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Research Article

OPTIMIZATION OF PHENOL DEGRADATION BY *STREPTOCOCCUS EPIDERMIDIS* USING ONE FACTOR AT A TIME METHODOLOGY

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ABSTRACT. This study was aimed at studying the effects of culture conditions of degradation of phenol by *Streptococcus epidermidis* isolated from soil.Confirmation of the isolate was carried out using standard microbiological procedures. Morphological and biochemical characterizations were employed to identify the isolate. Quantitative phenol was estimated via measure of its absorbance at 510nm wavelength by using UV–visible spectrophotometer and 4-aminoantipyrene as colour indicator. The experiment was repeated with different phenol concentrations to determine the effect of temperature, pH, and inoculum size on degradation of phenol. Percentage degradation (%) was calculated by dividing the reduced amount of phenol with the initial phenol concentration and multiplied by 100. An optimization study that takes into consideration effect of pH, temperature, contact time and inoculum size on the rate of degradation of phenol was carried out. The optimal conditions for the degradation of phenol were found to be pH of 7.0, temperature, of 35°C, inoculum size of 10% and optimum contact time of 24 hours after incubation. This study shows culture conditions such as pH, temperature, contact time and inocula dosage can affect the specific rate of phenol degradation by *Streptococcus epidermidis.*

Keywords: *Bacteria, degradation, temperature, concentration, phenol.*

INTRODUCTION

Phenol is the common name of hydroxyl-benzene, an aromatic compound having one hydroxyl group attached to the benzene ring which makes it a synthetic organic compound [1]. Phenol has also been called carbolic acid, phenic acid, phenylic acid, phenyl hydroxide or oxybenzene [2]. Phenol is the basic structural unit for a variety of synthetic organic compounds. It is a white crystalline solid which is soluble in most organic solvents [3]. Hill and Robinson [4], mentioned a number of methods 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. Resting on health, Calabrese and Kenyon [5], reveals how it causes death amongst adults who have been reported with intake of

phenol amounting to 1.5 to 33g. Prepich and Dauguilis [6], reported how phenol volatility and its attraction to water can create oral absorption of infected water and how it causes most hazards to human being. Different procedures for phenol removal such as chemical oxidation, adsorption, liquid membrane permeation, coagulation, incineration and other nonbiological treatment procedure have disadvantages like their prices, manufacture some hazardous byproducts and furthermore, some of these treatments use energy; ozone, radiation, etc. and chemical reagents; oxidizers and catalysts [7,8,9].

Wide varieties of microorganisms that can aerobically degrade phenol include pure bacterial culture such as: *Acinebacter spp*. [10], *Alcaligens eutrophus* [11], *Arthrobacter spp*. [12], *Bacillus stearothermophilus* [13], *Norcardiodess spp*. [14], *Pseudomonas aeruginosa [15]*, *Pseudomonas spp*. NBM11 [15] *Pseudomonas cepacia* G4 also known as *Burkholderia cepacia* G4 [16], *Pseudomonas fluorescens* [17]. Amongst the microorganism listed above, the genus *Pseudomonas* comprises an important group of bacteria with environmental application in bioremediation and biological control [1]. Studies by Mohanty and Jena [15] revealed how *Pseudomonas spp*. NBM11 was able to biodegrade phenol under different parameters.

MATERIALS AND METHODS

Confirmation of Identity and Biochemical Characterization of Isolate

The bacterium used in this study was obtained from Usmanu Danfodiyo University Microbiology Laboratory. Phenol-containing mineral salt medium (MSM) was used for the isolation of bacteria. The predominant colonies were again transferred to fresh MSM composed of KH_2PO_4 (0.5g/L), K_2HPO_4 (1.5g/L), NaCl (0.5g/L), $MgSO_4.7H_2O$(0.5g/L), NH_4NO_3(1g/L), $FeSO_4.7H_2O$(0.01g/L), $CaCl_2.2H_2O$ (0.01g/L) and NH_4SO_4 (0.5g/L) agar plates twice to ensure culture purity. The bacterium was tested for the ability to degrade phenol at varying concentrations. The bacterium was identified based on morphological observation and biochemical characterization using cell shape, colony shape, sucrose fermentation, citrate utilization, oxygen requirement, lactose fermentation, motility, catalase, etc. In identifying the isolates, Bergey's Manual of Determinative Bacteriology was used.

