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Research article

Isolation and Characterization of Phenol-Degrading Microorganism: Recent Advances

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ABSTRACT

Phenol is among the common toxic environmental pollutants that occur naturally and also originated from industrial effluents. Biodegradation is a major mechanism for the removal of pollutants from a contaminated site. The focal point of this review was on the recent researches on isolation and characterization of phenol degrading microorganisms mainly by bacteria, fungi and yeast from places around the world. Scientists are interested to have their own isolates because there are no individual organisms or groups of organisms that were universally appropriate and applicable for bioremediation since the place of origin does play a role in determining the important properties of microorganisms. The compatibility of the application site as well as the biochemical and physiological potential of the microbes should be considered. Overall, bacteria were generally capable as biodegradation agents in aquatic systems, while fungi play a greater role in terrestrial system.

INTRODUCTION

Environmental biology is an area that cover any biological organism, product or process that are beneficial to the environment especially in controlling or reducing of pollutant and power use. One of the commonly discussed topics in environmental biology is bioremediation that is related to a technology that can be applied into the biological system, usually about microorganisms which can clean up a polluted contaminated site. The bioremediation industry promise a turnover around 20 billion dollar globally [1].

Updated on Thursday, July 25th, 2013, The United State Environmental Protection Agency (EPA) had published the current list of 126 Priority Pollutants. It was a set of chemical pollutants that were being regulated and have several developed analytical test methods. Among the commonly found wastes are arsenic, benzene, chloroform, cadmium, chromium, lead, phenol, PCB's, trichloroethylene and toluene. The pollutant penetrates the ecosystem by various situation either it has been accidentally released to environment through leaking of the containers, crashes of toxic waste tanker, landfill leachate and runoff the excessive pesticide from agriculture farm or it was resulted from the development activities of industries. The cost for hazardous waste removal is very high. A report prepared by United States Government Accountability Office mentioned that their agencies spent almost about $30 billion from 1986 to 2008 for all the environmental cleanup and restoration activities [2].

Physical, chemical and biological methods have been widely applied to remove contaminant from the polluted area. However, amongst the technique, biological removal offers the cheapest and the safest way through bioremediation [3, 4].

There are various strategies in bioremediation which include; using native (indigenous) microbes at the place of the contaminants, improving microbes living conditions to the environments where there are only a few toxin-degrading microbes, so the condition will help the microbes to increase their numbers and by adding microbes which have been selected that have the capabilities to degrade contaminants to the polluted environment which is also known as bioaugmentation [5, 6]. The accomplishment of bioaugmentation relies on the survival of the microbes added and the cells could be more resistant to the new environment by immobilization in a carrier such as calcium alginate [7], gellan gum [8] and polyacrilamide [9].

Phenol pollution

Phenol is among the common toxic environmental pollutants that characterized as the first priority pollutants by European Union [10, 11]. Phenol occurs naturally and also originated from the industrial effluents such as pharmaceutical, food industries, oil refineries and coal conversion process [12, 13]. The phenolic compound was also released to be wastewater by steel industries, petrochemicals, polymeric resins and dye manufacturing units. It is an aromatic molecule that is soluble in water and organic solvents and could diffuse across a cell membrane of an organism. The studies showed that constant administration of phenol by animals could cause pathological changes. Phenol that was discharged to the water body will endanger aquatic life even at concentration of 500-2500 mgL-1 [14]. Phenol can cause health effects in both short-term (less than 14 days) and long term (after 365 days) exposure [15]. A human could suffer anorexia, weakness, headache and muscles pain in long-term exposure to phenols vapors, and meanwhile, acute or chronic ingestion of phenol may cause the burning of mouth, diarrhea, sweating, sore throat, vomiting and abdominal pain [16, 17]. The toxicity may depends on the degree of hydroxylation (-OH) and methoxylation of the ring. Based on the toxicity and its risk to the organism and environment, phenol requires appropriate treatment for waste containing phenol before being released to the nature. The ideal solution for pollution abatement nowadays is bioremediation, the most helpful technology that uses biological systems for the treatment of contaminants [18]. The biodegradation of phenol reduction is chosen as the cost of this technique is lower and offers complete mineralization [19, 20]. Even though this recent technology is a multidisciplinary approach, its central thrust depends on microbiology.

The use of genetically modified organisms which are capable to degrade phenol or newly designed phenol is still limited. Hence, exploration of the biodiversity of wild strains from natural ecological environments is currently the most preferable and the most appropriate approach to search for the capable candidate. Phenol had a bactericidal action and thus causes problem in microbial action. For that reason, research on the screening of strains and the isolation and characterization of microorganism that possess phenol degradation characteristic with better phenol tolerance is very crucial. A lot of researchers from various counties tried to discover the suitable microorganism because of the local sources needed for different locations. This is because local microorganisms are better suited with local conditions and would not cause ecosystem disaster as in the case of imported untested microorganisms. The discoveries have led to the application of microorganisms in bioremediation of phenol by individual microorganism or consortium [21]. The microorganisms have the possibilities to degrade phenolics by biological oxidation with the help of phenol oxidizing enzyme and microbial metabolism [22, 23]. Bioremediation technology has been applied in field pilot or full scale. Therefore, in order to reconditioning the polluted location, microbial bioremediation strategies can be used in-situ and ex-situ bioremediation. Both methods relies on the relative abundance, structure, catabolic versatility and biotic/abiotic interactions of the microbial communities (aerobic/anaerobic) that are indigenously present, amended or stimulated at contaminated sites such as industrial waste treatment plant [18]. This review summarizes the recent information on types of microorganism, its characterization, phenol degradation capabilities and procedures used for the research of this topic. Organisms endowed with phenol degrading capacities include the number of bacteria and fungi including yeast. Recently, various types of aerobic bacteria, fungi and yeast reported in phenol degradation. Whereas, only a few phenol degrading anaerobic organisms have been involved via one pathway, while, aerobic degradation is via meta or ortho pathway [24, 25, 26].

