed in landfill leachate and groundwater in comparison to other redox elements. That study also showed high concentrations of H_2S and Fe^{2+} in landfill leachate and groundwater, indicating that sulfate-reduction and iron-reduction were the dominant processes in the groundwater.

In the literature, for the biodegradation of contaminants in groundwater under anaerobic conditions, organic substrates (such as molasses, lactate, butyrate, methanol, ethanol, sodium benzoate) are often added as electron donor sources to enhance biodegradation rates. In addition, whey, vegetable oils, and compost are also used as organic substrates (EPA 2013).

Coastal groundwater contaminated by leachate–seawater mixtures at high organic contaminant and high saline concentrations creates high osmotic conditions. In such conditions, the addition of osmo-protective compounds (osmolytes) is important for protecting microbes (Slama et al. 2015). Several osmolyte compounds have been identified (Table 5) (Rhodes et al. 2002; Ashraf and Foolad 2007).

Factors to Consider

As noted earlier, the coastal groundwater status in some parts of low- and lower-middle-income ASEAN countries is in a critical condition because it has been contaminated. Cleaning up contaminated groundwater has become a mandatory task of governments to be implemented by government self or by offering to collaborated partners (practitioners or companies) but still under governments supervision.

Bioremediation appears to be a feasible cleanup option. It involves relatively low-cost, low-technology techniques that generally have high public acceptance and can often be carried out on site. However, there are some challenges that should be considered before applying bioremediation technology to coastal groundwater pollution. According to Zurbrügg (2002), in many cities of Asia, deficiencies in the provision of waste removal services are the result of inadequate financial resources, an absence of management, and lacking technical skills of municipalities and government authorities to deal with the rapid growth in the demand for services. The Asia Development Bank Institute (1998) reported that the main challenges facing local authorities in low-income Asian cities are as follows:

- Unplanned growth and increasing pressure to provide services
- Lack of adequate authority to address human, infrastructure, and resourcing problems
- Bureaucratic confusion and delays due to a multitude of agencies (local, provincial, and national levels) operating within the same municipal boundaries
- Lack of accountability
- Limited communications within the city administration and, more importantly, between the city administration and the various stakeholders
- Political interference: elected representatives often do not confine themselves to strategic planning, policymaking, and oversight of performance but instead become involved in daily operations
- Lack of skills among municipal workforces; training is often reserved to senior staff and seen as a reward for good work and as a chance to break away from daily obligations

These challenges are important to know about and should be minimized to ensure that the local authority's (government's) bioremediation strategic policy will continue to implemented.

Another factor is site characterization, as we know that site characterization is an initial step toward making a plan for the application of bioremediation techniques. A failure to take this step will affect subsequent planning. It should be noted that the main factor in the characterization of groundwater sites in coastal areas is the intrusion of saline seawater into groundwater due to tidal effects and the rise in seawater levels. In such conditions adequate techniques involving the selection of the appropriate microbes that are capable of degrading contaminants under high osmotic conditions. Recently, *haloarchaea* and *halobacteria* species have been used for the bioremediation of groundwater containing nitrogenous and aromatic hydrocarbon compounds (Bonete et al. 2015).

Further research on anaerobic haloarchaea and halobacteria will be necessary in order to find the appropriate microbes to use as biological agents in bioremediation in coastal groundwater contexts. It is conceivable that, someday, organic contaminants in groundwater will be removed by means of biodegradation, but whether saline groundwater can be remediated remains an open question. It seems that intrinsic bioremediation technology (natural attenuation) is suitable for application in the low-income ASEAN context because relative low-cost of operational and do not requires many expert people, as far as operational system was established. Some studies in the literature have reported on the attenuation of organic and inorganic compounds separately. Thus, it is necessary to further study processes of natural attenuation of organic and inorganic compounds in coastal groundwater contaminated by landfill leachate–seawater mixtures.

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