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GROWTH KINETICS OF *ALCALIGENES* SP d_2 DURING PHENOL BIODEGRADATION IN MINERAL SALT PHENOL MEDIUM

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ABSTRACT

Biodegradation is always considered as the safe and acceptable method for the degradation of a specific pollutant. The present study focused on the biodegradation of phenol with the bacteria *Alcaligenes* sp d_2. The strain has been reported earlier as a potent phenol degrading bacteria and was also found to be efficient in the treatment of phenolic industrial effluent. The process of phenol degradation by *Alcaligenes* sp d_2 was analysed in mineral salt phenol medium where phenol alone acted as the sole source of carbon and energy. The efficiency of degradation is determined by the rate of growth of the strain in the respective media. The rate of growth in terms of specific growth rate was obtained for *Alcaligenes* sp d_2 at various concentrations of phenol from 7.5 mM to 37.5 mM. The specific growth of the bacteria was found to decrease from 0.982 to 0.484 along with the increase in the phenol concentration from 7.5 mM to 37.5 mM. The percentage of phenol degradation obtained through the organism in the same medium was also found to be decreasing from 98.2% to 72.6% upon progressively enriching the phenol concentration from 7.5 mM to 37.5 mM. As phenol itself is toxic to the bacterial species, eventhough the organism is capable of degrading phenol at lower concentrations higher concentration was found to be growth limiting as the specific growth rate was considerably reduced upon increasing phenol concentration from 7.5mM to 37.5 mM.

KEYWORDS: Mineral salt phenol medium, kinetics, biodegradation, phenol. *Alcaligenes* sp d_2.

INTRODUCTION

Phenol and its derivatives are used in many industrial applications including paper, pharmaceuticals, resins and dyes industries hence found in the effluents of these industries (Bandhyopadhyay K et al, 1998). Phenol is considered as a priority pollutant as it is toxic to living organisms in every concentration. Phenol is a major environmental pollutant, being released from industries as effluent. The toxicity of phenol is wide and deep. It is harmful to environment as well as human-life when in large amounts. It causes high burning to the skin when exposed concentrated, and in persistent exposure can cause variety of cancers too. Systemic effects to the eyes are also prevalent. Phenol is produced from coal tar, by fractional distillation. It is also synthesized chemically. It is made use in many products, but its main use is in production of resins.

Eradication of such pollutants from the environment is necessary for our natural ecosystem. The removal of phenol is an uphill task. Among the various methods available, biodegradation has been an emerging alternative to physical/chemical methods, as it is cost effective and environmental friendly (Lee. S, 2017). The degradation is been carried out by certain bacteria capable of utilizing the carbon available in phenol. This property has been reported in quite a few bacteria, but is not a virtue shown by many, as, phenol is in fact a disinfectant. Degradation of phenol requires unique machinery in their proteome, especially, the enzyme composition as well as, its genome content and its transcription machinery. The bacteria been observed to degrade phenol include, *Pseudomonas putida*, *Alcaligenes sp.*, *Acinetobacter sp.*, etc.

Several micro organisms are reported so far, that have the capability for degrading phenol. The present work encompasses a detailed study on the growth kinetics of *Alcaligenes* sp d_2 on the biodegradation of phenol in mineral salt phenol medium. The work was conducted to investigate the capability of the strain to degrade and utilize the phenol provided by the given medium. The bacteria was grown in minimal medium with phenol as the only carbon source and allowed to degrade it *Alcaligenes* sp d_2, isolated from soil through soil enrichment culture, was earlier reported to be potent phenol degrading one. (Nair and Shashidhar 2004). The same organism has been reported to accumulate PHB intracellularly under phenol stress (Nair et al). *Alcaligenes* sp d_2 could also be used for the treatment of treat phenolic paper factory effluent (Nair et al). In the present work attempts are being made to explore the growth kinetics of the organism under phenol stress.