Phenol-Degrading Bacteria

Since 1975, the diversity of bacteria that can aerobically decompose phenol has been reported such as: *Bacillus stearothermophilus* [27], *Pseudomonas putida* [28, 29, 30, 31, 32,

33], *Pseudomonas cepacia* G4 or *Burkholderia cepacia* G4 [34, 35, 36, 37], *Pseudomonas resinovorans* [38], *Arthrobacter* [39], *Acinebacter* sp. [40, 41], *Alcaligenes eutrophus* [42], *Ralstonia eutropha* [43], *Nocardioidess* [44], *Pseudomonas pictorum* [45], *Pseudomonas* aeruginosa, *Pseudomonas fluorescens* [46, 47]. A study on *Desulfobacterium phenolicum* sp. that degrades phenol anaerobically was reported by Bak and Widdel, 1986 [48]. The studies on phenol-degrading bacteria were being continued until the recent years.

An *Acinetobacter* sp. isolated from wastewater and activated sludge from a petroleum chemical plant, showed the abilities of phenol degradation in a research done by Ren et al., 2008 [49]. With 16S rDNA sequence, out of ten isolates, four (PD4, PD5, PD8 and PD9) were being identified as *Acinetobacter* sp. Other five isolates (PD1, PD2, PD6, PD7 and PD39) known as *Pseudomonas* sp., and one (PD3) as *Comamonas* sp. The researchers choose *Pseudomonas* sp. PD39 as the representative to be studied in details. In medium containing 800 mgL-1 of phenol, the strain grown better in pH 7.0, at temperature 30 ºC. It could also metabolize phenol at up to 1200 mgL-1 and removed 99.96% of 637 mgL-1 in industrial wastewater within 72 hours

Using batch culture with synthetic phenol (100 to 500) mgL-1, Agarry et al. [50] studied the capabilities of an indigenous *Pseudomonas fluorescence* in bioremediation. Observation at multi cultivation times revealed that, phenol was totally degraded at varies cultivation times. The lag phase increased from 0 to 66 hours when the initial phenol concentration was increased from 100 mgL-1 to 500 mgL-1. Monod kinetic model has been used to show the inhibition effects of phenol with 500 mgL-1 as the initial phenol concentration. The *Ks* increased and *Rsmax* decreased when phenol was increased. Haldane model estimated the biokinetic constants and showed that the *Pseudomonas fluorescence* had a good potential in the bioremediation of phenol waste effluents.

A strain *Acinetobacter* sp. ISTPCP-3 was identified based on 16S rDNA sequence analysis and phylogenetic characteristics as well as morphology and biochemical tests. This strain was isolated from paper mill effluent and sediment core of pulp. It has high pentachlorophenol (PCP) degradation capability. *Acinetobacter* sp. ISTPCP-3 was mesophile because the best growing temperature was 30°C. It prefers pH 6.5–7.5, and pH 7 was the optimum and degradable 200 mgL-1 PCP while 250 mgL-1 was inhibitory to its growth. Application of gas chromatograph–mass spectrometric (GC–MS) revealed that this strain degrade the PCP via ortho ring-cleavage with the formation of 2-chloro-1,4-benzenediol and 2,3,5,6-tetrachlorohydroquinone [51].

According to the biochemical characteristics and 16S rRNA sequence analysis, Shourian et al. [52] had isolated *Pseudomonas* sp. assigned as *Pseudomonas* sp. SA01 from pharmaceutical disposal wastewaters plant. The strain was able to degrade 700 mgL-1 and up to 1000 mgL-1 of phenol with short lag phase within 30 hours of incubations. However, higher phenol concentrations (>1000 mgL-1) inhibit the growth of this bacterium. The optimization experiments resulted with the optimum degradation at pH 6.5 and with addition of casein and mannitol as auxiliary carbon and nitrogen sources, it could remove phenol within 20 hours of incubation. The SA01 strain metabolizes the phenol via a meta-cleavage pathway.

Cordova-Rosa et al. [53] reported that indigenous consortium of phenol-degrading bacterial with *Acinetobacter calcoaceticus* var. anitratus, was originated from wastewater treatment plant of an industrial coal. The consortium was able to survive in high phenol concentrations (1200 mgL-1) with faster degradation rate compared to a single culture of the A. *calcoaceticu*s. Using continuous and batch systems of a bioreactor, a high phenol biodegradation (above 95%) was obtained but the incubation in coke gasification wastewater showed that phenol was still in the degradation after 10 days of incubation at pH 9–11.

Majority isolation of phenol-degrading bacteria were done from the industrial waste, but Sandhu et al. [54], isolated ten bacteria from leaves of *Fraxinus pennsylvanica* that was able to mineralize phenol. The isolate was originated from trees near to (5–6 m) of a swine production facility in Hamilton County, Iowa. *Rhodococcus, Alcaligenes* and *Acinetobacter* were the identified genera according to 16S rDNA sequence analysis. The amplified fragments of the large subunit of a multicomponent phenol hydroxylase from these isolates signify it class as a single kinetic class, which have a moderate affinity for phenol that is consistent with the predicted levels of phenol in the phyllosphere. The degradation pathway was determined by the amplification of catechol 1,2-dioxygenase and catechol 2,3-dioxygenase genes via PCR supported with a functional assay for catechol 2,3-dioxygenase activity. The results proved that the gram-negative strains and also *Rhodococcus* degrade the phenol by ortho pathway. The results exhibited that the leaf surface has a taxonomically distinct communities of phenol-degrading bacteria based on the identified genes of phenol catabolic pathways.

