RESEARCH ARTICLE

EFFECT OF TEMPERATURE ON DEGRADATION POTENTIAL OF SELECTED BACTERIAL STRAINS FROM EFFLUENT TREATMENT PLANT OF COIR INDUSTRY.

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Abstract

Phenol-degrading bacteria exist widely in the environments and they are usually isolated from phenol contaminated site. Phenol and its components are majorly toxic to the environment. It is hazardous when exposed to the organisms’ surface. The aim of the study was to isolate and identify the phenol degrading bacteria from effluent treatment plant of coir industry and to analyse the effect of temperature on the degradation potential. From the isolated bacterial strains, 5 most potent strains were selected for the study (\textit{Brucella} sp, \textit{Aquaspirillum} sp, \textit{Erwinia} sp, \textit{Aeromonas} sp and \textit{Moraxella} sp). To determine the effect of temperature the experiments were carried out at different temperatures such as 27˚ C, 35˚ C and 47˚ C. The selected bacterial strains were grown in medium with different phenol concentrations (200, 400, 600, 800 and 1000ppm). The growth of bacteria and phenol concentration in the media were observed for the three selected temperatures. The decrease in phenol concentration accompanied with increase in biomass. From the preliminary analysis, the most potent strains were found to be \textit{Brucella} sp and \textit{Aeromonas} sp. The medium with 800ppm phenol showed better degradation for all the selected strains. At the same time when the prescribed temperature was changed, the degradation potential was reduced and was unstable.

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Introduction:

Phenol and its derivatives are the most common toxic organic pollutant of various industrial wastewaters and therefore it requires proper treatment before being discharged (Shewtha et al., 2013). Phenol and phenolic compounds are of general use in many industries such as polymeric resin production and oil refining. As a result, these compounds are commonly encountered in industrial effluents and surface water. The low volatility of phenol and its affinity for water make oral consumption of contaminated water the greatest risk to humans(D. Hank et al., 2010). Toxicity of phenolic compounds inhibits biological treatment or even eliminates sensitive micro-organisms from biological wastewater treatment process and significantly reduces the biodegradation of the other components.
(Yan et al., 2006). The presence of phenol in water imparts carbolic odour to receiving water bodies and can cause toxic effects on aquatic flora and fauna (Ghadhi and Sangodkar, 1995). Hence, it is necessary to remove phenol from industrial effluents before discharging them into the environment. Many technologies exist for the treatment of phenols, but their use is intermediates particularly from non biological process. Many aerobic bacteria have been confirmed to use aromatic carbon as a pure source of carbon and energy (Paller et al., 1995) which suggests using phenol as nutrient to the organism and thereby converts phenol to nontoxic component. Growth and biodegradation of any microorganism depends on various physiochemical parameters. Aim of this study was to optimize the temperature of incubation. Study on growth of bacteria at different temperature was carried out and the optimum temperature for the better biodegradation of phenol was found out.

Materials and methods:-
Samples from effluent treatment plant of paper industry were collected and serially diluted. Microbial enrichment was done using nutrient broth with different phenol concentrations (10, 20, 30, 40, 50 ppm). From the 50 ppm culture, organisms were collected and added to sorbitol agar medium with varying concentrations of phenol (200, 400, 600, 800 and 1000 ppm).

Estimation of Total Phenol:-
Estimation of total phenol was carried out the method of (Bray et al., 1954).

Observation of Total Growth:-
The growth rates of the microbes were observed by spectrophotometric analysis.

Identification of Isolates:-
Biochemical characterization was done based on Bergy’s manual of Determinative Bacteriology (1994) and Cappuccino et al., (1999, 2000)

Effect of Temperature on Growth and Total Phenol:-
The effect of temperature on total growth and phenol degradation of the selected strains were studied.