MATERIALS AND METHODS

Source of Bacteria

Alcaligenes sp d2, the microorganism which have the ability to degrade phenol was collected from culture collection centre of Bioprocess and Biotechnology lab, School of Biosciences, M. G. University, Kottayam. The strain was then sub -cultured on nutrient agar plates and was preserved and maintained.

Medium Used

Miineral salt phenol medium (MSPM) with various concentrations of phenol were inoculated with the organism (Nair et al 2007). The composition of MSPM is as follows.

MSPM for 200ml

- KH_2PO_4 – 0.2g
- $(NH_4)_2SO_4$ – 0.2g
- $MgSO_4.7H_2O$ – 0.1g
- $CaCl_2$ (anhydrous) – 0.0001g
- phenol at 7.5 to 37.5 mM

Estimation of Phenol

Phenol estimation in the present study was carried out through the method proposed by Mordocco, *et.al* (1999) with 2% of 4-amino antipyrine in presence of 8% potassium ferricyanide and liquid ammonia.

Analysis of the growth curve of *Alcaligenes sp d2*

Alcaligenes sp d2 was grown on LB broth which was then kept for 16-24 hours of incubation for attaining optimum growth. The culture broth was then centrifuged to obtain the pellets. The pellets were saline washed and a 3% of inoculums were added on to mineral salt phenol medium (MSPM) containing phenol as the sole source of carbon and energy. Different concentrations of phenol were used for the study. The sample is then kept for assay under definite incubation conditions and the bacterial growth at various incubation periods were expressed as the optical density measured at 600nm from 4-36 hours on a time interval of 4. A standard graph was plotted with optical density on Y axis and incubation time in hours on X axis.

Specific growth rate of the strain

The bacterial concentration obtained at various time intervals were used to calculate the generation time and also the specific growth rate at each phenol concentration. The following equation was used for tracing the specific growth rate (μ).

Number of generations= (log number of cells in end-log number of cells in beginning)/log 2

Generation time = 60minutes/hour* number of generations

$\mu = 0.693/t_d$

Analysis of the Percentage of Phenol Biodegradation.

The medium contained Phenol alone as the sole source of carbon and energy. The culture media was incubated from 4 to 36 hours at a definite time interval of 4. The samples collected at various incubation periods, centrifuged at 10,000 rpm for 10 minutes. The pellet was discarded and the supernatant was subjected to phenol assay (Mordacco et al, 1999). A graph was plotted by placing percentage of biodegradation on y-axis and incubation time in x-axis.

RESULT AND DISCUSSION

Phenol degradation is often treated as a challenging requirement as it is very difficult to get phenol degrading organism from nature. Phenol is toxic to organisms and usually act as growth limiting substrate. Eventhough it is very easy to get phenol tolerating organism from surroundings phenol catobilising microorganism rarely exist in nature. Phenol degradaing organisms act generally at low concentration of phenol and usually follows a diauxic nature. Phenol degradation is also found to be significantly influenced by external factors like pH, temperatue, incubation time and temperature. Even at the optimized situations, the degradation rate is greatly determined by the biomass formed and also on growth rate. Hence analysis of the terms of growth kinetics becomes crucial in the cases like phenol degradation where the substrate ultimately turns out to be inhibitory.

Evaluation of the terms of microbial growth kinetics is important in the design of the biodegradation process (Mizzouri, 2017). This will help in predicting the performance of the system and also will help in the improvisation of the process.

On analyzing the growth **o**f *Alcaligenes* sp d_2 in MSPM at **7.5** mM concentration of phenol an initial extended log phase could be observed from 5 hour to 9 hour. The second log phase continued from 12 hour to 16 hour (Fig.1 to Fig. 5). This diauxic nature was exhibited in all

concentrations of 15 mM, 22.5 mM, 30mM also in 37.5 mM indicating promising efficiency of the organism in the biodegradation of phenol even at higher concentrations.

Analysis of growth curve of *Alcaligenes* sp d_2 on different concentrations of phenol within 4 to 36 hours of incubation on a time interval of 4.