Liu et al. [55], reported about phenol-degrading bacterium from the activated sludge and phenol-contaminated soils in Northwest of China. The high biodegradation capability strain was known as strain XA05 and FG03. XA05 was identified as *Acinetobacter* sp. and FG03 was closely related to the Sphingomonas sp according to DNA sequencing and homologous analysis of 16s rRNA gene. Both strains were immobilized in polyvinyl alcohol (PVA) at the ratio of 1:1, via the repeated freezing and thawing. The experiments indicated that the immobilized and free cells have 95% of degradation efficiencies with 800 mgL-1 of phenol within 35 hours. The immobilized cells showed higher degradation rate constant in wider pH range and less temperature effectcompared to free cells. The immobilized cells can be reused in not less than 20 cycles and can still acquire better storage stability.

Also in China, microbes isolated from soil of natural forest of bamboo and pine, were able to decomposed phenolics compounds based on their abilities to grow in a medium containing p-coumaric acid, a phenolic allelochemicals. Identification process was done through biochemical analysis and 16S or 18S rDNA sequencing. Both methods recognize four isolated strains as *Pseudomonas putida* 4CD1, *Pseudomonas nitroreducens* 4CD2, *Pseudomonas putida* 4CD3 and *Rhodotorula glutinis* 4CD4 [56].

Li et al. [57] discovered the ability of a psychrotrophic organism, *Pseudomonas putida* LY1 to degrade 200 mgL-1 phenol at a range of temperature from 25 to 35ºC, with 25ºC as the optimum temperature. High initial phenol concentrations (800 mgL-1) inhibited the growth of this strain. Haldane model was used to correlate the growth kinetics at low concentrations of phenol.

Based on the report of other organisms, the Haldane parameters of psychotropic *P. putida* LY1 were almost within the range.

The research on *Pseudomonas* aeruginosa to remove phenol and the benzoic acid was done by Razika et al. [58]. The microbe prefers phenol compared to benzoic acid as the carbon source but within a range below 80 mgL-1. Besides, Hank et al. [59] and Zheng et al. [60] also reported the biodegradation activitis of *Pseudomonas aeruginosa*. Zheng et al. [60] reported that *Pseudomonas aeruginosa* strain HSD38 able to degrade 500 ppm initial concentration of phenol but was incapable of tolerating the concentration which is higher than 700 ppm.

Chakraborty et al. [61] did an investigation on the native bacterial isolated from the waste of a coke processing plant for phenol degradation. One strain namely as ESDSPB2 was the highly effective for the removal of phenol, as sole carbon and energy source. From an initial concentration of 200 mgL-1 it degraded to 79.84 ± 1.23 mgL-1 The optimal conditions for phenol elimination were at pH 7, 30ºC and 0.25 % glucose.

A strain TW1 originated from industrial wastewater treatment plant of Coke company (Cairo, Egypt) were isolated by Essam et al. [62]. Morphological and physiological analysis revealed that it was gram negative, single and cluster short-rod, motile and strictly aerobic, and partial 16S rRNA sequencing classified TW1 in the beta group of Proteobacteria as *Alcaligenes*. Strain TW1 tolerated various PAHs and monocyclic aromatic compounds. Interestingly, the Haldane model finely described it as it utilized up to 1200 mg phenol as a sole source of carbon and the growth kinetics.

Castillo et al. [63], explores the potential of the bacterial communities in cork-processing wastewaters. Minimal numbers of bacteria was found when four pool samples were analyzed by DGGE but there were similar bands in all profiles were showing some common microorganisms presence in all pools. The cultivable aerobic bacteria were isolated and further tested for their tolerance towards phenol and two chlorophenols. 16S rDNA sequencing identified the most resistant strains had been grouped as gram negative (*Enterobacter, Acinetobacter, Serratia* and *Stenotrophomonas*) and gram positive bacteria (*Arthrobacter* and *Bacillus*) that were common (chloro) phenol degraders. From the identified strains, three of them were capable tolerating up to 10 mM phenol and 1.5-2.0 mM chlorophenols. All strains had phenol hydroxylase activity. Some of the strains seem to used meta fission pathway to degrade phenol whereas the others might use both pathways. Acridine staining, epifluorescence microscopy and SEM were applied to detect the formation of biofilms onto the surface of residual small cork particles.

Mohite et al. [64] reported the isolation of two bacterial strains OCS-A and OCS-B from an oil-contaminated soil. Both were aerobic, phenol tolerance and had been degraded about 90% of 100 mg/L phenol in 80 hours via meta cleavage pathway. The isolates were identified as *Proteus mirabilis* and *Citrobacter freundi* according to the morphological, physiological and biochemical characteristics referred to Bergey's manual of systematic Bacteriology.

Isolation of microorganism from wastewater of coking industries attracted many scientists in China. Cao et al. [65] also identified bacteria that are responsible for phenol biodegradation from the activated sludge of aerobic and anaerobic pools. 28 species from 20 genera obtained belongs to beta and gamma-Proteobacteria. Based on 16s rDNA sequence, the efficient degraders was known as *Pseudomonas monteilii* GCS-AEJ-1 and *Pseudomonas plecoglossicida* GCS-AN-J-3, with a degradation ability of 94.6% and 92.2% respectively.

