

**QUANTIFYING THE PARTICULAR GROWTH RATE OF
*LYSINIBACILLUS SP. STRAIN GAKVM ON PHENOL***

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Article Received on 15 Feb. 2026,
Article Revised on 05 March 2026,
Article Published on 10 March 2026,

<https://doi.org/10.5281/zenodo.19046610>

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How to cite this Article: Anoop M.*¹, Gayathri Premkumar². (2026). Quantifying The Particular Growth Rate of *lysini bacillus Sp. Strain Gakvm* on Phenol. World Journal of Pharmaceutical Research, 15(6), 1107-1114.

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ABSTRACT

The *Lysini bacillus sp. strain GAKVM* (MT476862) was mineralized at the maximum concentration 67.5µm within 72hrs in a mineral salt medium at 37⁰C. The effect of initial phenol concentration on the biodegradation rate was studied using five different concentrations of phenol. Experiments performed for the time duration of 24hours-72hours while daily samples were withdrawn. Results of the specific growth rate in the initial concentrations presented increase in phenol concentration that amounts to decrease in the biodegradation and growth due to the inhibitory action of phenol. The bacteria showed efficient specific growth rate in 7.5µm and 22.5 µm and the specific growth activity of *Lysini bacillus sp strain GAKVM* at phenol concentration 7.5µm is calculated as 0.84 and those in 22.5µm phenol concentration is 0.495.

INTRODUCTION

Phenol and its derivatives are a major source of environmental pollutants (Said et al. 2013; Varma and Gaikwad 2008). The pollution of the aquatic environment by phenols could modify the biota of this environment because most of these compounds exhibit a high degree of toxicity (Lika and Papadakis 2009).

Biological processes using microbial systems provide an alternative to the existing physical/chemical technologies (expensive and commercially unattractive) because they are more cost-

effective, environment friendly and do not produce large quantities of sludge (Rajani V.2015). Microbial degradation is a useful strategy to eliminate organic compounds and detoxify wastewaters and polluted environments (Gallego et al. 2003). Because of widespread occurrence of phenol in the environment, many microorganisms utilize phenol as the sole carbon and energy source for their growth and metabolism which includes both aerobic and anaerobic microorganisms (Basha et al. 2010).

The assay on various kinetic parameters may provide better understanding of complex interactions between various phenol concentrations and the bacterial growth (Jayachandran 2018).

Therefore, metabolic and kinetics studies of pure or exactly defined mixed cultures is necessary for estimating the kinetic parameter of growth and modeling bioprocess running in a suitable type of bioreactor, besides this the performance of biological treatment systems is largely depend on the fundamental understanding of toxic substrate utilization which is essential for defining operational conditions for effective removed compounds during wastewater purification (Rajani V.2015). A variety of factors are known to influence the kinetics of microorganisms including temperature, pH, availability of dissolved oxygen and toxic strength (V. Arutchelvan et al. 2006).

Determination of growth and degradation kinetics of organism has been one of the main issues considered in these studies. The studies of the growth kinetics are essential for the understanding of the capacity of the microorganisms for the degradation and operations of the units.

MATERIALS AND METHODS

Source of The Bacteria

Lysinibacillus sp. strain GAKVM (MT476862.1), was collected from coir retting place on the coastal areas of Cherthala, Alappuzha district. Bacterial strain was aerobically grown at 37⁰c under shake flask condition at 70rpm. Mineral salt phenol medium with various concentrations of phenol were inoculated with the organism (Nair et al 2007). The culture conditions were procured and prepared as per standard reference protocol (Jayachandran et al 2018)

Growth curve of *Lysinibacillus sp. strain GAKVM* on various phenol concentrations

The bacterial species were inoculated on LB broth and incubated. The overnight culture was centrifuged and washed with 0.85% saline solution. 3% of inoculums was added into MSPM containing different concentrations of substrate and incubated. The OD value was taken at 600 nm for each 24hrs of time interval from 24-72hours of incubation and a graph was plotted.