Preparation of Stock Solution of Phenol

The stock solution of phenol was prepared by adding 10g of phenol to double distilled autoclaved water and the volume was made up to 1000ml. The final concentration of the stock solution was 10000ppm (10000mg/L) and the stock solution was diluted to the required concentration for its use in the experiments. The stock solution was filter-sterilized by passing it through a 0.25µM syringe filter.

Morphological Characteristics

The isolated bacterial strains were then examined for colony morphology, the cell shape and likewise, the cell size and these were carried out using the light microscope.

Gram Staining

The gram staining procedure for the isolates was as follows; smear was prepared from overnight viable cultures. The slide was heat fixed and also treated with crystal violet for

a period of thirty seconds. The slide was then rinsed gently in a stream of water and allowed to air dry and then later flooded with grams iodine solution for one minute and also washed with 95% alcohol for twenty seconds and then rinse with water. It was then allowed to air dry then counter stained with safranin for a period of thirty seconds and rinsed with water gently, it was allowed to air dry and then observed under the microscope [18].

Biochemical Characterization

Catalase Test: Test organism was inoculated into nutrient slants and incubated at 30°Cfor 24 hours. After the period of incubation, the tubes was flooded with 1ml of 3% hydrogen peroxide and observed for gas bubbles. If gas bubbles are observed, then it will be taken as catalase positive and its absence is taken a s catalase negative.

Indole Test : A loopful of test organism was inoculated into the tryptone broth and incubated for 48 hours. A few drops of indole reagent were added to the broth culture. A positive result will have a red layer at the top, while a negative result will have a yellow or brown layer [19].

Citrate Utilization : A loopful of test organism was streaked onto a citrate agar slant and incubated for 24 to 48 hours with a loose cap. A positive result is shown by a slant having a prussian blue colour, while a negative result is shown by a slant with no bacteria growth and will also remain green[19].

Oxidase Test: A small amount of organism was transferred from an overnight grown plate onto a sterile swab; a drop of the reagent was placed on the culture on the swab. Positive reaction will turn the bacteria violet to purple immediately, that is, within 10 to 30 seconds. Reactions that are delayed will be ignored.

Nitrate Reduction : The isolate was inoculated into the nitrate broth and incubated. After incubation, a dropful of sulfanilic acid and α-napthylamine was added. The result is said to be positive if the medium turns red and if it doesn't, a small amount of powdered zinc will be added. If it turns red after addition of the powdered zinc, this shows that the unreduced nitrate is present and this shows a negative result, but if the medium does not turn red it is a positive result.

Triple Sugar Iron (TSI): The test organism was inoculated onto the TSI medium slant by stabbing the butt down to the bottom of the slant. After incubation period of 24 hours at 37°C, acid butt, alkaline slant (yellow butt, red slant) indicated glucose has been fermented but not sucrose or lactose. Acid butt, acid slant (yellow but, yellow slant) indicates lactose and/or sucrose has been fermented. Alkaline butt, alkaline slant (red butt, red slant) will imply that neither glucose nor sucrose or lactose has been fermented [19].

Methyl Red - Voges-Proskauer Test (MR-VP): A loopful of bacteria was inoculated in MR-VP broth and incubated for a time period of 3 to 5 days. After incubation the result was analyzed from the broth. A clear broth indicated that the organism did not grow and cannot be tested. 1 ml of broth was removed and placed into a sterile tube before performing the methyl red test. 3-4 drops of methyl red was added to the original broth. A positive result has a distinct red layer at the top of the broth. A negative result has a yellow layer. To 1 ml of the culture from the MR-VP brot,h 15 drops of the VP A

(Napthol) reagent and 5 drops of the VP B reagent (Potassium Hydroxide) was added. This reaction takes few minutes before a color change. With a positive reaction the medium changes to pink or red, while a negative reaction, the broth will not change color or will be copper colored [20].