16SrDNA gene sequence analysis and also the physio-biochemical characteristics were applied by Yu et al. [66] to classify the phenol-degrading bacterium. The identified *Bacillus cereus* B3, was originated from the coking wastewater of Coking Plant of Shougang in Beijing. *B. cereus* achieved the optimum growth conditions at 37ºC, pH 9.0 with 10% of inoculum. 800 mg L^{-1} was the maximum concentration of phenol for the strain. The addition of glucose inhibits the degradation of phenol.

Banerjee et al. also isolated *Bacillus cereus* that identified as AKG1 MTCC9817 and AKG2 MTCC 9818 for biodegradation of phenol by free and immobilized cells in Ca-alginate gel. pH 6.7 and 6.9 was the optimal phenol degradation of AKG1 and AKG2 in 3% alginate. The immobilized cells of AKG1 and AKG2 improved tolerance toward 2000 mgL-1 phenol with 50% degradation within 26 and 36 days, respectively. Haldane and Yano model represented the degradation kinetics of phenol by free and immobilized cells of these two strains [67].

Bacteria designated as *Acinetobacter lowffii* strain UW7 according to morphological, physiological and biochemical properties, supported by 16S rRNA gene sequence was originated from wastewater treatment plant in a coking chemical factory. The best growth and phenol degradation was achieved at pH 7 and 30 °C with 25 g/L as the effective degradation rate but could not tolerate up to 4.0 g/L [68].

Another Asia country that was involved in the isolation of phenol-degrading bacterium was Malaysia. In 2011, a research group reported about 115 isolation of bacterial from industrial sites and farms. The isolated strains were tested for phenol biodegrading capability in minimal salt media with 500 mgL-1 phenol. Out of thirty seven bacterial isolates exhibited phenol degrading capability but the best isolates was identified as *Acinetobacter* sp. strain AQ5NOL 1 according to partial 16S rDNA sequencing as well as BiologTM GN plates. High degradation rate were achieved in an optimum condition, 30oC, pH 7 and with the addition of 0.04% (w/v) of $(NH_4)_2SO_4$ and 0.01% (w/v) NaCl in the optimum condition, the strain could tolerate until 1500 mgL^{-1} [69].

Another work done by Arif et al., 2012 [70] reported the isolation of phenol-degrading *Rhodococcus* sp. strain AQ5NOL 2, identified via 16S rDNA analysis. Optimization of cultural requirement revealed that *Rhodococcus* sp. AQ5NOL 2, degraded phenol in broad optimum temperature (20°C-35°C) and preferred ammonium sulphate, as nitrogen source for growth in phenol containing medium. Strain AQ5NOL 2 tolerate up to 2000 mgL-1 phenol using meta-pathway and was inhibited by 1 mgL-1 of Zn^{2+}, Cu^{2+}, Cr^{6+}, Ag^{+} and Hg^{2+}. The growth of this strain was modeled using Haldane kinetics with maximum specific growth rate (μmax) of 0.1102 per hour, K(s) (half-saturation constant)=99.03 mgL^{-1}, and K(I) (substrate inhibition constant) = 354 mgL^{-1}.

In Vietnam four strains of aerobic bacteria (designated D1.1, D1.3, D1.4, and D1.6) isolated from soil contaminated with dioxin (Da Nang airport's area) were isolated. Their potential to degrade phenol was tested and the results revealed that these bacteria were highly effective for phenol removal. Strain D1.4 degraded 54.84% of 100 mg/L and 44.19% from the initial concentrations of 1000 mg/L phenol within 120 hours and the other strain D1.6 degraded 66.45% of phenol from the initial concentration of 1500 mg/L. A positive effect on the phenol degradation activity also observed with the combination of those bacteria in the same medium [3].

Mohanty [71] isolated *Pseudomonas* sp. NBM11 from phenol-contaminated soil from hospital waste. Taguchi method was applied to study the physiological parameters resulted with 30ºC as the optimum temperature and 7 as the best pH. Free cells of *Pseudomonas* sp. NBM11 completely degrades up to 1000 ppm of phenol within 168 hours while more than 1100 ppm inhibited the growth. While using immobilized cells, the isolated strain was able to degrade up to 1000 ppm of phenol within 48 hours. The microbe that has been reported has 199 bp genes that encode the 60.523 kD of phenol hydroxylase P3 component, a component of the enzyme phenol hydroxylase.

A Thermoanaerobacterium-rich sludge dominated by *Thermoanaerobacterium thermo-saccharolyticum, Thermoanaerobacterium aciditolerans, Desulfotomaculum* sp., *Bacillus coagulans* and *Clostridium uzonii* were used to test phenol degradation ability in palm oil mill effluent (POME) with 100 to 1000 mgL^{-1} of phenol concentration. 65% of 400 mgL^{-1} phenol was removed with acetic acid and butyric acid as the main metabolites. Response surface methodology (RSM) was applied to study the phenol removal efficiency and the effects of oil palm ash, NH_4NO_3 and Fe^{2+} concentration on hydrogen production and the result shown that 200 mgL^{-1} Fe^{2+}, 300 mgL^{-1} NH_4NO_3 and 20 gL-1 oil palm ash in POME will increased phenol degradation efficiency, with predicted 3.45 L hydrogen production and 93% phenol degradation efficiency. High reproducible results were obtained in a confirmation experiment under optimized conditions with 3.43 L hydrogen production and 92% phenol degraded. Meanwhile, continuous stirred tank reactor was used and 4.2 L hydrogen was produced with 92% phenol degraded [72].