Results and Discussion:-
Serially diluted effluent of coir industry was transferred to 10, 20, 30, 40 and 50 ppm phenol having minimal media. From the 50 ppm medium, they were inoculated into selective sorbitol agar media with varying phenol concentrations (200, 400, 600, 800, 1000ppm). In this study, five most potent bacterial strains were isolated and identified as Brucella sp, Aquaspirillum sp, Erwinia sp, Aeromonas sp and Moraxella sp. They were further used to investigate the growth and total phenol content in varying phenol concentrations. They were also used to study the effect of temperature. To determine the effect of temperature the experiments were carried out at different temperatures such as 27˚C, 35˚C and 47˚C. The selected bacterial strains were grown in medium with different phenol concentrations (200, 400, 600, 800 and 1000ppm). Neumann et al., 2004, adapted Pseudomonas strains to high concentrations of phenol (1000 mg/L) and further biodegradation was carried out at a concentration of 800 mg/L. The total phenol and growth of selected bacterial strains, during different temperatures (27°C, 35º C and 47) are given in the tables 1 to 15.

At 27ºC ( tables 1 to 5) , phenol degradation in 200 ppm concentration was detected to be highest at 48 hrs of incubation by Aeromonas sp (0.0191) then decreased by Brucella sp (0.0376), Erwinia sp (0.0411) ,the least degradations were showed by Aquaspirillum sp and Moraxella sp (0.0465). The growth rates of the bacterial strain that was noted to be the highest was at 24hrs of incubation by Aeromonas sp (0.0349) followed by Aquaspirillum sp(0.0269),Erwinia sp(0.0251), Brucella sp(0.0188) and Moraxella sp(0.0154) . In 400ppm concentration ,the highest degradation was at 72 hrs of incubation by Aeromonas sp (0.0207) followed by Erwinia sp (0.0246), Aquaspirillum sp (0.0292) , Moraxella sp (0.0302) and Brucella sp (0.0356) .The growth rate was maximum for Aeromonas sp at 24 hrs of incubation (0.0300) ,then Aquaspirillum sp (0.0269) , Erwinia sp (0.0251), Brucella sp (0.0188) and Moraxella sp( 0.0154).

Table 1:- Total phenol and growth of coir effluent sample 200ppm

| Strains     | Total Phenol (720nm) | Growth (600nm) |
|-------------|----------------------|----------------|
|             | 24 | 48 | 72 | 96 | 24 | 48 | 72 | 96 |
Growth rate was highest for Aeromonas sp at 24 hrs of incubation and then decreased by, In 800ppm the phenol degradation was noted highest for Moraxella sp (0.0349) followed by Brucella sp (0.0083) and Erwinia sp (0.0184), followed by Aeromonas sp at 48 hrs of incubation (0.0137) and followed by Brucella sp (0.0133) and Aeromonas sp (0.0103). Growth rate was noted the highest for Erwinia sp at 48 hrs of incubation (0.0137) and followed by Brucella sp (0.0103), Aeromonas sp (0.0133) and Aquaspirillum sp (0.0083), and Moraxella sp (0.0082).

In 800ppm the phenol degradation was noted highest for Moraxella sp (0.0168) at 96 hrs of incubation and the decreased by, Aeromonas sp (0.0238), Erwinia sp (0.0280), Aquaspirillum sp(0.0333), and the Brucella sp (0.0350). Growth rate was highest for Aeromonas sp at 24 hrs of incubation (0.0349) and then Aquaspirillum sp (0.0269), Erwinia sp (0.0251), Brucella sp (0.0188), and Moraxella sp (0.0154).

| Strains    | Total Phenol (720nm) | Growth (600nm) |
|------------|----------------------|----------------|
|            | 24       | 48       | 72       | 96       | 24       | 48       | 72       | 96       |
| Brucella sp| 0.0278   | 0.0359   | 0.0356   | 0.0309   | 0.0188   | 0.0286   | 0.0097   | 0.0058   |
| Aquaspirillum sp | 0.0354 | 0.0503   | 0.0292   | 0.0403   | 0.0269   | 0.0144   | 0.0103   | 0.0094   |
| Erwinia sp | 0.0669   | 0.0308   | 0.0246   | 0.0264   | 0.0251   | 0.0213   | 0.0100   | 0.0088   |
| Aeromonas sp | 0.0349 | 0.0410   | 0.0207   | 0.0265   | 0.0300   | 0.0242   | 0.0106   | 0.0080   |
| Moraxella sp | 0.0269  | 0.0498   | 0.0302   | 0.0213   | 0.0154   | 0.0233   | 0.0099   | 0.0090   |

