ISSN No:-2456 –2165

Shake Flask Studies of Phenol Biodegradation using Candida Sloofi and Sacchromyces Cervaciae

P. S. Sagar[1], Ch. V. R. Murthy[2]
[1]Department of Chemical Engineering, GMR Institute of Technology(A), Rajam, Srikakulam Dist.,532127, Andhra Pradesh, INDIA
[2]Department of Chemical Engineering, College of Engineering(A), Andhra University, Visakhapatnam-530 003, Andhra Pradesh, INDIA

Abstract:-The accumulation of organic carbon in many freshwater locations and the effluents from industrial plants joining the rivers and seas has great concern. It is becoming increasingly important to investigate how to degrade many troublesome contaminants and their biological oxidations in waste. The kinetic and study state degradation of Phenol with solution suspended Candida Slooffi , Sacchromyces cervasiae & mixed culture bacteria for the effect of operating parameters initial concentrations of Phenol and % degradation of Phenol at lower concentrations both the bacteria are suitable the percentage degradation is comparable with the literature values therefore these industrially available low cost bacterium may be used for the effluent treatment of phenolic waste waters.

Keywords:-Biodegradation, F/M Ratio, Inhibition, Growth Rate, Acclimatization.

I. INTRODUCTION

The accumulation of organic carbon in many freshwater locations and the effluents from industrial plants joining the rivers and seas has great concern. The origin of phenol in the environment is both anthropogenic as well as xenobiotic (Sagar.)**.** Anthropogenic sources are from forest fires, natural run-offs from urban area where asphalt is used as binding material and natural decay of lingo-cellulosic materials.

Stringent regulations put forward by the regulatory bodies on Phenol concentrations in wastewater to be discharged in seas, has made the removal of Phenol from wastewater essential before discharge.

Wastewater having lower Phenol concentration in the range 5-900ppm is suitable for biological treatment in a bioreactor. Important bioreactors which are in process are activated sludge bioreactor, trickling bed bioreactor, oxidation ponds, lagoons etc. The design of a reactor for the process catalyzed by immobilized cells is in principle similar to that for a conventional chemical process involving a heterogeneous catalyst, apart from the fact that sterile and aseptic conditions might be a necessity. So it is very essential to understand how biological reactors work, and also we should have complete idea about

- The biological reactor operation
- The fundamental phenomenon with the gas and liquid mixing and contacting patterns.
- The different design and scale up procedures required for reactors with different flow.

II. MATERIALS AND METHODS

A. Microorganism

Candida Sloofi bacteria which is widely available as Baker Yeast In the food production is chosen as it is widely available and able at affordable cost and Sacchromyces cervaciae bacteria is taken from the ethanol fermentation industry for the present study of its effectiveness degrade high concentration organic loads, these two bacteria are chosen as they are able to workable at high concentration organic loadings.

B. Maintenance and Sub Culturing

The strain was periodically sub cultured once in a fifteen days on agar slants. The agar slants were prepared by suspending separately 2.8gms of nutrient agar and 1.5gms of bacteriological agar in 100ml distilled water and were boiled to dissolve the medium completely (Sagar et al 2008). This solution were distributed in 10 test tube and sterilized in autoclave at 1.5Kg/cm^2 at 121^{0}C for 20min.They are kept in a slant position under aseptic conditions and medium was allowed to solidify while cooling. A loop (3mm) full of microorganism was taken from the stock culture and streaked on agar slants medium in a zigzag way, so that microorganism can consume medium easily and hence can grow and multiply.

This was then incubated at 30^{0}C in a bacteriological incubator for 48hrs. After observing satisfactory growth and for any contamination visually, the sub cultured slants were stored at 4^{o}C and sub cultured regularly for further experimentation.

C. Media Preparation

Organisms were grown on phenol as the sole carbon energy as defined in the medium wich is explained as minimal media M6 is used. To prevent precipitation of $Ca^{++}$and Mg^{++} the medium was prepared as two solution A & B ,which were separately steam sterilized. The solution was adjusted to pH 7.0 by 0.1N NaOH. Detailed minimal mineral media details are available in (Sagar et al 2008).

D. Acclimatization Procedure

The first step of the experiment is adaptation of microorganism to the phenol substrate. For the mixed cultures it is sufficient to acclimatize for few hours, whereas in the case of the monocultures acclimatization is as follows. About 99ml of mineral media were taken in the conical flask and sterilized in autoclave at 1.5Kg/cm^2 at 121^0c for 20min and cooled to room temperature (Shetty et al 2007). To this 1ml of 10,000ppm phenol solution was added to get an initial phenol concentration of 100ppm and the total volume of 100ml.Aloop full of test organism from the freshly sub cultured slant was inoculated into the above medium. The culture was incubated in a shaker for 48hrs. This formed the primary acclimatized culture.