Chen et al. [73] isolated *Ochrobactrum* sp. CH10 from soil of wetland in Beijing. Within 24 hours, the strain grows and efficiently degrades 100% of phenol in medium containing 400 mgL-1 phenol with pH 7.0 as the initial pH at 30 ºC and with 5% of inoculum. For 900 mgL^{-1} phenol, 92.3% has been degraded within 44 hours and 82.2% for 1000 mgL^{-1} phenol in 48 hours. The degradation kinetics was done according to Haldane's model with parameter; (maximum specific rate) upsilon (max), = 0.126 per hour, K(s) (half-saturation constant) = 23.53 mgL-1 and K(I) (inhibition constant) = 806.1 mgL^{-1}.

In another part of China, Liu et al. [74], successfully isolated a bacterial strain as an excellent bacterial candidate for the biotreatment of high-strength phenol-containing industrial wastewaters. Samples of activated sludge were taken from a coke plant in Wuhan, China. The bacteria known as *Pseudomonas aeruginosa* WUST-C1 based on the 16S rDNA sequence alignment. Beside phenol, the strain could grow on pyrocatechol, alpha-naphtol, hydroquinone, naphthalene, iso-quinoline and indole in an aerobic condition. *P. aeruginosa* WUST-C1 was unaffected by ampicillin and chloromycetin. In 36 hours, WUST-C1 has completely degrades 1200 mgL^{-1} phenol. The cell growth kinetics of WUST-C1 was investigated at 35 °C with a range of 50 to 1600 mgL^{-1} as the initial phenol concentrations ranging as described by Halden equation. The Haldane parameters are suitable to grow WUST-C1 on phenol were, μmax = 2.47 h per hour, *K(s)* (half-saturation constant) = 48.7 mg L^{-1}, and K(I) (inhibition constant) = 100.6 mg L^{-1} (R^2 = 0.988).

Sun et al. [75], had isolated phenol-degrading bacteria from activated sludge from an aeration tank of the cooking wastewater treatment plant in China. Five strains were identified as *Pseudomonas* spp. through physiological, morphological and biochemical test. The strains are capable to degrade 55.5% to 79.6% of initial 500 mgL-1 phenol within 24 hours incubation at 28ºC and 170 rpm agitation.

Another group of scientist from China screened and isolated four phenol degraders from activated sludge in coking plant. The strains were identified as *Sphaerobacter*, *Acinetobacter baumannii, Comamonas testosterone* and *Novospingobium naphtalenivorans* based on morphological, physiological and 16S rDNA identification. The highest degradation efficiency was owned by *Comamonas testosterone* with high tolerance up to 2000 mgL^{-1} of phenol and degrades 100% of 1000 mgL^{-1} of phenol within 48 hours. The optimum conditions for phenol degradation by the best strain was in initial pH 7.5-8.5, at 30-40ºC and 150r x min^{-1} of shaking rate [76].

Meanwhile, He Xi-Pu et al. [77], isolated a strain named F5-2 that has high capability to utilized phenol as the sole source of carbon was isolated from the wastewater of a paper mill plant. According to the morphology, biochemical characteristics, and the 16S rDNA sequence, this strain was known as *Arthrobacter* sp. 1500 mgL^{-1} is the highest phenol concentration for the strain which could tolerate temperature within a range 20-40 ºC, pH 5.0-9.0, salinity of 0-40 gL^{-1} with shaking rate of 200 r x min^{-1}. The *Arthrobacter* sp. F5-2 degrades 96.13% of the phenol within 57 hours with the initial phenol concentration of 800 mgL^{-1} and 2% inoculum size of the culture medium via ortho-pathway.

A paper reported by Gracioso et al. [78], explained their work on isolation and characterization of a phenol-degrading bacterium, *Achromobacter* sp. strain C-1, a gamma Proteobacteria from an industry in Cubatao, Brazil. 16S rRNA gene sequences showed that it has 99% similarity to *Achromobacter xylosoxidans*. Physiology and morphological characteristics showed that it is a Gram negative, short-rod or coccobacillary bacterium, non-motile and strictly aerobic on medium containing phenol, catechol, m-cresol and o-cresol. The phenol degradation pathway of phenol or glucose for *Achromobacter* sp. strain C-1 was determined by two-dimensional SDS-polyacrylamide gel (2D SDS PAGE). Nine proteins spotted on the gel were exclusively induced from phenol-cultures of strain C-1. Using peptide mass fingerprinting, three phenol-degrading enzymes (4-hydroxy-2-oxovalerate aldolase, hydroxymuconic semialdehyde dehydrogenase and phenol degradation meta-pathway protein) were identified. The result supported by tandem mass spectrometry of selected peptides and also analysis of the metabolite produced that suggests the phenol degradation was via the β-ketoadipate pathway using catechol 2,3 dioxygenase enzyme.

Using physiological, biochemical and 16S rDNA methods, Liu et al. [79], identified a strain that used phenol as carbon source known as *Achromobacter*. The strain could completely degrade 600 mgL-1 phenol within 67 hours and tolerates up to of 1000 mgL-1 of initial concentration. The degradation rate was maximized at pH 7, 30ºC with 0.3% salinity.

Bhattacharya et al. [80], demonstrated that the three from 28 isolates identified by 16S rDNA sequencing as *P. aeruginosa* MTCC 1034, *P. fluorescens* MTCC 2421 and B. cereus ATCC 9634 isolated from Bangalore industrial effluent were capable of degrading phenol. The optimum conditions for growth and phenol degradation were 30°C, pH 7.0, 0.1% of glucose and peptone. A degradation of 74%, 76% and 83% was obtained for B. cereus ATCC 9634, *P. fluorescens* MTCC 2421 and *P. aeruginosa* MTCC 1034 respectively within 48 hours.