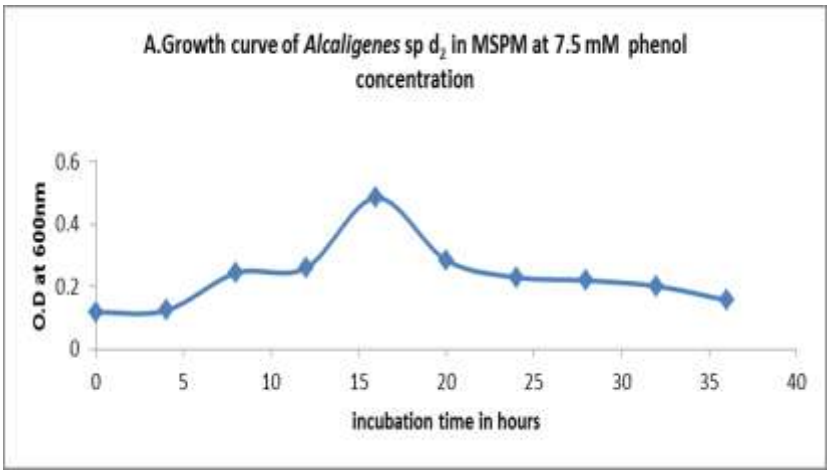


Fig.1.

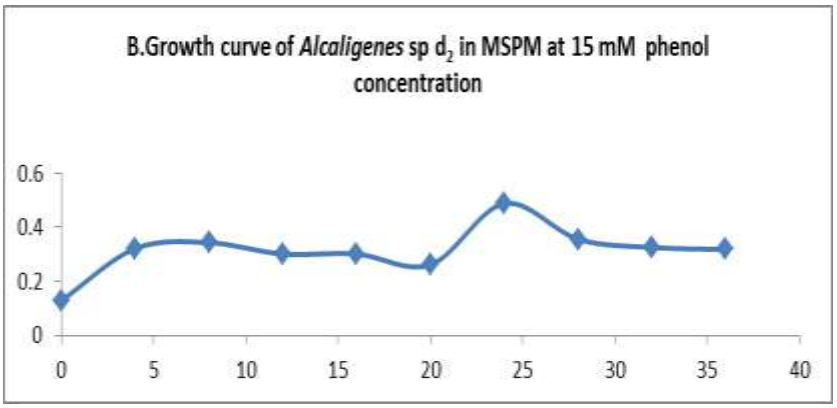
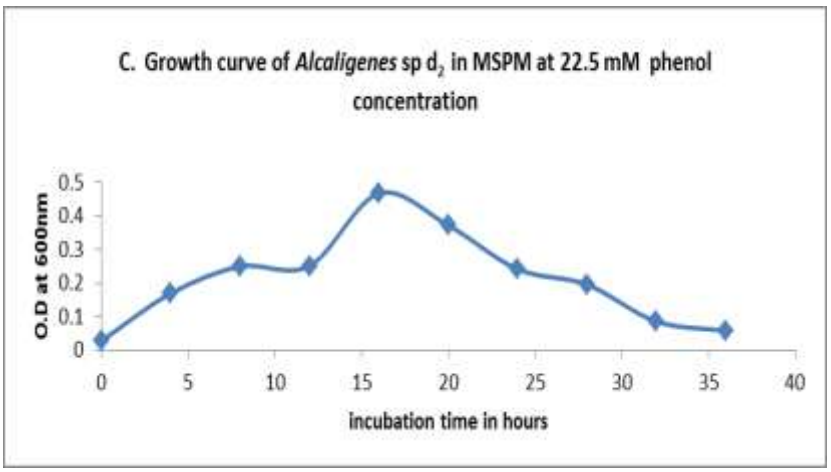


Fig 2.