Table 2: Total phenol and growth of coir effluent sample 400ppm

The phenol degradation at 600ppm was highest during 96 hrs of incubation by Aquaspirillum sp (0.0183) followed by Moraxella sp (0.0184), Aeromonas sp (0.0224), Erwinia sp (0.0238) and Brucella sp (0.0253). Growth rate was noted the highest for Erwinia sp at 48 hrs of incubation (0.0137) and followed by Brucella sp (0.0133), Aeromonas sp (0.0106), Aquaspirillum sp (0.0083), and Moraxella sp (0.0082).

In 800ppm the phenol degradation was noted highest for Moraxella sp (0.0168) at 96 hrs of incubation and the decreased by, Aeromonas sp (0.0238), Erwinia sp (0.0280), Aquaspirillum sp (0.0333), and the Brucella sp (0.0350). Growth rate was highest for Aeromonas sp at 24 hrs of incubation (0.0349) and then Aquaspirillum sp (0.0269), Erwinia sp (0.0251), Brucella sp (0.0188), and Moraxella sp (0.0154).
In 1000ppm concentration the highest phenol degradation was obtained by *Erwinia* sp (0.0170) at 96 hrs of incubation and then followed by *Moraxella* sp (0.0275), *Aeromonas* sp (0.0300), *Aquatirillium* sp (0.0332), and *Brucella* sp (0.0358). Growth rate was the highest for *Erwinia* sp at 48 hrs of incubation (0.0249) and then decreased with *Brucella* sp (0.0127), *Aquatirillium* sp (0.0124), *Moraxella* sp (0.0116), and *Aeromonas* sp (0.0111).

At 35°C (tables 6 to 10), in 200 ppm medium, it was observed that *Brucella* sp showed highest phenol degradation at 48hrs hrs (0.0057) followed by *Aquatirillium* sp (0.0119), *Erwinia* sp (0.0205), *Aeromonas* sp (0.0312) and *Moraxella* sp (0.0162).The least degradation was showed by *Aeromonas* sp for 24 hrs (0.0880) incubation. The growth rate was observed highest for *Aquatirillium* sp at 72 hrs of incubation (0.0817) followed by *Aeromonas* sp (0.0777), *Brucella* sp (0.0768), *Moraxella* sp (0.0757) and *Erwinia* sp (0.0725).

**Table 6:** Total phenol and growth of coir effluent sample (200ppm)

| Strains         | Total Phenol (720nm) | Growth (600nm) |
|-----------------|----------------------|----------------|
|                 | 24  | 48  | 72  | 96  | 24  | 48  | 72  | 96  |
| *Brucella* sp   | 0.0144 | 0.0057 | 0.0220 | 0.0101 | 0.0631 | 0.0063 | 0.0768 | 0.0708 |
| *Aquatirillium* sp | 0.0461 | 0.0119 | 0.0190 | 0.0086 | 0.0634 | 0.0072 | 0.0817 | 0.0752 |
| *Erwinia* sp    | 0.0687 | 0.0205 | 0.0153 | 0.0094 | 0.0630 | 0.0068 | 0.0725 | 0.0618 |
| *Aeromonas* sp  | 0.0880 | 0.0132 | 0.0265 | 0.0153 | 0.0579 | 0.0054 | 0.0777 | 0.0739 |
| *Moraxella* sp  | 0.0737 | 0.0162 | 0.0206 | 0.0135 | 0.0540 | 0.0051 | 0.0757 | 0.0755 |