A strain RTE1.4, originated from effluents by a chemical industry was grown well in a medium containing phenol and its derivative, named guaiacol, 2,4-dichlorophenol and pentachlorophenol. This bacterium was identified as *Acinetobacter* sp. by 16S rRNA gene analysis supported with biochemical, morphological and physiological characteristics. It decomposes phenol at 200 to 600 mg/L at wide pH range from 5 to 9 and temperature 25-37 ºC. The phenol degraded by the ortho-pathway [81].

Halophilic microorganism for biotreatment of phenol was identified as *Modicisalibacter tunisiensis, Halomonas organivorans* and *Arhodomonas aquaeolei*. These three bacteria grown on hypersaline media with a 100 gL^{-1} of total salts. The strains habor the genes encoding catechol 1,2-dioxygenase and protocatechuate 3,4-dioxygenase that have been cloned from *Halomonas organivorans* [82].

Elahwani et al. [83] isolated six phenol degrading bacteria and identified one of them as *Alcaligenes* sp. AM4 using 16S rDNA sequencing. The Plackett Burman, a statistical experimental design was applied to optimize the medium and culture conditions. The optimal predicted formula was 240 mgL^{-1} of phenol, 0.15 of inoculum for 37.5 ml of culture volume containing 500 mgL^{-1} NH_4SO_4, 750 mgL^{-1} K_2HPO_4 and KH_2PO_4, 300 mgL^{-1} $MgSO_4$ and 250 mgL^{-1} of NaCl.

Kumari et al. [84] isolated *Pseudomonas aeruginosa* from an activated sludge plants in South Africa. It was identified based on morphology, biochemical as well as 16S rRNA analysis. 600 mgL^{-1} was the maximum concentrations for the isolated *P. aeruginosa* with a spesific growth rate at 0.209.

Awan et al. [85] collected soil samples from industrial area of Lahore, Jahang, and from Gomal University D.I.Khan. The locally isolated soil bacteria were tolerated up to 2.5 g/100 ml phenol. The degradation ability of these bacteria was optimum at 35 ºC and pH 7 with shaking speed of 120 rpm.

A phenol degrading *Alcaligenes faecalis* was isolated from coke oven wastewater of Durgapur steel plant, India. It was identified via 16s rDNA analysis, biochemical, and morphological study. This isolate tolerated high phenol concentration (2100 mgL^{-1}) [86].

Finally, Maza-Marquez et al. [87] recently reported that, the bacterial community of an olive washing water (OWW) storage basin was characterized and cultivated. In order to select high phenol-degrading strains, effluent pollutants reduction media have been applied containing the increasing amounts of polyphenols, Two isolate, *Raoultella terrigena* and *Pantoea agglomerans* were identified through 16S rRNA gene sequencing and shows the ability to degrade 93% of phenol while at the same time reduce the BOD by 91%, COD by 89% and colour by 62%.

For anaerobic system, few investigation has been done such as an investigation of phenol degradation in the strictly anaerobic iron-reducing deltaproteobacterium *Geobacter metallireducens* $GS^{-1}5$. Enzymatic, metabolite, transcriptome and proteome analyses has been applied to characterize the strain [88]. Another investigation by Qui et al. isolated anaerobic bacteria that identified by 16S rRNA as Syntrophorhabdus aromaticivorans also in the class of Deltaproteobacteria [89]. All the isolation and characterization of phenol degrading bacteria was summarized in Table 1.

Phenol-Degrading Fungi

Mycobioremediation of phenol by eukaryotes have been reported by a number of fungal and mold strains. Among the commonly genera that was isolated from soil are known as *Fusarium, Alternaria, Penicilium* and *Graphium* [90, 91]. Within the recent five years, a few other works have been described (Table 2).

In 2008, the isolation of fungi for biodegradation of phenol has been done from crude oil contaminated soil at Rio Grande harbor area, in southern Brazil [7, 13]. The isolated *Aspergillus* sp. LEBM2 has been applied in phenol degradation experiments. In 72 hours 3.76 mg L^{-1} h^{-1} of biodegradation rate achieved with addition of 500 mgL^{-1} glucose and 20% inoculum using 200 rpm agitation. Aspergillus sp. LEBM2 was able to consume up to 989 ± 15 mg L^{-1}.

A novel phenol degrading fungi, *Paecilomyces variotii*, JH6 has been identified by standard morphological and phylogenetic analysis. The strain was isolated from activated sludge. *P. Variotii* JH6 capable to degrade 1800 mgL^{-1} of phenol at 37 ºC in pH 5 medium containing 100 mg L^{-1} glucose. It also tolerates other compound that was commonly found in wastewater such as m-cresol and quinoline [92].

Beside outdoors such as soil and effluent, a study on indoor fungi isolated from paint-coated walls of sick-building has also been done. Researcher from Jordan assumed that the paint was containing toxic aromatics compounds, and therefore, the fungi that are able to survive, probably could degrade the compounds. Using cellotape and PDA as the growth medium, two genera of fungal have been isolated. Strain that have pale brown, cylindrical and catenulate conidiospores identified as *Alternaria* sp. whereas Penicilium sp. was identified based on hyaline and straight conidiospore. The capability of both strains to utilize phenol demonstrated by the increasing in mycelial biomass in cultures containing 10 μgL^{-1} of phenol with 0.46 g for Alternaria and 0.48 g for Penicilum. The Alternaria could also tolerate up to 20 μgL^{-1} of phenol with 0.45 g of mycelial. Analysis by gas chromatography parallel with mycelial growth verified the phenol consumption over time of incubation of these two fungi [93].