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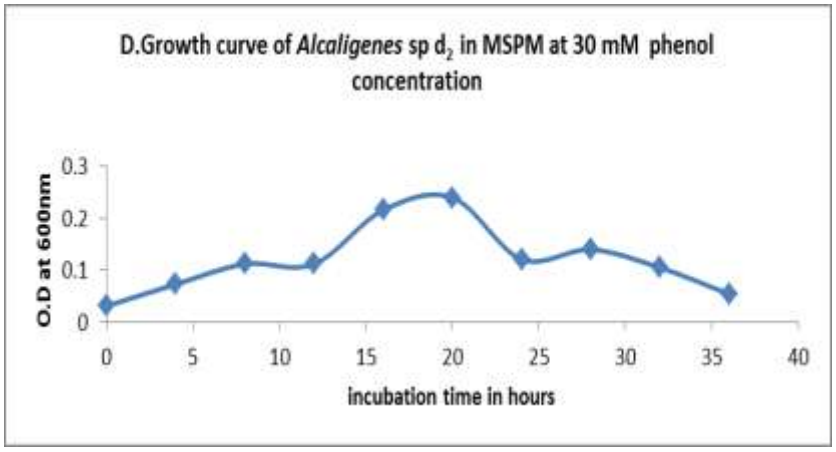


Fig 4.

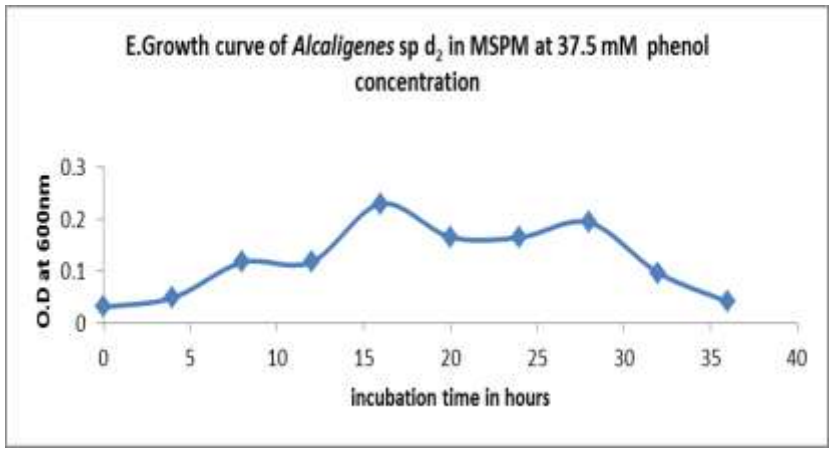


Fig 5.

The results of the growth analysis of the organism at different substrate concentrations was used for the calculation of specific growth rate at respective phenol concentrations (Table 1). The growth rate was found to be decreasing along with the increase in substrate concentration from 7.5 mm to 37.5 mM establishing the fact that phenol at higher concentrations are growth limiting (Fig.6).

Table 1: Specific growth rate (μ) of *A lcaligenes sp* d_2 during growth in MSPM at different concentrations of phenol.

Concentration of phenol	Generation time Hours/generation	μ
7.5 mM	0.705	0.982
15 mM	0.74	0.936
22.5 mM	0.931	0.744
30mM	1.015	0.682
37.5mM	1.43	0.484

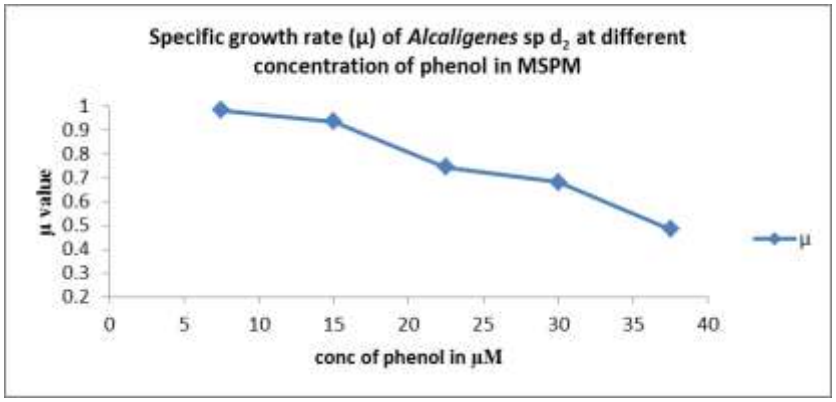


Fig 6.