**Table 7:** Total phenol and growth of coir effluent sample (400ppm)

| Strains         | Total Phenol (720nm) | Growth (600nm) |
|-----------------|----------------------|----------------|
|                 | 24  | 48  | 72  | 96  | 24  | 48  | 72  | 96  |
| *Brucella* sp   | 0.0647 | 0.0139 | 0.0168 | 0.0087 | 0.0477 | 0.0064 | 0.0658 | 0.0324 |
| *Aquatirillium* sp | 0.0482 | 0.0103 | 0.0144 | 0.0093 | 0.0576 | 0.0067 | 0.0744 | 0.0694 |
| *Erwinia* sp    | 0.0863 | 0.0100 | 0.0218 | 0.0108 | 0.0598 | 0.0056 | 0.0713 | 0.0329 |
| *Aeromonas* sp  | 0.0888 | 0.0065 | 0.0178 | 0.0134 | 0.0587 | 0.0054 | 0.0754 | 0.0355 |
| *Moraxella* sp  | 0.0067 | 0.0052 | 0.0277 | 0.0120 | 0.0623 | 0.0051 | 0.0709 | 0.0574 |

**Table 8:** Total phenol and growth of coir effluent sample (600ppm)

| Strains         | Total Phenol (720nm) | Growth (600nm) |
|-----------------|----------------------|----------------|
|                 | 24  | 48  | 72  | 96  | 24  | 48  | 72  | 96  |
| *Brucella* sp   | 0.0772 | 0.0170 | 0.0181 | 0.0106 | 0.0534 | 0.0083 | 0.0755 | 0.0672 |
| *Aquatirillium* sp | 0.0194 | 0.0021 | 0.0169 | 0.0132 | 0.0613 | 0.0079 | 0.0774 | 0.0706 |
| *Erwinia* sp    | 0.0832 | 0.0126 | 0.0259 | 0.0172 | 0.0554 | 0.0065 | 0.0680 | 0.0673 |
| *Aeromonas* sp  | 0.0702 | 0.0089 | 0.0209 | 0.0078 | 0.0582 | 0.0078 | 0.0688 | 0.0488 |
| *Moraxella* sp  | 0.0507 | 0.0093 | 0.0096 | 0.0080 | 0.0566 | 0.0090 | 0.0694 | 0.0434 |

**Table 9:** Total phenol and growth of coir effluent sample (800ppm)

| Strains         | Total Phenol (720nm) | Growth (600nm) |
|-----------------|----------------------|----------------|
|                 | 24  | 48  | 72  | 96  | 24  | 48  | 72  | 96  |
| *Brucella* sp   | 0.0971 | 0.0108 | 0.0250 | 0.0111 | 0.0577 | 0.0051 | 0.0734 | 0.0681 |
| *Aquatirillium* sp | 0.0314 | 0.0153 | 0.0230 | 0.0103 | 0.0574 | 0.0080 | 0.0740 | 0.0630 |
| *Erwinia* sp    | 0.0163 | 0.0092 | 0.0187 | 0.0124 | 0.0557 | 0.0052 | 0.0833 | 0.0762 |
| *Aeromonas* sp  | 0.0890 | 0.0226 | 0.0248 | 0.0192 | 0.0561 | 0.0065 | 0.0686 | 0.0647 |
| *Moraxella* sp  | 0.0820 | 0.0230 | 0.0188 | 0.0140 | 0.0555 | 0.0058 | 0.0715 | 0.0488 |

| Strains         | Total Phenol (720nm) | Growth (600nm) |
|-----------------|----------------------|----------------|
|                 | 24  | 48  | 72  | 96  | 24  | 48  | 72  | 96  |
| *Brucella* sp   | 0.0923 | 0.0140 | 0.0149 | 0.0091 | 0.0554 | 0.0076 | 0.0718 | 0.0694 |
In 400ppm medium, *Moraxella* sp showed highest phenol degradation at 48 hrs (0.0052) followed by *Aeromonas* sp (0.0065), *Erwinia* sp (0.0100), *Aquaspirillum* sp (0.0103) and *Brucella* sp (0.0139). The least degradation was observed for *Aeromonas* sp (0.0888) at 24hrs incubation. The growth rate was observed highest for *Aquaspirillum* sp at 72 hrs of incubation (0.0817) followed by *Aeromonas* sp (0.0777), *Brucella* sp (0.0768), *Moraxella* sp (0.0757) and *Erwinia* sp (0.0725).