Specific Growth rate of the bacterial strain

The samples represented different optical density from 0.1- 1.0 were collected. The serial diluted sample of MSM containing different phenol concentration was pour plated on nutrient agar plates and incubated for 24 hours. The number of colonies were counted and recorded. The following equation was used for tracing the specific growth rate (Jayachandran et al, 2018).

RESULT

Effect of initial concentration

The degradation behavior of *Lysinibacillus sp. strain GAKVM* at 37⁰C on various phenol concentrations were ranging from 7.5 μ m to 67.5 μ m was presented (Fig 1). These result shows, that the time taken for degradation is more accordance with initial phenol concentrations.

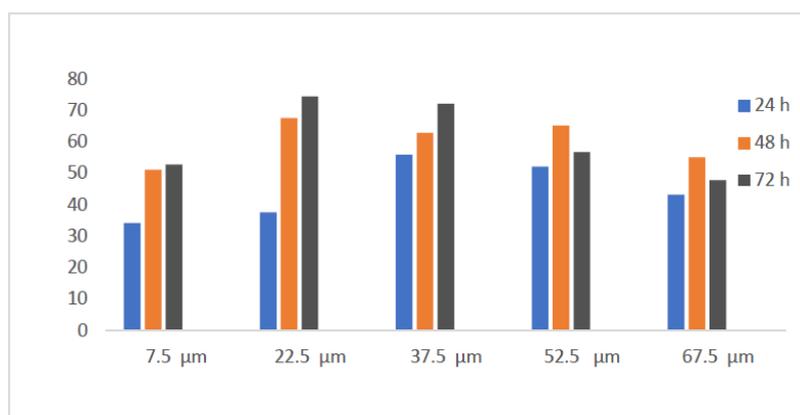


Fig. 1: Analysis of degradation of different concentration of phenol by *Lysinibacillus sp. strain GAKVM* at different periods of incubation time.

Growth kinetics of *Lysinibacillus sp. strain GAKVM* at different concentrations of Phenol

On observing the growth of *Lysinibacillus sp. strain GAKVM* in mineral salt phenol medium

contains 7.5 μ m concentration of phenol there is an initial extended log phase could be observed in between 24 hours and 48 hours (Fig 2). When comparing with other initial concentrations of phenol, the prolonged log phase could be observed from 48hours to 72hours in 22 μ m phenol concentration (Fig 3). The diauxic growth nature was exhibiting in all the higher substrate concentrations of 37.5 μ m, 52, 5 μ m and also in 67.5 μ m shows the efficiency of the organism in the biodegradation of phenol at its higher concentration.

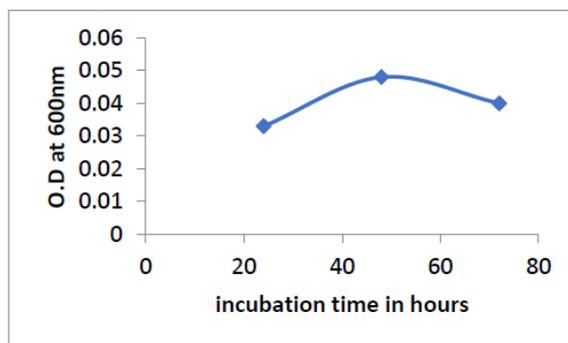


Fig. 2: Growth curve of *Lysinibacillus sp* in MSPM at 7.5 μ m phenol concentration.

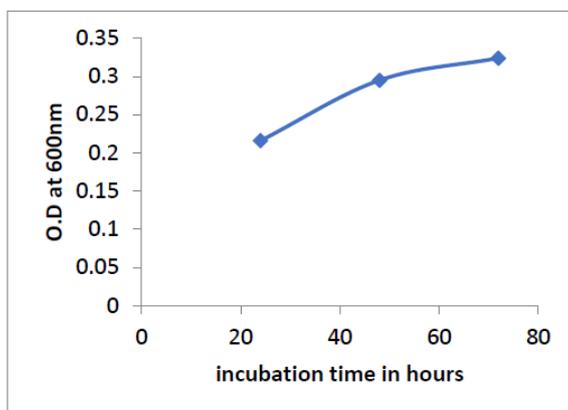


Fig 3: Growth curve of *Lysinibacillus sp* in MSPM at 22.5 μ m phenol concentration.