When the ability of the organism to bring reduction in the phenol content was traced, more than 90% reduction could be observed in 7.5 mM which subsequently reduced to 81% at 15 mM, to 73.2% at 22.5 mM and 30mm and finally to 72.64% at 37.5 mM (Fig.7 to Fig.11).In all the cases of phenol degradation, maximum removal could be observed at 36 hour.

Percentage of Phenol biodegradation by *Alcaligenes* sp d_2 at different concentrations of phenol at various incubation periods in MSPM

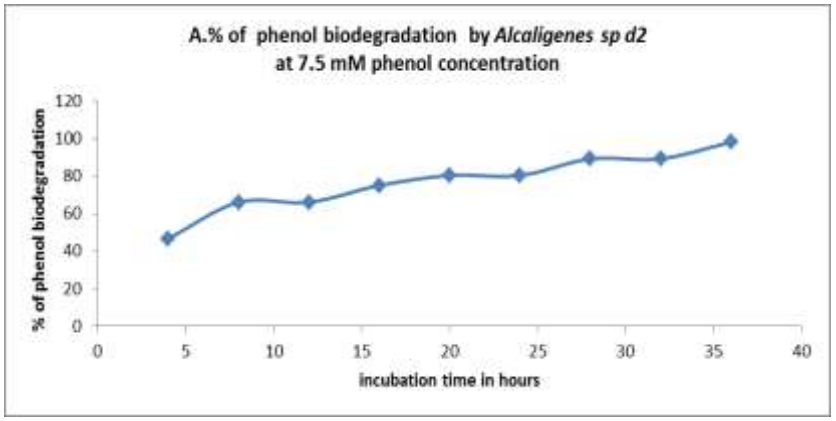


Fig. 7.

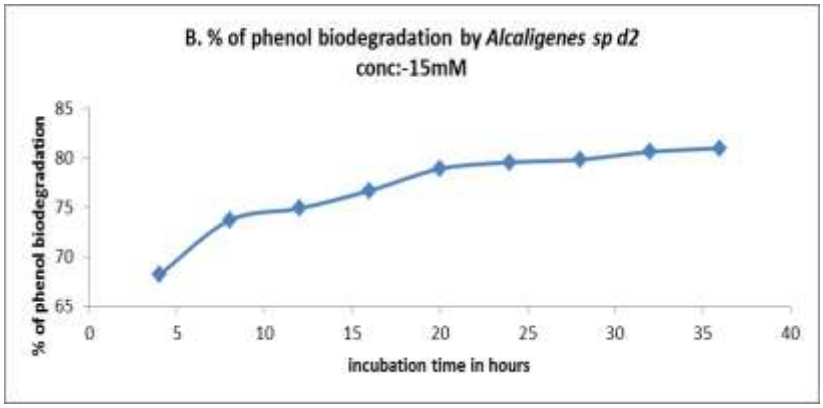
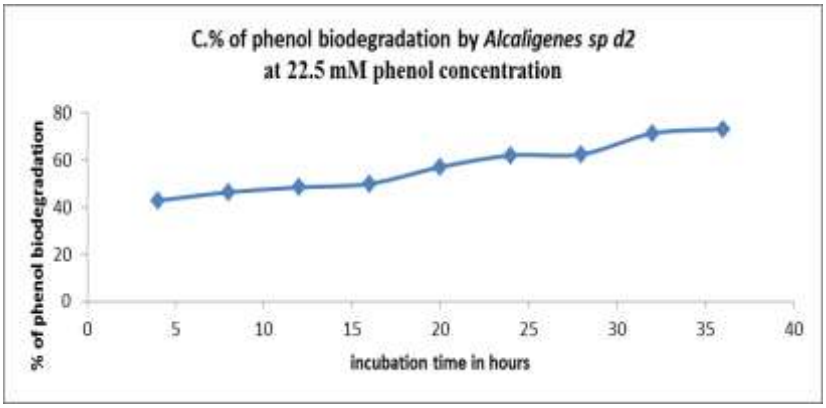


Fig 8.



