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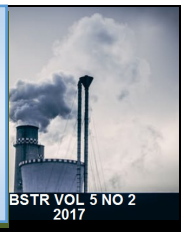

Phenol Removal Via Cellular Immobilization: A Review

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ABSTRACT

Environmental pollution is one of the major concerns in the 21st century; where billions of tonnes of harmful chemicals are produced by industries such as petroleum, paints, food, rubber, and plastic. Phenol and its derivatives infiltrate the ecosystems and have become one of the top major pollutants worldwide. This review covers the major aspects of immobilization of phenol-degrading bacteria as a method to improve phenol bioremediation. The use of various forms of immobilization matrices is discussed along with the advantages and disadvantages of each of the immobilization matrices especially when environmental usage is warranted. To be used as a bioremediation tool, the immobilized system must not only be effective, but the matrices must be non-toxic, non-polluting and if possible non-biodegradable. The mechanical, biological and chemical stability of the system is paramount for long-term activity as well as price is an important factor when the very large scale is a concern. The system must also be able to tolerate high concentration of other toxicants especially heavy metals that form as co-contaminants, and most immobilized systems are geared towards this last aspect as immobilization provides protection from other contaminants.

INTRODUCTION

Industries across the world produce billions of tonnes of noxious chemicals into the environment that gets it way into the water, air, and Land. Combustion of fuels in the factories is the major way through which, toxic compounds such as hydrocarbons and heavy metal enter the environment [1]. The air pollution results an increase in sickness and premature deaths from asthma, bronchitis, emphysema, pneumonia [2], coronary artery diseases and abnormal heart rhythms [3,4].

Direct or indirect discharges of toxic pollutants into water bodies or land without sufficient treatment to eliminate these dangerous compounds are the primary source of water and soil pollutions. Industries without proper ways to control overflow also add the toxic toxicants such as polyaromatic hydrocarbons, heavy metals, and pesticides to the environment [5–7]. Oil spills, deck overspill leakages from vessels, pipelines and storage tanks and offshore disposal of waste are also major sources of water pollution [8,9]. Hydrocarbon and heavy metal pollutions can increase the vulnerability to disease and upset reproductive processes and give harmfully affect to plant and aquatic lives [10,11]. Water and soil pollutions are a universal problem and require continuous monitoring and remediation [12].

Phenol

Phenolic compounds or phenols are a group of aromatic compounds that comprises a hydroxyl group (OH) that is directly bonded to an aromatic ring [13]. **Fig. 1.** Phenols are injurious to organisms even at even low concentrations with many of them are categorized as dangerous pollutants because of their likely harm to human well-being [14]. Some of the phenolic compounds include chlorophenols, nitrophenols, methyl phenols, alkylphenols, aminophenols, butylhydroxytoluene, nonylphenol and Bisphenols A.

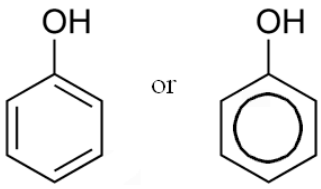

Fig. 1. Chemical structure of phenol [15].

Phenol was first isolated in 1841 from coal tar by Ferdinand Runge, a German Scientist [14]. Phenol is one of the most significant industrial effluents discharged by the processing industries such as oil refineries, dye, pesticides, plastic plants and pharmaceutical industries [16].

Sources of phenol

The primary source of natural phenol is from the decomposition of organic materials and other fossils. Coal is one of the major minerals aside from oil and gas. Coal tars are produced from coal mining. Coal from the fossil is far more plentiful than oil or gas, with around 119 years of coal remaining worldwide. World annual coal production was estimated at 6.7 billion tons in 2009 with China producing almost 40% and became the highest global producer followed by The United States with 15% of the annual coal production [17]. Malaysia had an estimated coal resources of about 1.72 billion tons [18], with 99% is from East Malaysia namely Sarawak 80% and Sabah 19 % while three in the Malaysian Peninsular states of Perak, Perlis and Selangor having only 1 % [19].

Toxicity of phenol

Human

Phenol is the critical environmental pollutant that is noxious to livings even at low concentration [20]. The approved level of phenol in water is < 0.002 mg/L. Lethal dose is 50-500 mg/kg [21]. Acute exposure to phenol could result in many health problems such as the central nervous system disorders and can potentially lead to coma, muscle weakness, burning effects on skin and other effects including renal damage, irritation of the eye, headache, liver damage, gastrointestinal disturbance. In addition, it is also suspected that the exposure to phenol may cause cancer and tumour [22]. Chronic exposure to phenol is associated with an increased risk of insufficient blood to the heart and also coronary artery diseases [23].