Urease Test: Using a sterile bacteriological loop, three to four isolated colonies from a pure culture were picked and streaked over the surface of the urea agar slant without stabbing the butt. It was incubated aerobically at 35 - 37°C overnight. A positive urease reaction was indicated by a colour change from pale to red-pink [19].

Optimization Experiments and Estimation of Phenol Degradation

The quantitative phenol was estimated via measure of its absorbance at 510nm wavelength by using UV–visible spectrophotometer and 4-aminoantipyrene as colour indicator [17]. Standard solution of phenol was prepared with known quantity of phenol starting from 0.5g/100mL, adding few drops of 4-aminoantipyrene as indicator until the colour of phenol becomes colorless to red, noting the absorbance values of phenol at 510nm. The absorbance values of phenol from supernatant with the absorbance values of standard were compared to estimate the degraded phenol. The whole experiment was repeated with different phenol concentrations to determine the effect of temperature, pH, and inoculum size on degradation of phenol. Percentage degradation (%) was calculated by dividing the reduced amount of phenol with the initial phenol concentration and multiplied by 100 [21].

RESULTS AND DISCUSSION

In this study of phenol degradation by bacteria *S. epidermidis*, optimum degradation was recorded at between pH 7.0 and 7.5 (Fig. 1 and Fig. 2). This may be due to the ability of this bacterium to thrive within the pH range. pH have been reported by Annadurai *et al*. [22] to have effect on surface charge of the absorbent, bacteria in this instance, and the degree of its ionization. Further increase in pH showed reversal of degradation potential of the bacteria suggesting reduction in efficiency of the bacteria. Wang *et al*. [23] revealed in their experiment, *Streptococcus* showed maximum phenol degradation rate at pH of about 6.5 and 32°C temperature. Mohite *et al*. [24] revealed that *S. epidermidis* isolated from soil was able to degrade 200mg/l of phenol and confirmed by both spectrophotometric and HPLC analysis. In a similar study by Mohite [24] on biotransformation of phenol and its derivatives, it was found that 4-nitrophenol was best transformed by *S. epidermidis* at pH 7.0 and 32°C temperature.

Furthermore, results obtained from this study showed optimum degradation was recorded at temperature 35°C. Similar to other studies, phenol degradation might be temperature dependent as reported by Mohanty and Jena [15] and Cordova-Rosa *et al*. [25] in their studies on phenol degradation by *Pseudomonas spp*. This contrasted sharply with the study of Moghadem *et al*. [26] in which temperature does not show significant effect on phenol biodegradation. The effect of contact time on the degradation of phenol experimented in this study revealed that maximum contact was attained at between 18-24 hours. Initial contact and rapid adsorption might be due to the surface area of the cell and this may get exhausted on attaining equilibrium [7]. Similarly, as observed in this study, optimum degradation was recorded at 10% (v/v) inoculum size. Higher dosage of

inoculum has been shown to maintain the biomass proliferation in culture. Similarly, larger inoculum size was reported to have impact on lag phase of growth by reducing its duration [27]. Phenol degradation by *S. epidermidis* increases linearly with the increase of inoculum size.

Table 1. *Morphological and Biochemical Characteristics of Isolate*

TEST	RESULT
Gram Staining	+
Cell shape	Cocci
Motility	-
Colony elevation	Raised
Surface texture	Smooth
Colony color	Pale yellow
Oxygen requirement	Aerobic
Indole test	-
Catalase test	+
Citrate test	-
Urease test	+
Methyl red test	-
Lactose	+
Sucrose	+
Glucose	+
H_2S production	+