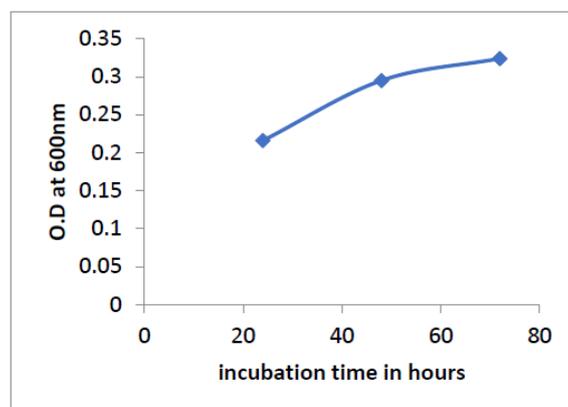


Fig 4: Growth curve of *Lysinibacillus sp* in MSPM at 37.5 μ m phenol concentration.

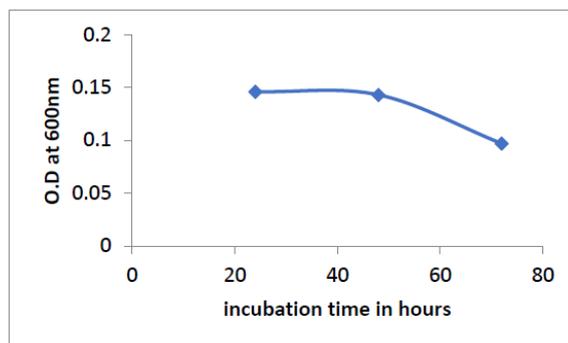


Fig. 5: Growth curve of *Lysinibacillus sp* in MSPM at 52.5 μM phenol concentration.

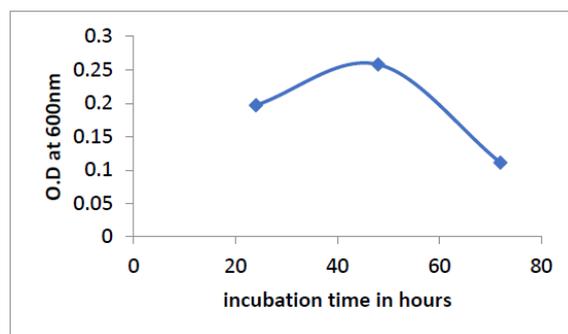


Fig. 6: Growth curve of *Lysinibacillus sp* in MSPM at 65.5 μM phenol concentration.

Specific Growth Rate

The growth rate was found to be decreasing along with the increase in substrate concentration from 7.5 μM to 65.5 μM establishing the fact that phenol at higher concentrations is growth limiting (Fig 6). The plot of specific growth rate with respect to initial substrate concentrations which is shown in Figure (7). The specific growth rate decreases as the phenol concentration progressively increases. At a high initial concentration of phenol, the specific growth rate decreased, which is shown by phenol inhibition after a certain concentration.

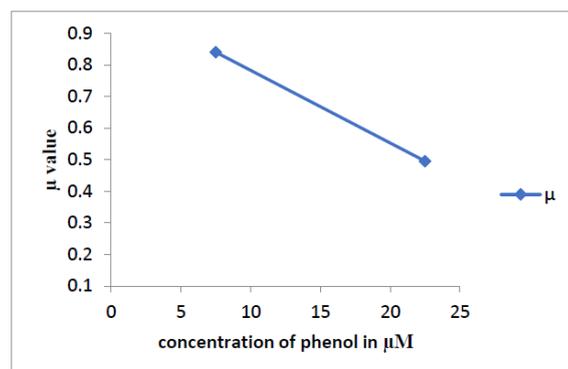


Fig. 7: Specific growth rate of *Lysinibacillus sp* strain GAKVM on Phenol.

