Microbial degradation of phenol by locally isolated soil bacteria

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Phenol compounds are extensively sprinkled in nature so phenol is normally liberated because it is intermediate in the metabolism of microorganism through the decay of wood materials. The normal delivery of phenol containing substances become gravely enhanced as a result of the discharge of manufacturing by-product into the surroundings. Wastes delivers from textile, petrochemical as well as coal industries contain phenol derivatives in very lofty attention consequently it is an obligation to get rid of phenol containing compounds from the surroundings. Among a variety of procedures existing for elimination of phenols, biodegradation is an atmosphere gracious and outlay efficient method. The present study was conducted to take advantage of the potential of locally isolated soil bacteria to degrade phenol. The isolates obtained from different soils were grown on nutrient agar plates, nutrient broth and mineral salt media. Mineral salt media was used for the degradation of phenol. Phenol degradation was examined along with dry cell weight. The locally isolated soil bacteria were made potent up to 2.5 g/100 ml phenol concentration. These bacteria were examined for their degrade ability by revealing them to different temperatures and 35°C was found as the optimum temperature for the degradation of phenol and 20°C or 50°C were minimum. The ability of bacterium to degrade phenol at different pH was also observed. Maximum degradation was recorded at pH 7 and the pH below 4 or above 9 was examined as minimum. Ability to degrade phenol by locally isolated soil bacteria was also examined by trying different shaking speeds compared to stationary phase. The speed of 120 rpm was found to be the optimum. Shaking speed of 80 rpm and 160 rpm were no more effective.

Keywords: Agar plates, degradation, phenol, soil bacteria.

INTRODUCTION

Phenol is a poisonous and dangerous substance still at low concentration and it is necessary to minimize the phenol concentration in wastewater to satisfactory levels. There are number of method accessible for handling of phenol, biological handling is particularly attractive as it has likely to approximately involve in the degradation of phenol entirely by producing harmless last yield and least derivative dissipate production (Hill and Robinson, 1975).

Resting on the health be sound familiar (Calabrese, and Kenyon, 1991) causes deaths amongst adults have
reported with intake of phenol with the series starting 1.5 to 33 g (Prpich and Daugulis, 2005). The little phenol volatility and its attraction to water create oral absorption of infected water is the most hazards to human being. (Prepich and Daugulis, 2005).

Biologically phenol degradation have studied broadly and several investigations have reveal that phenol may be aerobically tarnished via a widespread diversity of microorganisms as well as pure bacterial culture like Acinetobacter calcoaceticus, Bacillus stearothermophilus, Burkholdearia cepacea G4 (Folsum et al., 1990; Schroder et al., 1997; Solomon et al., 1994) Alcaligines eutrophous (Hughies et al., 1984) Nocardia species, Pseudomonas picketti, Pseudomonas putida Pseudomonas resinvorans and Ralstania eutrapha.

Among microorganisms listed as excellent biodegraders of phenol, the uncontaminated culture of Pseudomonas are the mainly utilize knowingly for metabolic passageway studies and their ability to utilize or degrade many other aromatic compounds. The genus Pseudomonas comprises an important group of bacteria with environmental application in bioremediation and biological control. A large diversity of microorganisms is known to be competent of metabolizing many of the organic pollutants or chemicals generated and discharged (Kobayashi and Rittman, 1982). Metabolic processes are governed by the action of enzymes. Enzymes are precise for each type of reaction, thus microbial metabolism is a procedure of energy conversion continual by oxidation reduction reactions, providing the crucial resource of energy. The nutritional requirements of microorganisms normally comprise nitrogen, phosphorus, potassium, sodium, calcium, magnesium, iron, trace elements and carbon. Dissolved oxygen is required for the respiration of the microbes under aerobic conditions. Dissolved oxygen can be considered as a nutrient related to other dissolved nutrients. Soil types affect the rate of mass transport of nutrients, pollutants of air, water and pH adjuster. This consequence on mass transportation in go back affect the degradation process.

The current study was designed with main aim to screen out soil bacteria which have phenolic degradation ability, to increase phenol degradation potency by the development of resistant potency in isolated strains and optimization of cultural condition for phenol degradation by various physical and chemical methods.

MATERIALS AND METHODS

Collection of samples

Soil samples were collected from industrial area of Lahore, Jahang, and from Gomal University D.I.Khan. Isolation of bacteria was carried out in the microbiology research laboratory, department of Biological sciences, Gomal University Dera Ismail Khan and then all the isolates were screened for biodegradation of phenol.