In 600ppm medium, *Aquaspirillum* sp showed highest phenol degradation at 48hrs incubation (0.0021) followed by *Aeromonas* sp (0.0089), *Moraxella* sp (0.0093), *Erwinia* sp (0.0126) and *Brucella* sp (0.0170). The growth rate was observed to be highest for *Aquaspirillum* sp at 72 hrs of observation (0.0817) followed by *Aeromonas* sp (0.0777), *Brucella* sp (0.0768), *Moraxella* sp (0.0757) and *Erwinia* sp (0.0725).

In 800ppm medium, *Erwinia* sp showed highest phenol degradation at 48hrs incubation (0.0092) followed by *Brucella* sp (0.0108), *Aquaspirillum* sp (0.0153), *Aeromonas* sp (0.0226) and *Moraxella* sp (0.0230). The least phenol degradation was shown by *Brucella* sp (0.0971) at 24 hrs of incubation. The growth rate was observed to be highest for *Aquaspirillum* sp at 72 hrs of incubation (0.0817) followed by *Aeromonas* sp (0.0777), *Brucella* sp (0.0768), *Moraxella* sp (0.0757) and *Erwinia* sp (0.0725). Shen and Wang (1993) conducted experiments on simultaneous chromium reduction and phenol degradation in a co culture of *Escherium coli* and *Pseudomonas putida*. They also reported similar growth pattern with a short lag period, followed by an exponential growth phase and then a declining growth phase.

In 1000ppm medium the phenol degradation rate was highest for *Erwinia* sp (0.0061) followed by *Aeromonas* sp (0.0064), *Aquaspirillum* sp (0.0076) *Brucella* sp (0.0091) and *Moraxella* sp (0.0106). The growth rate was observed highest for *Moraxella* sp at 72 hrs of incubation (0.0738) followed by *Brucella* sp (0.0718), *Aquaspirillum* sp (0.0592), *Erwinia* sp (0.0649) and *Aeromonas* sp (0.0640). Mrs. C H Supriya and Devaneehr, 2014 carried out a study to determine the effect of temperature the experiments were carried out at different temperatures such as 25°C, 35°C, 40°C,50°C and 60°C for 120 hrs of incubation period. The data showed that there was maximum phenol degradation takes place at room temperature of 35°C and on further increase in temperature. The rate of biodegradation decreases because the catalytic activity of the enzymes starts to decrease beyond that temperature. So, the optimum temperature for the maximum enzymatic activity is 35°C.

At 47°C (tables 11 to 15), 200ppm concentration of phenol showed highest degradation at 24 hrs of incubation by *Brucella* sp (0.0114) followed by *Moraxella* sp (0.0246), *Erwinia* sp (0.0373), *Aquaspirillum* sp (0.0403), and *Aeromonas* sp (0.0570). The growth rate was indicated to be highest at 48hrs of incubation by *Aeromonas* sp (0.0563), then decreased by *Aquaspirillum* sp (0.0470), *Brucella* sp (0.0392), *Erwinia* sp (0.0314), and *Moraxella* sp (0.0292).

**Total phenol and growth at 47°C**

| Strains        | Total Phenol (720nm) | Growth (600nm) |
|----------------|----------------------|----------------|
|                | 24                   | 48             | 72             | 96             | 24          | 48          | 72          | 96          |
| *Brucella* sp  | 0.0117               | 0.0140         | 0.0350         | 0.0416         | 0.0209      | 0.0392      | 0.0222      | 0.0094      |
| *Aquaspirillum* sp| 0.0403               | 0.0202         | 0.0438         | 0.0344         | 0.0185      | 0.0470      | 0.0126      | 0.0120      |
| *Erwinia* sp   | 0.0373               | 0.0372         | 0.0435         | 0.0315         | 0.0119      | 0.0314      | 0.0153      | 0.0092      |
| *Aeromonas* sp | 0.0570               | 0.0368         | 0.0332         | 0.0301         | 0.0062      | 0.0563      | 0.0222      | 0.0125      |
| *Moraxella* sp | 0.0246               | 0.0350         | 0.0457         | 0.0404         | 0.0077      | 0.0292      | 0.0175      | 0.0157      |