E. Phenol Analysis

a). Direct Photometry Method

In this method phenol was directly taken to finding out the absorbance. The absorbance was measured at 410nm using UV-spectrophotometer (Busca, Guido, et al. 2008)..

- *Preparation of Phenol Solution:*

1,000 ppm Phenol solution stock was prepared by dissolving 1 ml of concentrated Phenol in l000 ml of distilled water.100 ppm Phenol solution was prepared from 1,000 ppm Phenol stock.

- *Preparation of Standards:*

To obtain a calibration curve a series of 100ml Phenol standards with reagent are prepared. 1ml, 2ml, 3ml, 4ml of 1,000ppm Phenol were pipette out in 1,000 ml standard flasks and made up to the mark with distilled water so that the final concentration obtained be 1, 2, 3,4ppm respectively (Sagar et al 2008). The solution was shaken well. After 15 minutes the standards were transferred to the cell and the absorbance was read against blank (Distilled water) at 403nm using UV-spectrophotometer (UV-160 A spectrometer-Hitachi)

F. Experimental Procedure

To study the kinetics of degradation of Phenol in batch bio reactor, For Effect of initial Phenol concentration (200ppm, 400ppm and 800ppm)on % degradation, prepare the solutions in the reactors directly. For 200,400,600 and 800ppm solutions pour 200,400,600,800 of 1000ppm solutions respectively, add 50ml of solution A, 50ml of solution B (nutrient media) and make it to 1 liter by distil water. And now sterilize the reactors in autoclave at 1.5kg/cm^2 pressure. Now bring the reactors out and cool it to normal temperature. Add 2ml of acclimatized (bacteria) solutions to the respective concentration reactors (Sundar Kumar et al 2010). Now collect 5 ml of sample from every reactor for every 2hrs and analyze it individually by the following way in UV-Spectrophotometer. Filter the sample by using filter paper and collect sample. After collecting sample make up 100ml solution by adding distilled water, after that finding the absorbance by using spectrophotometer (Busca, Guido, et al. 2008)

III. RESULTS AND DISCUSSIONS

A. Batch Studies Phenol Biodegradability: Shake Flask Batch Studies

Is evident that both the bacteria are capable of phenol bio degradation and from the graphical analysis of data it is find that the reaction can be best assumed as first order reaction (Kumar et al 2005) within the working rage up to 800 ppm. Key parameters of effluent concentrations and percentage degradation of individual bacteria are discussed in detail in sub topics

a). Effect of Initial Phenol Concentration on Phenol Biodegradability With Saccharomyce cerevisiae

Figure 1 represents the effect of inlet concentration on sample concentration of during the startup and steady state with *Saccharomyce cerevisiae*at initial phenol concentration of 200,400&800ppm. The phenol concentration has lagged at 2hrs during the start up phase from but it has increased after lag phase, the growth of microorganism is increased exponentially hence phenol concentration in shake flask are reduced drastically during the experimental study period of 40 hr the fallowing results are obtain. At 40 hrs the out let phenol concentration was found to decrease continuously to the levels of 12,35, 65.5and 86.5ppm respectively they are truly fitted for exponential decay equations with 90, 98 and 97 percent fitment

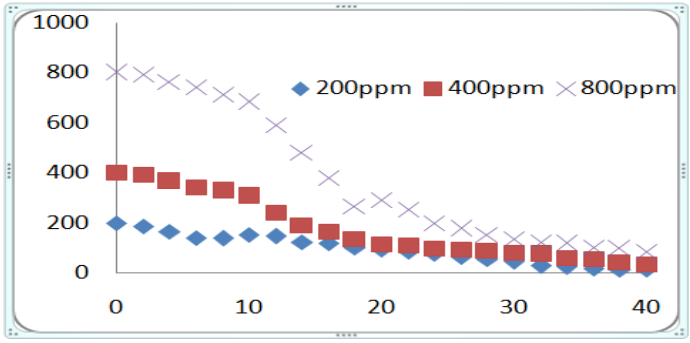

Figure 1:-Study of kinetic behavior Phenol by varying concentration with respect to time by using *Saccharomyce Cervasiae.*

ISSN No:-2456 –2165

b). Effect of Initial Phenol Concentration on Phenol Biodegradability with Candida Sloofi

Figure 2 represents the effect of in let concentration on out let phenol concentration during the start up and steady state with *Candida Sloofi* at initial phenol concentration of 200,400, 600 &800ppm. The phenol concentration has lagged at 2hrs during the start up phase from but it has increased after lag phase, te growth of microorganism is increased exponentially hence phenol concentration in shake flask are reduced drastically during the experimental study period of 40 hr the fallowing results are obtain. At 40 hrs the sample concentration of was found to decrease continuously to the levels of 12,35, 65.5and 86.5ppm respectively they are truly fitted for exponential decay equations with 90, 98 and 97 percent fitment.