A year before that, a highly phenol-degrading fungi, *Aspergillus flavus*, originated from polluted soil of a workshop at Makkah, Saudi Arabia has been isolated. The strain degraded 100 mgL^{-1} of phenol in 72 hours and also tested in 300, 500 and 700 mg L^{-1} phenol that took 96 hours, 120 hours and 240 hours respectively. It tooks more than 240 hours to degrade 900 and 1000 mgL^{-1} whereas 2000 and 3000 mgL^{-1} was the lethal concentration for the growth of *Aspergillus flavus*. The optimizations of the medium and growth conditions were done using Plackett-Burman and Box-Behnken statistical design [94].

Another report by this genus was an *Aspergillus niger* a filamentaous fungi isolated from waste of paper manufacture in India was capable to degrade phenol because of its ability to tolerate up to 268 mgL^{-1} of the effluent. In this study, a mixture of 0.5 mL Folin-Ciocalteau reagent and 1 mL sodium carbonate (200 mgL^{-1}) was added to 10 mL sample for phenol determination analysis at 725 nm. After five days of incubation, phenol concentration using free and entrapped cells system in calcium alginate decreased to 110 mgL^{-1} and 119 mgL^{-1} respectively. Meanwhile, the experiment using synthetic wastewater containing 250 mgL^{-1} phenol revealed that *Aspergillus niger* reduced the phenol concentration until 150 mgL^{-1} using free cells and 28 mgL^{-1} by the immobilized cells in five days of incubation. Cells entrapment of *Aspergillus niger* showed a better capability as it reduce the cell's adaptation time. The cells were more resistance towards high phenol concentration because the capsule forms a network to protect the cells against the toxicity of phenol [95].

Phenol-Degrading Yeast

Yeast, a single cell mold which also involved in phenol degradation [96]. Some of the examples that were isolated and characterized were *Trichosporon cutaneum* [97, 98, 99], *Candida* spp. [100, 101, 102] and *Fusarium* sp. [103]. The effort was prolong until the current years as listed is Table 3.

A research group isolated *Aureobasidium pullulans* FE13 which has the ability to degrade phenol. The yeast was originated in stainless steel effluents. Research using free and alginate-immobilized cells proven that the strains functioning in both system with a similar degradation rate of 16 mM and 18 mM phenol with 18.35 mgL^{-1} and 20.45 mgL^{-1} respectively [7].

Patel and Raikumar, 2009 [104] isolated a phenol degrading yeast from the soil sample collected from a landfill site, in Ahmedabad, India. The isolate was identified as *Saccharomyces cerevisiae* based on growth charactristics on Sabouraud's Dextrose Agar, microscopic studies and biochemical tests. *S. cerevisiae* tolerates phenol up to 800 mgL^{-1}. Phenol degradation rate range achieved from 8.57 to 100% for the concentration of phenol from 800 mgL^{-1} to 200 mgL^{-1}).

A soil yeast involved in phenol biodegradation at an agricultural field site was identified as *Trichosporon multisporum* by an amplification of the 18S-28S internal transcribed spacer (ITS) region using fungi-specific PCR and morphological characteristics. High-performance liquid chromatography and turbidometeric analyses showed that the culture was able to metabolize and grow on 200 mgL^{-1} phenol in an aqueous mineral salts medium within 24 hours at room temperature [105].

Zhou et al. [106] optimized the phenol degradation process by *Candida tropicalis* Z-04 with statistical experimental design, Plackett-Burman and Central Composite Design as well as Response Surface Analysis. Correlation between predicted and experimental values showed the removal of 99% of phenol achieved using 410 mgL^{-1} of yeast extract, 1.03 gL^{-1} phenol, 1.43% of inoculum size (v/v) at 30.04 ºC. Involvement of Candida albicans in phenol degradation activity have also been reported [107, 108, 109].

A latest work applied using thermotolerant *Candida tropicalis* isolated from a chemical contaminated soil sample. The strain was capable of degrading 100 mgL^{-1} phenol completely from 20-42 °C. In a minimum mineral salt medium, it completely degrades phenol at an initial concentration up to 1000 mgL^{-1}. The optimum temperature and initial pH were 35 °C and 8, respectively. Most tested sugars such as sucrose, glucose, maltose, fructose, galactose, mannose and sorbitol at 10 mM concentration, inhibited phenol degradation completely and similarly when oxygen in the medium was gradually decreased. Mean while, most of the organic acid such citric, lactic and succinic acids of 10 mM concentration, showed no effect. Metal ions such as Co^{2+}, Cu^{2+} and Ni^{2+} showed strong inhibition to phenol degradation whereas Zn^{2+} showed a slight inhibition. The strain-metabolized phenol via catechol ortho-clevage pathway and the catechol 1,2-dioxygenase specific activities was comparable to other *Candida* spp. [110].

Phenol-Degrading Algae

Although algae have been reported to be able to bioremediate phenol but inadequate information about algae were found in recent literature. Lika and Papadakis [111], reported about the development of a mechanistic model for photosynthetic algae. Application of this model may help photosynthetic algae to degrade phenolic compound under heterotrophis, photoautotrophic and mixotrophic conditions. Isolation on phenol-degrading algae will give benefit to the tropical countries especially in Asia that obtains sunlight around the year as phenols are frequently found in many near-shore marine systems.