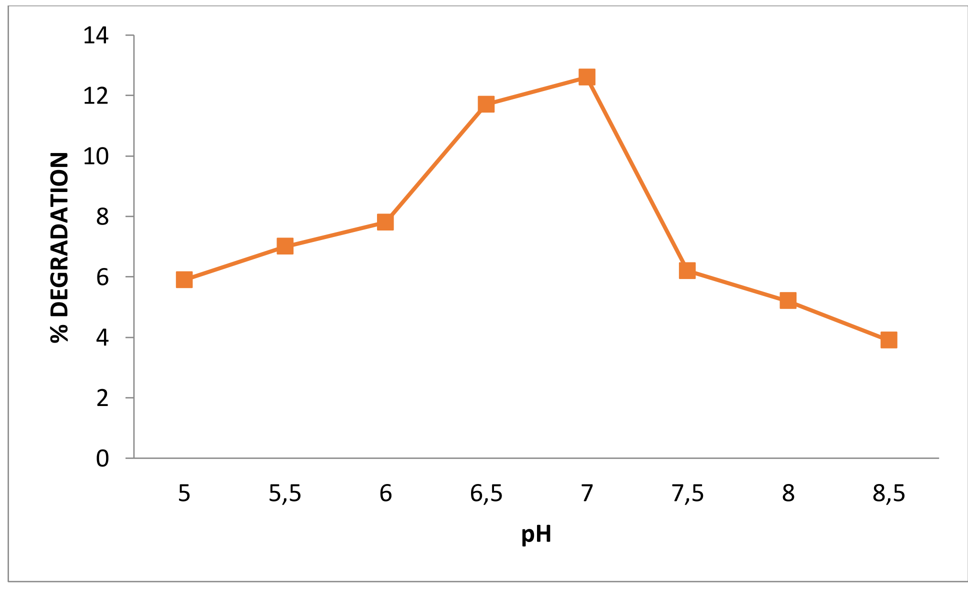

Fig. 1. *Effect of pH on degradation of phenol at 5ml concentration.*

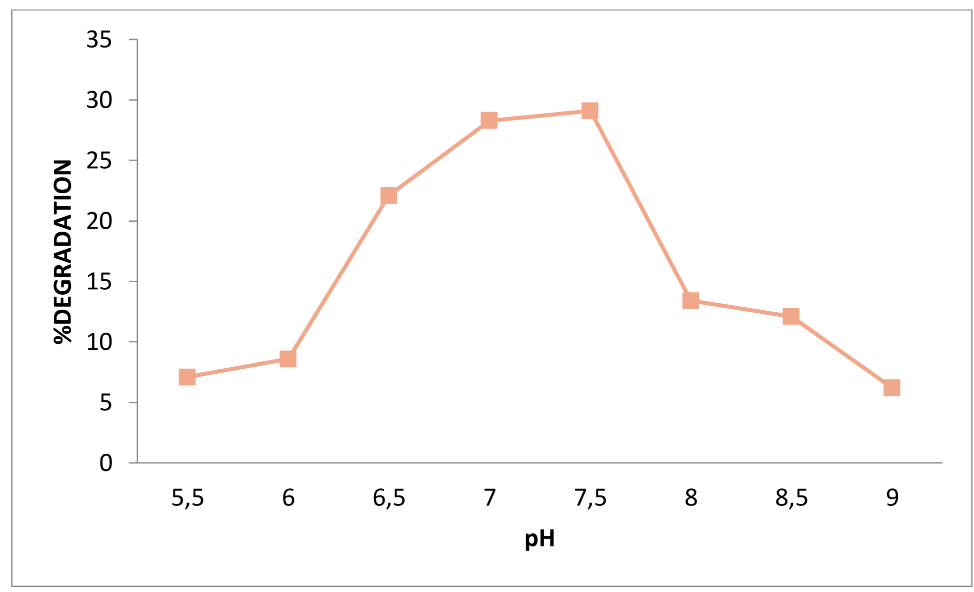

Fig. 2. *Effect of pH on degradation of phenol at 2ml concentration.*

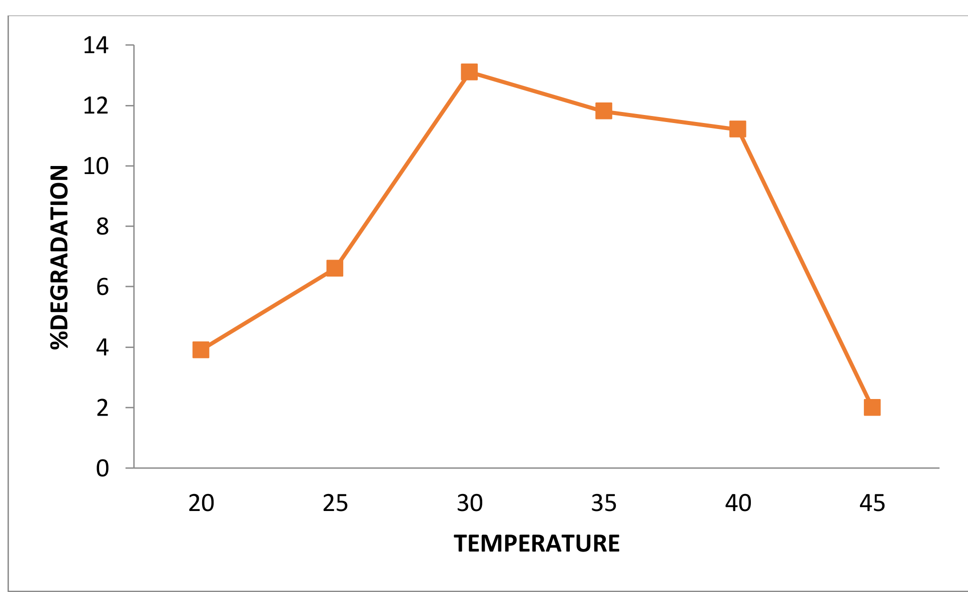

Fig.3. *Effect of temperature on degradation of phenol at 5ml concentration.*