Exposure to phenol can be through the skin, inhalation, and ingestion. Inhalation accounts for almost 80% of the case of phenol exposure [24]. Exposure through inhalation or dermal route is highly irritating to skin, eyes and mucous membranes Systemic effects include the gastrointestinal tract (GIT) irritation [25] and dermal necrosis [26]. In water it caused diarrhoea [27], mouth sores, burning of the mouth and dark urine. Short exposure causes irritation of skin and eyes, headache, diarrhoea and vomiting [28]. Phenol and hydroquinone have been suggested as factors in producing leukaemia connected with benzene exposure. Phenol produces the haematotoxicity effects associated with benzene, triggering DNA and chromosomal damages observed in leukaemia, inhibits topoisomerase II and clonal selection process [29,30].

Fish

Phenol is toxic to fish, a sub-lethal concentrations of phenol causing different types of organs and system disorders such as nervous system disorders that result in paralysis and convulsion, interference with respiration causing asphyxia [31], increased necrosis of gills and mucus production [32], destruction of erythrocytes [31], histopathological changes in skin, liver, spleen and heart.

Fish exposed to sublethal concentration (1.5 mg/L) of phenol for more than 10 days has been reported to have a marked increase in the activities critical liver enzymes (GPT, GOT, LDH, and ALP). The elevation in the transaminases activities may be due to liver injury [33]. Hypocholesterolemia has also been associated with the toxicity of phenol in fish [34]. The African catfish have been reported to have a lethal concentration of phenol (LC_{50}) at 35 mg/L by immersion for [35], *Oreochromisniloticus* (Tilapia) has an LC_{50} of 29 – 28 mg/L [20], [36] and tilapia (*Oreochromis mossambicus*) has an LC_{50} of 28.49 mg/L [35]. It can also lead to suffocation at higher dose after 72 to 96 hr exposure [32].

Plants

Phenol is less toxic to many plants; some plants have the ability of phenol bioaccumulation [37]. However, high concentrations of phenol can inhibit the growth index of many plants [38]. Some plants that are exposed to 1000 mg/L phenol cannot survive; they eventually died [39]. Phenol may be accumulated into the plants part such as roots, fruits, and leaves and may be toxic to human and animals if they consume the affected plant [40]. Phenol concentration of 500 mg/L can affect the seed germination of some plants such as *V. Sativa* [38]. Research using a *Brassica napus* hairy root showed that phenol is not toxic to the plants at the concentration of 10-50 mg/L. However, signs of toxicity appeared when a concentration of 100 mg/L was used [41] while *Brassica juncea* hairy root can remove 97% 1000 mg/L phenol without any signs of toxicity [42].

Phenol pollution

Due to the wider use of phenol by the industries, phenol has been found to be a major environmental pollutant discharged in industrial effluents such as from the pesticide making industries [43], paper and pulp [44], textile, plastic [45], gas and coke and steel and oil refineries [46,47]. Phenol as a component of oil refinery waste also acts as a by-product of coal conversion to gaseous and liquid fuels. There are various report cases of phenol contaminations in soil, water, and air from these sources [48]. Phenol can also be introduced into our environment by being discharged from the municipal waste treatment plants and spills [49].

In soil, phenol may remain or degrade by microorganisms; it can be broken down in the air within 1-2 days and may persist in water for weeks. Several pollutions by phenol and its derivatives have been reported around the world as shown in **Table 1.**

Table 1. Some Incidences of phenol and phenolics pollution around the world.

Year	Location	Phenol concentration	Source of pollution	Reference
1961 and 1979	Linggi River, Malaysia	>0.02 mg/L		[50]
2000	Indonesia	230 tons	Tankers	[51]
2000	Volga River, Russian			
2006	Azerbaijan	-	Oil spill	[52]
2002	River Dee, UK			[53]
2008	River, South Korea	11 kg	leaked into river	[54]
2013	Lake Maryut, Egypt	0.5 ppm	spillage from oil refinery	[55]
2010	River, India	7 mg/L	coke oven processing wastewater	[56]
2013	Okrika River, Nigeria	-	oil refinery	[57]
2014	Tap water in Lanzhou, China	5 mg/L	leaked into river water	[58]