CONCLUSION

The biotreatment of phenol in domestic and industrial effluents is very crucial due to its persistent and toxic effect. Information from this review has made a list of works done by scientists across the globe on the isolation and characterization of microorganisms that are capable to degrade phenol. A lot of attempts were made to isolate, screen and select the most efficient microorganisms capable of degrading phenol. Most of the isolated microbes either bacteria, fungi or yeast were originated from phenol contaminated samples. The process parameters for maximum degradation by the efficient isolates were optimized. All reports demonstrated that the isolated microorganisms were capable of degrading phenol and the degradation was greatly influenced by the cultural conditions like pH, agitation rate and temperature. Certain biodegradation process induced with the presence of auxiliary carbon and nitrogen sources. Optimum conditions for growth and phenol degradation for the majority of mesophilic microorganisms were 30-37 °C and in neutral pH with 150 rpm agitation. 0.1% of glucose and peptone also needed by certain bacteria especially *Pseudomonas*. The degradation rate varies among the

microorganism between 75% to 100% depending on the concentration within a specific incubation period. Some bacterial isolates were stable even at higher concentrations (100-500 mgL^{-1}). The degradation kinetics also varies among the microbes and conditions. The excellent achievement of the research lies in the fact that the native isolates from the industrial waste or effluents contaminated with phenol and its derivatives do not need much attenuation to degrade phenol. Majority of the studies on biodegradation of phenol

degradation have been performed aerobically, and there are only some organisms could degrade phenol anaerobically. Based on the findings, we confers that microorganisms have a remarkable potential for its application in bioremediation and waste management, including the detoxification of phenolic wastes.

Table 1. Phenol-degrading bacteria.

Bacteria	Type of sample	Maximum phenol concentration tested (mgl^{-1})	Refer-ences
Acinetobacter Pseudomonas Comamonas	Waste water and activated sludge of petroleum chemical plant	1200	[50]
Psedomonas flurescence	NA	500	[51]
Acinetobacter	Paper mill effluent and sediment core of pulp	200	[52]
Pseudomonas sp.	Pharmaceutical disposal wastewater	1000	[53]
Acinetobacter calcoaceticus	Waste water of coal industry	1200	[54]
Alcaligenes Acinetobacter Rhodococcus	Trees of fraxinus pennsylvanica near of a swine production facility	NA	[55]
Acinetobacter sp. *Sphingomonas* sp.	Activated sludge and phenol contaminated soil	800	[56]
Pseudomonas putida Pseudomonas nitroreducens Rhodotorula glutinis	Soil of natural forest of pine and bamboo	NA	[57]
Pseudomonas putida	NA	80	[58]
Pseudomonas aeruginosa	NA	NA	[59]
Pseudomonas aeruginosa	NA	700	[60]
NA	Waste of coke processing plant	NA	[61]
Alcaligenes	Waste water of coke company	1200	[62]
Enterobacter Acinetobacter Serratia Stenotrophomonas Arthrobaster Bacillus	Waste water of cork manufacturing	941	[63]
Proteus mirabilis Citrobacter freundi	Oil contaminated soil	100	[64]
Pseudomonas monteilii Pseudomononas plecoglossicida .	Activated sludge	NA	[65]

Table 1 (Continued). Phenol-degrading bacteria.

Bacteria	Type of sample	Maximum phenol concentration tested (mgl^{-1})	Refer-ences
Bacillus cereus	Waste water of coking industry	800	[66]
Bacillus cereus	Na	2000	[67]
Acinetobacter lowffii	Waste water of chemical factory	4000	[68]
Acinetobacter sp.	Industrial site and farm	1500	[69]
Rhodococcus sp	NA	2000	[70]
Pseudomonas sp.	Phenol-contaminated soil from hospital waste	1000	[71]
Thermoanaerobacterium thermosaccharolyticum Thermoanaerobacterium aciditolerans Desulfotomaculum sp. *Bacillus coagulans Clostridium uzonii*	Palm oil mill effluent	1000	[72]
Ochrobacterium	Soil of wetland	1000	[73]
Psedomonas aeruginosa wust-c1	Coke plant	1200	[74]
Pseudomonas spp.	Activated sludge of coking waste water treatment plant	500	[75]
Sphaerobacter Acinetobacter baumannii Comamonas testosterone Novospingobium naptalenivorans	Activated sludge in coking plant	2000	[76]
Arthrobacterer	Waste water of paper mill plant	1500	[77]
Achromobacter xylosoxidans	Industry	NA	[78]
Achromobacter	NA	1000	[79]
Pseudomonas aeruginosa Pseudomonas fluorescens Bacillus cereus	Industrial effluent	NA	[80]
Acinetobacter sp.	Effluents of chemistry industry	600	[81]
Modicisalibacter tunisiensis Halomonas organivorans Arhodomonas aquaeolei	NA	NA	[82]
Alcaligenes sp.	NA	240	[83]
Pseudomonas aeruginosa	Activated sludge plants	600	[84]
NA	Industrial area	250	[85]
Alcaligenes faecalis	Coke oven wastewater of steel plant	2100	[86]
Raoultella terrigena Pantoea agglomerans	Olive washing water storage basin	NA	[87]
Syntrophorhabdus aromaticivorans	NA	NA	[88]
Geobacter metallireducens	NA	50	[89]

Table 2. Phenol-degrading fungi.

Fungi	Type of sample	Maximum phenol concentration tested (mgl^{-1})	References
Aspergillus sp.	Crude oil contaminated soil	500	[13]
Paecilomyces variotii	Activated sludge	1800	[92]
Alternaria sp. *Penicilium* sp.	Paint-coated walls of sick building	0.02	[93]
Aspergillus flavus	Polluted soil of workshop	1000	[94]
Aspergillus niger	Waste of paper manufacture	250	[95]

Table 3. Phenol-degrading yeast.

Yeast	Type of sample	Maximum phenol concentration tested (mgl^{-1})	References
Aeroebasidium pullulans	Stainless steel effluents	1506	[7]
Saccharomyces cerevisiae	Soil of landfill site	800	[104]
Trichosporon multisporum	Agriculture field	200	[105]
Candida tropicalis	NA	1030	[106]
Candida albicans	NA	NA	[107, 108, 109]
Candida tropicalis	Chemical contaminated soil	1000	[110]

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