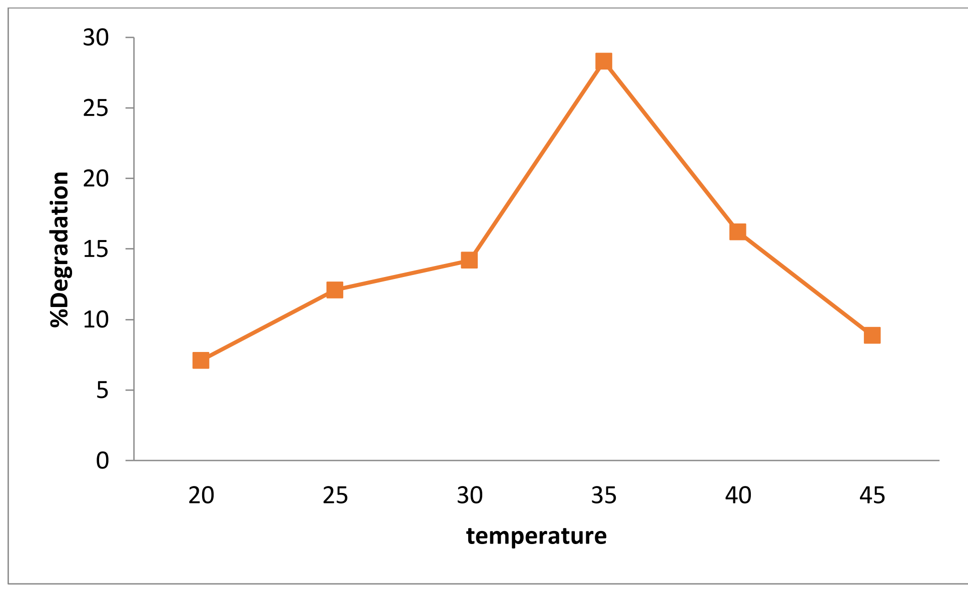

Fig.4. *Effect of temperature on degradation of phenol at 2ml concentration.*

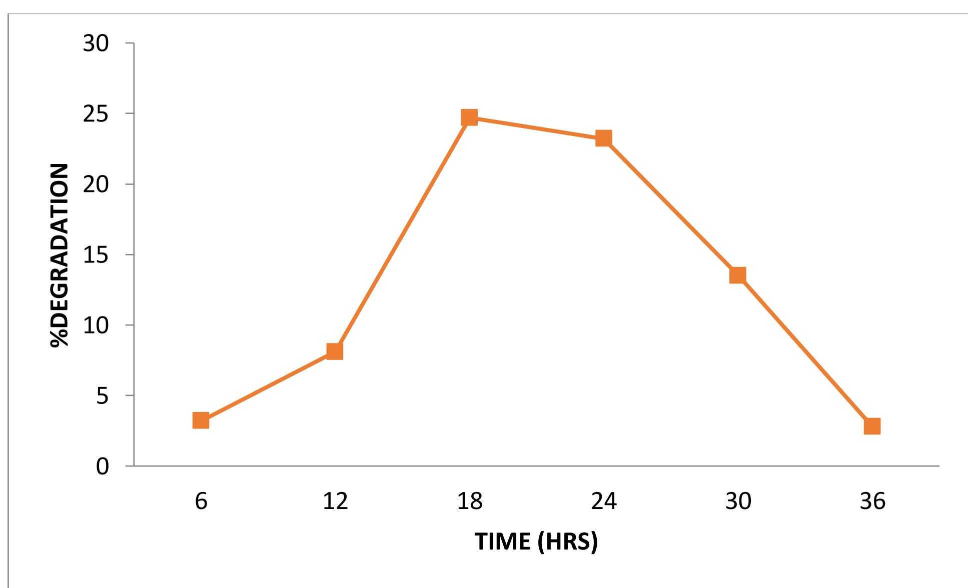

Fig.5. *Effect of contact time on phenol degradation at 5ml concentration.*

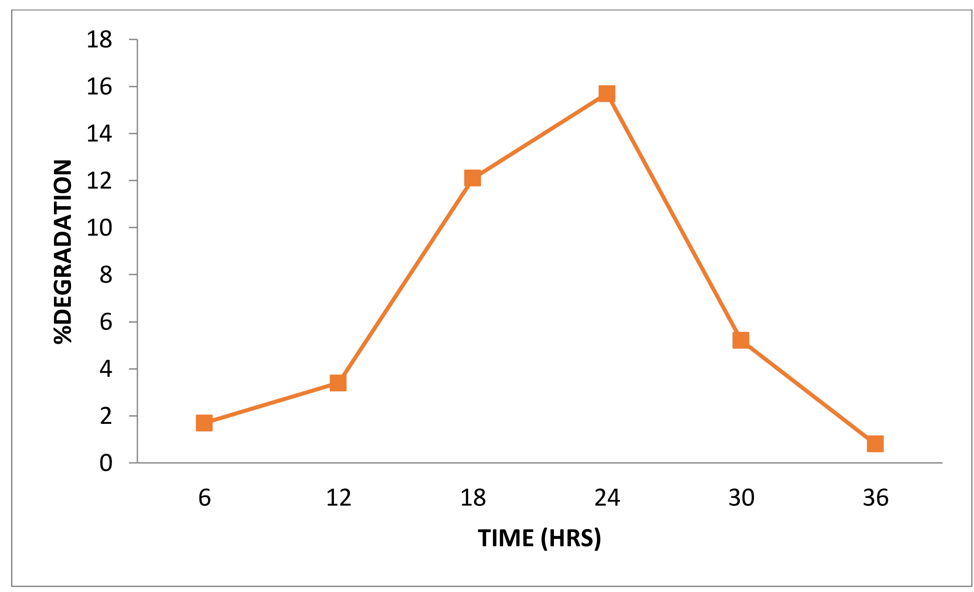