Cells immobilisation

Immobilisation is a term used to describe a broad range of the cell or particle attachment or entrapment methods [59]. It can be utilized to principally all types of biocatalysts including enzymes, cellular organelles, animals and plant cells. Cell immobilization can also be defined as the physical imprisonment or localization of viable microbial cells to a certain defined region of space in such a way as to limit their free relocation and exhibit hydrodynamic characteristics, which is in contrast to those of the neighbouring environment while being retentive of their catalytic activities for repetitive and continuous use [60–62]. When compare with immobilized enzymes, immobilized cells gives a new potential outcome since they can be utilized as natural, water-insoluble carriers of required enzyme activities [63]. Through the immobilization of microbial cells, their field of utilization spreads from industrial to environmental processes. When retained on supportive carriers, microorganisms can be used and reused continuously permitting for the decrease in cost, as the biocatalyst does not need to be filled up [64–66].

Since the early 70s, when Chibata's group announced a successful process of continuous fermentation of L-aspartic acid [67], numerous research groups had attempted various microbial applications with immobilised cells [68]. Currently, different kinds of immobilisation have found wide applications not only in the field of biotechnology but also in the pharmaceutical, environmental, biosensor and also food industries [69]. The cell immobilization materialized as a substitute for enzyme immobilization, Cheetham et al., (1979) reported cells immobilisation using calcium alginate gels [71], and immobilisation of yeast using gelatin [72] .

In the field of environmental studies, immobilization of microbial cells was first reported by Bettmann and Rehm [73] for the degradation of phenol using a polymer entrapment method, while Anselmo et al. [74] reported on the degradation of phenol by cells of *Fusarium flocciferum* immobilized by entrapment in agar, K-carrageenan, alginate, and polyurethane, and by adsorption on preformed polyurethane foams (Sahasrabudhe et al., et al 1988). *Pseudomonas* sp. B13 cells were successfully immobilised using calcium alginate for the dehalogenation of 3-chlorobenzoate, while pentachlorophenol degradation was successfully carried out using polyurethane-immobilized *flavobacterium*, [60,75]. [76] immobilised *Alcaligenes* sp. TK-2 using calcium alginate for 4- dichloro 2-nitrophenol degradation, while [77] immobilised *Alcaligenes* sp. A7-2 in granular clay for the biodegradation of 4-nitrophenol. [78] utilised polyvinyl alcohol as a carrier for biodegrading di-n-butyl phthalate (DBP). Wang et al. (2007) reported on the degradation of activated sludge using an improved polyvinyl alcohol, while phenol degradation by gellen gum-immobilised cells of *Acinetobacter* sp. strain AQNOL 1 has been reported [80]. The degradation of phenol by immobilized *Ralstonia eutropha* has also been reported [81].

Four major types of immobilization techniques

Covalent bonding/crosslinking

The mechanism involved in this kind of immobilization is based on the formation of covalent bond between inorganic support and cell in the presence of a binding agent. Chemical modifications of the surface is a necessary step crosslinking. The covalent attachment or cross-linking are efficient and long-lasting for enzymes, but it is seldom used for immobilization of cells but is very effective for enzymes, this is because most of the binding agents are cytotoxic and can cause cell damage when used for cells immobilization [68]. However, [82] reported a strong covalent binding of the yeast cells on porous silica beads. Successful immobilization of yeast (*Saccharomyces cerevisiae* and *Saccharomyces amurca*) by the method of covalent bonding/crosslinking on borosilicate glass and zirconia ceramics was also reported [83].

Entrapment

Entrapment is an irreversible entrapment of the cells within a support matrix or inside fibres, where immobilized cells are entrapped in a support matrix or inside fibres. The technique ensures a protective barrier around the immobilized microbes, creates prolonged viability during processing and storage in polymers [84]. Entrapment is the most widely considered strategy in cell immobilization [68,85]. In this strategy, a lot of permeable polymers can entangle microorganisms under encompassing conditions [86,87].

Generally speaking, the entrapment techniques depend on the inclusion of the cells inside a rigid system to prevent the cells from diffusing into the encompassing medium while at the same time permitting infiltration of the substrate. Although several matrices such as agar, carrageenan, cellulose and its derivatives, collagen, gelatin, gellan gum, photo-cross-linkable resins, polyacrylamide, polyester, polystyrene, and polyurethane have been used as support matrices, but alginate gel is the most widely used because its setup uses a simple procedure and also require mild conditions [88]. Entrapment permits high mechanical quality. However, there are few burdens; for example, cell spillage, expenses of immobilization, diffusion limitations, and deactivation after immobilization and abrasion of support materials during usage [89–91].