Media composition and Cultural Condition

Following media were used for the growth of microorganisms.

i. Nutrient agar

Nutrient agar consists of following composition. Peptone 0.5 g/100ml, Beef extracts 0.3 g/100ml, Agar 1.5 g/100ml and Distilled water 100 ml.

ii. Mineral salt medium

Following components were the basic ingredients in mineral salt medium (Banerjee et al., 2001).

KH₂PO₄ (0.5 g/l), K₂HPO₄ (1.5 g/l), NaCl (0.5 g/l), Mg SO₄.7H₂O (0.5 g/l), NH₄NO₃ (1 g/l), FeSO₄.7H₂O (0.01 g/l), CaCl₂.2H₂O (0.01 g/l) and NH₄SO₄ (0.5 g/l).

iii. Broth media

Broth media consist of following compositions in 100ml distilled water. Peptone 0.5 g, Beef extracts 0.3 g and NaCl 1g.

Analytical Method

The density of cells was observed spectrophotometrically by measuring the absorbance by using a Shimadzu ultraviolet spectrophotometer at a wavelength of 600 nm. The optical density values were then transformed to dry cell mass by means of a dry cell weight calibration curve. The dry cell mass density (mg/100ml) was found to follow the Regression equation: X (mg/100ml) = 434.8 (OD600) (Banerjee et al., 2001).

Phenol was analyzed through photometric method by using 4-aminoantipyrine like the colouring substance and measure the absorbance at 510 nm with a Shimadzu ultraviolet spectrophotometer (Yang and Humphrey, 1975; Oboirien et al., 2005).

Isolation of Bacterial Strains

Different soil samples were obtained from industrial areas of Lahore, Jahang and from the soil of Gomal University Dera Ismail Khan. Total 3 sets of experiments were carried...
out for the isolation of bacteria and whole the procedure was repeated for each set of experiment. A sample of 5g soil was taken and homogenized it. Dissolved the soil sample in 100 ml water. One ml of soil solution was taken and serially diluted in normal saline. A volume of 0.1ml of solution from normal saline was spread evenly over the surface of nutrient agar plates using sterile glass spreader to get a single colony. Whole the apparatus was kept in incubator at 30°C for 24h.

All media (except phenol) was autoclaved at 120°C for 20 minutes for sterilization before using. After 24h colonies were observed over the surface of nutrient agar plates. Counted the number of colonies, assign them names and shifted them separately into liquid broth media. Mineral salt media of 100ml was taken and sterilized then added 0.5g phenol and 10ml inoculums of single colony from liquid broth. The flasks were cap by means of cotton plugs and sited the whole apparatus in shaking incubator for 24h at 37°C at 120 rpm.

After 24h a sample of 10ml was taken from the flask of shaking incubator, note the optical density at 600nm at spectrophotometer and then centrifuge at 3000 rpm for 20 minutes. After centrifugation pellet was taken for dry cell weight and supernatant for estimation of phenol. Repeat the whole experiment with different phenol conc. in Mineral salt medium for each colony from each soil and then optimized different parameter.

**Estimation of phenol concentration**

The under grade, quantitatively phenol was estimated via measure of its absorbance at 510nm wavelength by using UV – visible Spectrophotometer (Perkin Elmer, Lambda 35, USA) and 4 amino Antipyrene as colour indicator (Oboirien et al., 2005; Yang and Humphrey, 1975).

Standard solution of phenol was prepared with known quantity of phenol started from 0.5 g/100ml, added few drops of 4 amino antipyrene as indicator until the colour of phenol becomes colorless to red. Noted the absorbance values of phenol at 510nm.Compared the absorbance values of phenol from supernatant with the absorbance values of standard to estimate the degraded phenol. Repeated the whole experiment with different phenol conc. in Mineral salt medium with each isolate from each soil and then optimized different parameters.

**Estimation of biomass**

Dry cell weight method was applied to estimate the biomass. A trial of culture broth (10 ml) was taken and centrifuged at 3000 rpm. intended for 20 minutes in centrifuge tubes. Supernatant was decant keen on small bottles and then stored at 5°C for successive estimation of phenol. Re-suspended the pellets in the deionizer water and subsequently re-centrifuged. Separated the supernatant and pellet was taken at filter paper. Dried the filter paper in an oven at 100°C for 15 h, then cooled and again weighted so that a constant dry weight was attained. The variation between the pre weighed filter paper and the final stable weight was used to estimate the dry weight of the biomass.