The kinetic data can bring out a more understanding of complex interactions between

substrate concentration, growth and the performance.

DISCUSSION

Phenol, an aromatic hydrocarbon, is degraded by various microorganisms which utilize phenol as the sole carbon source for the growth of the organisms (B, Peyton et al 2002). Phenol and its derivatives are not easily biodegradable because they are toxic to most microorganisms. In higher concentrations, they can even inhibit the growth of microbial strains that are capable of assimilating them (kahru et al 2002). Microbial degradation of phenol is depending on certain factors such as concentration of phenol present in growth medium for growth of the degrading microorganisms, nitrogen source, temperature condition, pH, salt concentration, buffer concentration etc. (Karigar, C et al 2006). Therefore, finding optimal condition for phenol biodegradation is important. Optimization of microbial growth conditions, particularly physiological and chemical parameters (medium components) are of primary importance in the development of any biodegradation process (Van scnte, P. M., & Young, L. Y. 2000). The efficiency of the biodegradation of the microbes will be maximum when the process is carried out under optimum condition. Kinetics study is essential for understanding of the capacities of the microorganisms for the degradation of phenol and understanding the kinetics of cell growth is essential for system optimization (He, F. et al 2004). The kinetic studies of *Raoultella sp SBS2* suggested that the organism exhibited diauxic growth in mineral salt phenol medium, which provides support for evidence regarding the degradation of phenol by bacteria and the typical growth kinetics presented by the bacterial species (Anoop 2018). Different Concentrations of Phenol presented different interaction with the *Lysinibacillus sp strain GAKVM*. The growth kinetics of *Lysinibacillus sp strain GAKVM* at different concentrations (Fig 2) from 24 to 72-hour time interval suggested that, the bacteria can effectively grow on phenol at lower concentrations of 7.5 μ M and was found to be less effective as the concentration of phenol is increased to 67.5 μ M. When the concentration of Phenol was increased, the specific growth rate of bacteria was found to be diminishing. This amounts to the concept that if the substrate is an inhibitor then the specific growth rate will be decreased as the concentration of substrates gets increased. The bacteria utilized phenol as its sole source of carbon and energy thereby facilitated biodegradation. From the above data, the bacteria *Lysinibacillus sp strain GAKVM* was proved to an eligible candidate in biodegrading 22.5 μ M concentrations of phenol effectively (Fig 3). A graph obtained after plotting specific growth rate and the specific concentration of substrate (fig 7), was used to evaluate the yield and death coefficient for the phenol

biodegrading microorganism. The yield coefficient (cell mass) in the present study found to be close to those reported in standard references (Jayachandran et al, 2018). The obtained data in aerobic culture shows the specific growth rate, which is the measure of bacterial growth, increases with increase in substrate concentrations. The fig 8 and Fig 9 shows the accurate generation of bacterial cells in the chosen phenol concentration when the time increases.

CONCLUSION

The present work evaluates the capability of the locally isolated bacteria *Lysinibacillus sp strain GAKVM* on biodegrading toxic aromatic compound phenol of coir retting contaminated sources. The work determines the growth and degradation kinetics of the bacteria using five different phenol concentrations at various incubation periods from 24-48 hours on a definite time interval of 24 hours. The isolated strain degrades the average of 45.75% of synthetic phenol up to the concentration of 67.5µm. The duration taken up by the species was about 72 hours. The organism shows a lag phase in high phenol concentration whereas as in low concentration there is a short lag phase. *Lysinibacillus sp GAKVM* possessed the maximum specific growth rate, phenol degradation rate, phenol affinity in the degradation, indicating the real time utilization of the strain for treatment of phenol compounds.

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