Gellan gum

Gellan gum is a linear heteropolysaccharide, and it is an anionic compound. Gellan gum is commercially produced by a gram negative bacteria *Sphingomonas elodea* [92,93], and its structure is consist of repeating unit of tetrasaccharide composed of the backbone 1,3-β-D-glucose, 1,4-β-D-glucuronic acid, 1,4-β-D-glucose and 1,4-α-L-rhamnose. Gellan gum in its natural form is esterified with acetate and L-glycerate at the C-2 and C-6 positions of the (1-3)-linked D-glucose [94]. Based on the acyl content, gellan gum forms diverse kinds of gels. The hard and brittle gels are formed by the deacylated type in the presence of cations, while the native type forms soft and elastic gels even in the absence of cations [95,96].

Encapsulation

In this method of immobilization, the whole cell or the enzymes are enclosed in a semi-permeable membrane in the form of a capsule. It is an irreversible method of immobilization similar to entrapment. Membrane nitrocellulose or nylon are commonly used (Górecka and Jastrzębska, 2011). In this process, the effectiveness depends on upon the stability of enzymes inside the capsule. The membrane ensures the free flow of nutrients and substrates while keeping the biocatalyst inside the membrane. It is cost effective, simple procedure and a significant number of microorganisms or enzymes can be encapsulated (Song et al., 2005). The method was used to immobilize the whole cell in a polymer-gel [97]. It is one of the most common methods employed in the laboratory but suffers from pore size limitations and one of the drawbacks of this method of immobilization is only small substrates can pass through the semi-permeable membrane [98].

Adsorption

Adsorption is a reversible method of cell immobilization and is probably the simplest method of immobilization [99,100]. In this approach, biocatalyst adheres onto porous and inert support materials similar to the adsorption of colloid particles [101]. Forces such as van der Waals forces, ionic and hydrophobic interactions and hydrogen bonds are involved in the process. Adhesion of the cells to the support are usually governed by both electrostatic and hydrophobic interactions, which is the most important process in controlling the cell immobilization on the support [102,103]. This technique is based on the physical interaction between the microorganism and the carrier surfaces and the microorganisms which is frequently reversible. The method is simple, cheap and efficient. The immobilization of microorganisms on suitable adsorbents preserves the physiological characteristics of the organisms, and also stimulates metabolic activities and protects cells from unwanted agents [104]. Adsorption allows direct contact between the immobilized cells and the nutrient, and thus this is an advantage over entrapment [105].

Recently, immobilization techniques have been receiving increasing attention for the treatment of phenol-containing wastewater [106]. Phenol degradation using immobilized cells could be less expensive since they can be utilized and reused several times without a significant loss of activity [107]). Thus, immobilized cell techniques have been considered as a promising tool for wastewater treatment in the past few decades and the near future [108]. Many support matrices have been reported by the various researcher for the degradation of phenol using immobilization techniques. **Table 2** shows some the microorganisms used and the type of matrices utilized in the process. The most widely reported support matrix is the calcium alginate.

Table Error! No text of specified style in document.**.** List of some bacteria and the matrices used for the degradation of phenol by immobilized cells.

Microorganism	Immobilised bead	References
Acinetobacter sp.	polyurethane	[109]
Arthrobactercitreus	ca- alginate and agar	[110]
Acinetobacter sp. strain AQ5NOL 1	gellan gum	[111]
Acinetobacter sp. *BS8Y*	polyvinyl alcohol	[112]
Bacillus cereus	ca-alginate	[113]
Comamonas acidovorans		[114]
Pseudomonas putida	ca-alginate	[115]
Pseudomonas putida	ca-alginate	[116]
Pseudomonas resinovorans	ca-alginate	[106]
Rhodococcus sp	gellan gum	[117]
Sphingomonas sp	polyvinyl alcohol	[118]
Candida tropicalis	ca-alginate	[119]
Trichosporoncutane	polyamide	[120]

To be used as a bioremediation tool, immobilization must cover these aspects which include; the matrices must be non-toxic, non-polluting and if possible non-biodegradable. In addition, other aspects such as mechanical, biological and chemical stability, high bacterial cell mass loading capacity and economic must be taken into account. Furthermore, the immobilized cells must easily be removed from the aqueous bioremediation site so that it can be recycled. Lastly, the immobilized matrix must be able to tolerate high concentration of other toxicants especially heavy metals that form as co-contaminants.

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