**Optimization of various parameters for degradation of Phenol**

Effects of temperature, pH, and agitation were observed for the degradation of phenol for all isolates isolated from each soil.

**Effect of Temperature on degradation of Phenol**

The experiments for degradation of Phenol were performed at different temperatures in shaking incubator at 120 rpm. Temperature maintained at 20°C, 25°C, 30°C, 35°C, 40°C, 45°C and 50°C in culture of mineral salt media at pH 7. Samples were collected and analyzed spectrophotometrically for Phenol concentration.

**Effect of pH on degradation of Phenol**

A sequence of experiments for degradation of Phenol was conducted to reveal effect of pH at 35°C at different time in shaking incubator at 120 rpm. PH maintained 1 to 9 in culture of mineral salt media. Samples were collected and were analyzed spectrophotometrically for Phenol concentration.

**Effect of Agitation on degradation of Phenol**

A series of experiments were performed to know the effect of agitation on the degradation of Phenol at 35°C and at pH 7 in shaking incubator. Shaking speed maintained to 80,100,120,140 and at 160 rpm. in culture of mineral media. Samples were collected and were analyzed spectrophotometrically for Phenol concentration.

**RESULTS**

Present research was conducted to exploit the potential of locally isolated soil bacteria for the degradation of phenol. These bacteria were isolated from the soil of industrial area of Lahore, Jahang and from the soil of Gomal University D.I.Khan. These isolates were grown on different media like nutrient agar plate, nutrient broth, and mineral salt media. The mineral salt media was used for the degradation of phenol.
Table 1. Screening of soil bacteria for the degradation of phenol from the soil of Lahore

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Strains</th>
<th>Degradation of phenol</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ABGD-L-3</td>
<td>+++</td>
<td>70%</td>
</tr>
<tr>
<td>2</td>
<td>ABGD-L-6</td>
<td>+</td>
<td>47%</td>
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<tr>
<td>3</td>
<td>ABGD-L-9</td>
<td>+</td>
<td>49%</td>
</tr>
<tr>
<td>4</td>
<td>ABGD-L12</td>
<td>+++</td>
<td>64%</td>
</tr>
<tr>
<td>5</td>
<td>ABGD-L-15</td>
<td>+</td>
<td>48%</td>
</tr>
<tr>
<td>6</td>
<td>ABGD-L-19</td>
<td>++++</td>
<td>71%</td>
</tr>
<tr>
<td>7</td>
<td>ABGD-L-23</td>
<td>+</td>
<td>45%</td>
</tr>
<tr>
<td>8</td>
<td>ABGD-L-27</td>
<td>+++</td>
<td>69%</td>
</tr>
<tr>
<td>9</td>
<td>ABGD-L-29</td>
<td>+</td>
<td>10%</td>
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<tr>
<td>10</td>
<td>ABGD-L-31</td>
<td>+</td>
<td>49%</td>
</tr>
<tr>
<td>11</td>
<td>ABGD-L-35</td>
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<td>67%</td>
</tr>
<tr>
<td>12</td>
<td>ABGD-L-38</td>
<td>+</td>
<td>6%</td>
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<tr>
<td>13</td>
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<tr>
<td>14</td>
<td>ABGD-L-44</td>
<td>+</td>
<td>29%</td>
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<tr>
<td>15</td>
<td>ABGD-L-46</td>
<td>+</td>
<td>15%</td>
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<tr>
<td>16</td>
<td>ABGD-L-47</td>
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<tr>
<td>18</td>
<td>ABGD-L-52</td>
<td>+</td>
<td>27%</td>
</tr>
<tr>
<td>19</td>
<td>ABGD-L-56</td>
<td>+</td>
<td>15%</td>
</tr>
</tbody>
</table>

Screening of soil bacteria for the degradation of phenol

Isolates obtained from different soil were screened for the degradation of phenol. Three sets of experiments were carried out for the isolation of bacteria and whole the procedure was repeated for each set of experiment. A sample of 5g soil was taken and homogenized it. Dissolved the soil samples in 100 ml water. One ml of soil solution was taken and serially diluted in normal saline. A volume of 0.1ml of solution was spread evenly over the surface of nutrient agar plates using sterile glass spreader to get a single colony and then plates were incubated in incubator at 30°C for 24h.

All media (except phenol) was autoclaved at 120°C for 20 minutes for sterilization before using. After 24h, colonies were observed over the surface of nutrient agar plates. Counted the number of colonies, assigned them names and shifted them separately into liquid broth media.