Fig.6. *Effect of contact time on phenol degradation at 2ml concentration.*

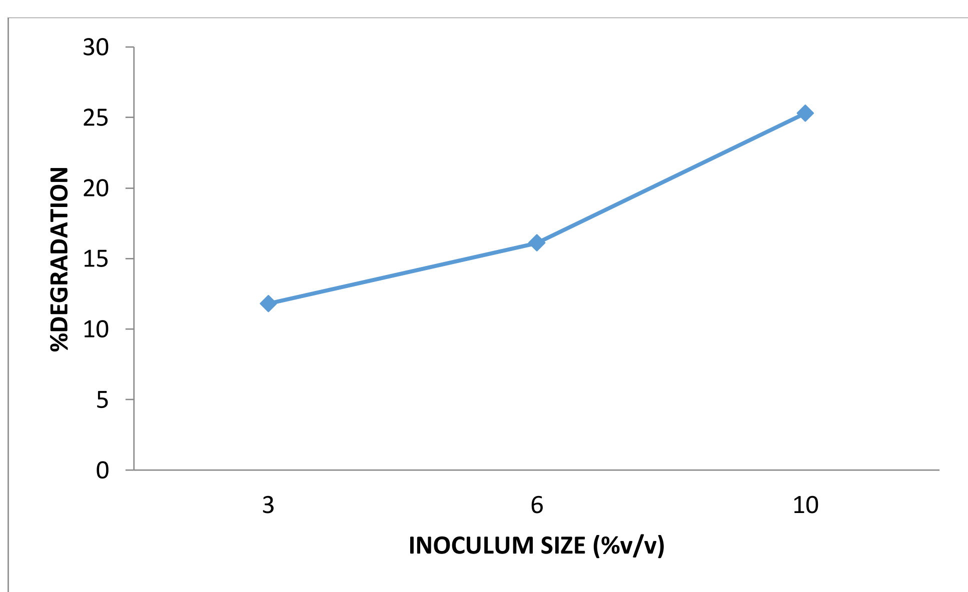

Fig.7. *Effect of inoculum size on phenol degradation at 5ml concentration*

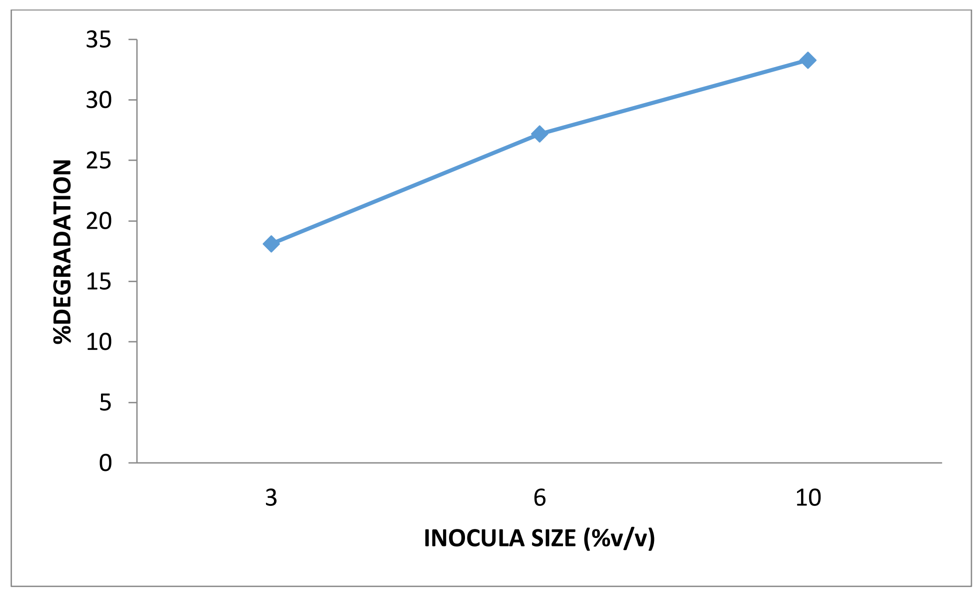

Fig.8. *Effect of inoculum size on phenol degradation at 2ml concentration*

CONCLUSION

This study showed that changes in the pH, temperature values of culture media, contact time and inocula size have influence on the outcome of phenol degradation by *S. epidermidis*. The isolates studied in this work were able to degrade phenol at varying optimum conditions under the parameters studied.

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