**Table 10:** Total phenol and growth of coir effluent sample (1000ppm)

**Table 11:** Total phenol and growth of coir effluent sample 200 ppm

| Strains        | Total Phenol (720nm) | Growth (600nm) |
|----------------|----------------------|----------------|
| *Brucella* sp  | 0.0117               | 0.0140         | 0.0350         | 0.0416         | 0.0209      | 0.0392      | 0.0222      | 0.0094      |
| *Aquaspirillum* sp| 0.0403               | 0.0202         | 0.0438         | 0.0344         | 0.0185      | 0.0470      | 0.0126      | 0.0120      |
| *Erwinia* sp   | 0.0373               | 0.0372         | 0.0435         | 0.0315         | 0.0119      | 0.0314      | 0.0153      | 0.0092      |
| *Aeromonas* sp | 0.0570               | 0.0368         | 0.0332         | 0.0301         | 0.0062      | 0.0563      | 0.0222      | 0.0125      |
| *Moraxella* sp | 0.0246               | 0.0350         | 0.0457         | 0.0404         | 0.0077      | 0.0292      | 0.0175      | 0.0157      |

**Table 12:** Total phenol and growth of coir effluent sample 400ppm
The phenol degradation at 600ppm concentration was greatest at 24 hrs of incubation by Moraxella sp (0.0215) followed by Aeromonas sp (0.0238), Brucella sp (0.0276), Erwinia sp (0.0485) and Aquaspirillum sp (0.0499). Growth rate was noted highest for Brucella sp at 24 hrs of incubation (0.0247) and then Aeromonas sp (0.0215), Moraxella sp (0.0206) and Aeromonas sp (0.0193).

In 800ppm the phenol degradation was noted highest for Aeromonas sp (0.0276) at 24 hrs of incubation and the decreased by , Erwinia sp (0.0453), Brucella sp (0.0523), Aquaspirillum sp(0.0569), and Moraxella sp (0.0733). Growth rate was noted to be highest for Brucella sp at 48 hrs of incubation (0.0472)and then Moraxella sp (0.0291) , Aquaspirillum sp (0.0284) , Aeromonas sp (0.0133) , and Erwinia sp (0.0127).

In 1000ppm the highest phenol degradation was obtained by Aeromonas sp (0.0220) at 24 hrs of incubation and then followed by Aquaspirillum sp (0.0298) , Erwinia sp (0.0439) Brucella sp (0.0464), and Moraxella sp (0.0538). Highest growth rate was obtained by Erwinia sp at 48 hrs of incubation (0.0201) and the decreased by Moraxella sp (0.0184), Aeromonas sp (0.0160) Aquaspirillum sp (0.0148) and Brucella sp (0.0104).
From this preliminary analysis, it was clear that the maximum degradation potential can be achieved only during the optimum temperature. When there occurs a change in temperature of incubation, the growth of strains as well as their degradation potential will be unstable.

**Conclusion:-**

Biodegradation is one of the cheapest methods with no production of hazardous by-products. This method is generally preferred due to lower costs and possibility of complete mineralization. The growth and phenol biodegradation study was carried out in sorbitol agar media with varying phenol concentrations. The effect of temperature on the rate of phenol degradation by the selected strains was carried out. It was observed that the rate of phenol biodegradation was significantly affected by temperature of incubation. From this, it was clear that when temperature is altered, growth also decreases and the degradation do not occur properly. Highly acclimatisable microbes are those which are able to degrade phenol at high concentration and at greatest rate will be the best phenol degrader candidates. The results of study on effect temperature revealed that the standard optimum temperature (35°C) was required for normal bacterial growth and phenol consumption as their energy source. Future studies should be carried out to isolate more potent useful microbes and can be used for the waste management.

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