Screening of soil bacteria for the degradation of phenol from the soil of Lahore

Total number of colonies observed in the most diluted soil of Lahore was 58. These strains were separately grown in mineral salt media containing phenol. Out of 58, 19 were phenol degrading while 39 were phenol non degrading. Out of 19 phenol degrading bacteria only 5 were found as the most potent which degraded phenol more than 50%. These were named as ABGD-L-3, ABGD-L-12, ABGD-L-19, ABGD-L-27, and ABGD-L-35.

Screening of soil bacteria for the degradation of phenol from the soil of Jahang

Total number of colonies observed in the most diluted soil of Jahang was 56. These strains were separately grown in mineral salt media containing phenol. Out of 56, 15 were phenol degrading while 41 were phenol non degrading (Table 2) Out of 15 phenol degrading bacteria only 3 were found as the most potent which degraded phenol more than 50%. These were named as ABGD-J-2, ABGD-J-15, and ABGD-J-32.

Screening of soil bacteria for the degradation of phenol from the soil of Gomal University D.I.Khan

Total number of colonies observed in the most diluted soil of Gomal University D.I.Khan was 54. These strains were separately grown in mineral salt media containing phenol.
Table 2. Screening of soil bacteria for the degradation of phenol from the soil of Jahang

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Strains</th>
<th>Degradation of phenol</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ABGD-J-2</td>
<td>+++</td>
<td>65%</td>
</tr>
<tr>
<td>2</td>
<td>ABGD-J-6</td>
<td>+</td>
<td>47%</td>
</tr>
<tr>
<td>3</td>
<td>ABGD-J-7</td>
<td>+</td>
<td>49%</td>
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<td>ABGD-J-12</td>
<td>+</td>
<td>44%</td>
</tr>
<tr>
<td>5</td>
<td>ABGD-J-15</td>
<td>++++</td>
<td>72%</td>
</tr>
<tr>
<td>6</td>
<td>ABGD-J-17</td>
<td>+</td>
<td>34%</td>
</tr>
<tr>
<td>7</td>
<td>ABGD-J-20</td>
<td>+</td>
<td>46%</td>
</tr>
<tr>
<td>8</td>
<td>ABGD-J-22</td>
<td>+</td>
<td>19%</td>
</tr>
<tr>
<td>9</td>
<td>ABGD-J-25</td>
<td>+</td>
<td>48%</td>
</tr>
<tr>
<td>10</td>
<td>ABGD-J-28</td>
<td>+</td>
<td>40%</td>
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<tr>
<td>11</td>
<td>ABGD-J-31</td>
<td>+</td>
<td>47%</td>
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<tr>
<td>12</td>
<td>ABGD-J-32</td>
<td>+++</td>
<td>68%</td>
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<tr>
<td>13</td>
<td>ABGD-J-37</td>
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<td>43%</td>
</tr>
<tr>
<td>14</td>
<td>ABGD-J-41</td>
<td>+</td>
<td>12%</td>
</tr>
</tbody>
</table>

Out of 54, 11 were phenol degrading while 43 were phenol non-degrading. (Table 3) Out of 11 phenol degrading bacteria only 2 were found as the most potent which degraded phenol more than 50%. These were named as ABGD-G-19, ABGD-G-21.

Comparative degradation of phenol by the strains isolated from the soil of Lahore.

The phenol degrading capacity of all bacteria isolated from soil of Lahore were compared in media, the most potent were ABGD-L-3, ABGD-L-12, ABGD-L-19, ABGD-L-27, and ABGD-L-35 that degraded phenol more than 50% (Figure 1).

Comparative degradation of phenol by the strains isolated from the soil of Jahang.

The phenol degrading capacity of all bacteria isolated from soil of Jahang were compared in media, the most potent were ABGD-J-2, ABGD-J-15, and ABGD-J-32 that degraded phenol more than 50% (Figure 2).
Comparative degradation of phenol by the strains isolated from the soil of Gomal University D.I.Khan.

The phenol degrading capacity of all bacteria isolated from soil of Gomal University D.I.Khan were compared in media, the most potent were ABGD-G-19 and ABGD-G-21 that degraded phenol more than 50% (Figure 3).

DISCUSSION

Biodegradation of phenol and associated phenol containing compounds by way of various microorganisms is to be the focus of scientific concentration since many years. A huge number of innate and artificial organic compounds are
degraded by microorganisms as tiny proportion of their ordinary metabolism for energy and development.