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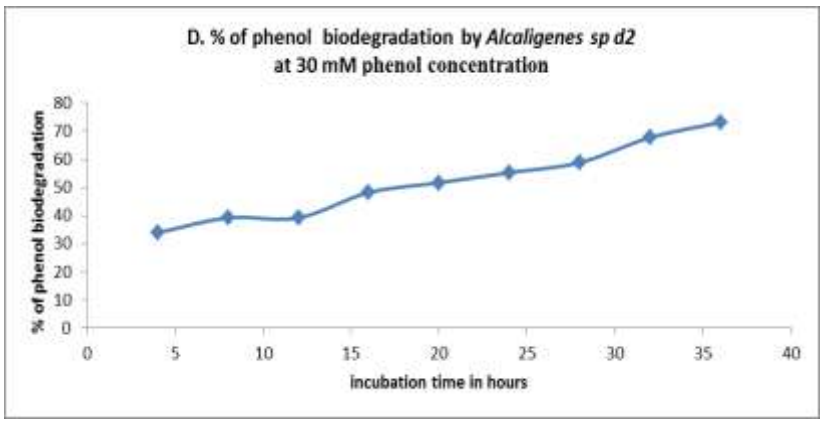

Fig 10.

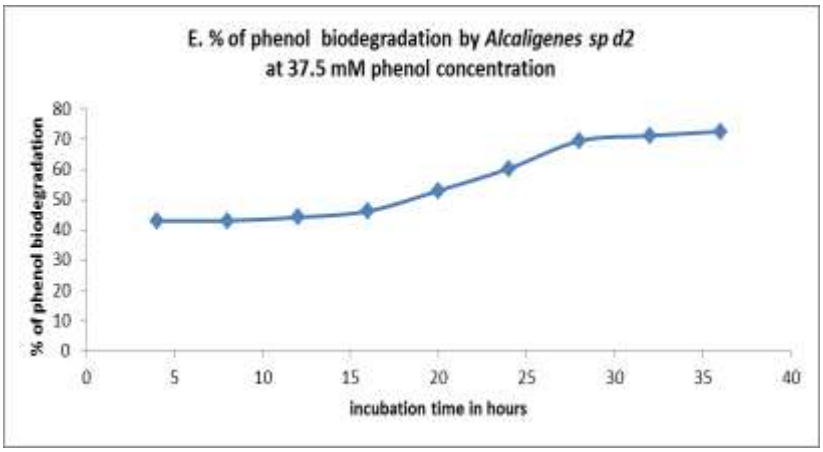

Fig 11.

The kinetics of phenol degradation were studied under optimised conditions biodegradation phenol concentrations of 7.5mM to 37.5 mM, pH6.8, temperature of 30^0C and incubation period upto 36 hours. The bacteria used phenol in MSPM as sole source of carbon and energy. The bacterial growth forwards from an initial lag phase to stationary phase through a logarithmic phase. During such a balanced growth, the growth mimics a first order chemical

reaction, where the concentration of cells is equated with definite time interval. As the incubation time is increased, the bacterium may show ability to project more metabolites from the parent compound. The specific growth rate, which is the measure of bacterial growth, increases with increase in substrate concentrations. On contrary, when the substrate is an inhibitor, the growth rates were found to be diminishing on increasing substrate concentrations. The kinetic data can bring out a more understanding of complex interactions between substrate concentration, growth and the performance.

When a laboratory designed process is taken for large scale studies the extend of predicting performance becomes significant. This is important for both steady and dynamic system. When a single substrate oriented growth process becomes growth limiting, the possibility of applying co metabolism arises (Juang and Tsai. 2006). In the case of phenol degradation by this selected strain, better phenol degradation at higher concentration could be effected through cometabolism. Growth in a more easily assimilable substrate can trigger the degradation of phenol in a better way.

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