There are factors that can control degradation ability or metabolism of microorganisms by either preventing or stimulating growth of the organisms and more carefully by affecting gene expression. Some of the factors that affect the biodegradation of phenols as well as other xenobiotic compounds are chemical structure and compound toxicity. Other are the environmental factors (Singleton, 1994.) such as pH, temperature, nutrient (mineral salt medium), oxygen ease of use (aeration and agitation), bioavailability and soil type (Talley and Sleeper, 1997). Fantastic values of the pH of the medium (pH values less than 3, or greater than 9 or 10) as well as rapid changes in pH in which the microbes are present can slow down its growth. However, studies on phenol degradation in the laboratory are usually carried out at/near neutral pH values (Annadurai et al., 2000; Bandyopadhyay et al., 1998). Each life form has a minimum, optimum and maximum temperature for growth.

Phenol were used as single limiting substrate in present study for locally isolated soil bacteria. Different initial phenol conc. of 0.5g/100ml to 2.5g/100ml was used. The degree of Phenol degradation was investigated by using different initial phenol conc. degradation proceeds with biomass growth. The growth curve has characteristic exponential and static phase with increasing lag phase.

The isolates obtained from different soil were grown on different media like nutrient agar plates, nutrient broth and mineral salt media. Mineral salt media was used for phenol degradation. Phenol degradation was examined by measuring the optical density at 600nm and dry cell weight method. Quantitatively Estimation of phenol was checked at 510nm. The locally isolated soil bacteria can grow up to 2.5g/100ml phenol conc. Furthermore increase in phenol conc. cease the growth because toxicity of phenol occurred and bacteria did not tolerate further increase.

The nutrient accessibility in the biodegradation of organic pollutants, temperature performs an important function. Phenol degradation was considerably reserved at 30°C. On the other hand, my laboratory study on phenol biodegradation has also been conceded out at temperature of 30°C. Chittra (1995) describe that while the temperature improved from series of 30°C, there was due to cell worsen, no phenol degradation is observed. So the phenol degradation is a temperature dependent procedure. It was noted that growth rates in universal approximately doubled meant for each 10°C ascendant in temperature in the typical mesophilic equipped series from 10 to 30°C. The increase tariff usually reaches optimum in the temperature of range between 35°C as well as 37°C, however protein denaturation on elevated temperatures slow down the development tariff for mesophiles. But unusual diverse cultures modified to thermophilic species had the optimum temperatures series from 50 to 65°C. Thermophilic observed decreased degradation of phenol at the intermediary temperature range from 40 to 45°C as mesophilic species. So we can conclude that the function at the lower mesophilic range is about around 35°C with an optimum temperature and in the thermophilic series it is temperature optimum ranges about 50 to 60°C.

In present study locally isolated bacteria were checked for their degradative ability by exposing them to different temperatures and it was found that 35°C was the optimum temperature for the degradation of phenol. Maximum percentage reduction was recorded at 35°C by different strains. The temperature of 30°C was also suitable for the
degradation of phenol but maximum percentage reduction at 30°C was observed 85% after this decline phase occurred due to cell worsen. At 40°C percentage reduction was 80%.

The interior atmosphere of entire existing cell was understood to be roughly impartial. The majority of organisms could not situate for pH range below from 4.0 or above from 9.0. At high or low pH values acid or base could burst during into cells further simply, because they have a affinity to survive in undissociated structure underneath these circumstances and electrostatic force cannot shun them from incoming cells (Alexander and Robertson, 1992). The optimum pH for phenol degradation is about 7.0 for majority of bacteria.

In present study the ability of bacterium to degrade phenol at different pH was also observed. Maximum degradation was at pH 7. Percentage reduction recorded at pH 7 was 96% after this decline phase occurred because most of bacteria have a affinity to exist in undissociated form at pH 7 but in acidic or basic surroundings and electrostatic force could not shun them from incoming cells. At pH 6 percentage reduction was 90% because it is somewhat acidic medium and some bacteria did not tolerate at this pH.

The effect of trembling on the phenol-degrading ability of locally isolated soil bacteria were also investigate by trying different shaking speeds compare to stationary conditions. A speed of 120 rpm. was institute to be the optimum. Maximum percentage reduction at 120 rpm was 90%. The biodegradation rate might be appropriate to sufficient lofty mass shift consequently more oxygen to be dissolved and become accessible for the metabolism of micro organism. Shaking speed of 80 rpm and 140 rpm were no more effective and percentage reduction was 80% then decline phase occurred because the decrease might be appropriate to higher shave stress effect so chief to cell thrashing or lower biomass concentration.

REFERENCES