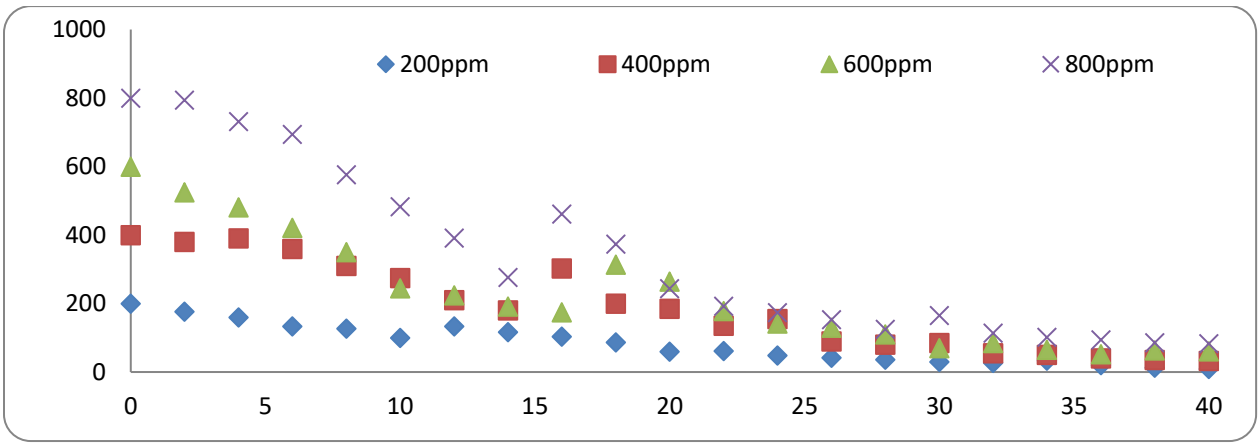

Figure 2 Study of Kinetic Behaviour of Phenol By Varying Concentrations (200ppm, 400ppm, 600ppm, 800ppm) By Using Candida Sloofi

B. Effect of Initial Phenol Concentration on Percentage Degradation With Candida Sloofi

Figure 3 showing that percentage degradation is strong function of initial phenol concentration the coefficent of this is almost constant wich is due to growth rate of microbes are constant it is indipendet of concentration growth rate may be lagged because of the for the initial phenol concentrations 200, 400, 600 and 800 ppm experiments conducted the percentage degradation is high for the lower concentrations that is for 200 and 400 ppm. It is also observed that percentage degradation is show some assymetry of graphs which is because of rate of degradation is also function of amout of bio mass present and which is it selfs function of initial concentration therefore amout of bio mass increses with initial phenol concentration hence they are symmetricall and very close to each other.

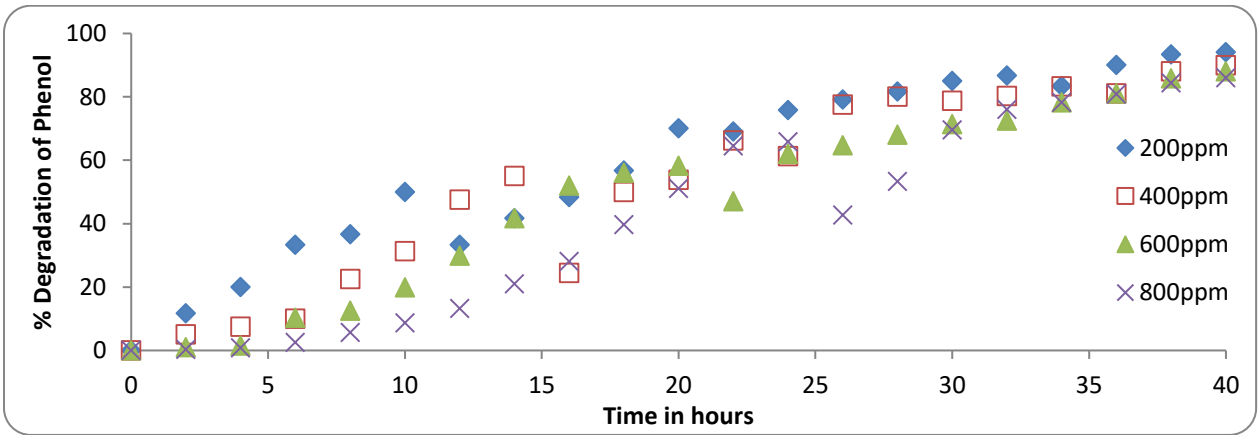

Figure 3:- Study of Kinetic Behavior of Phenol % Degradation Takes Place By Varying Concentration With Respect To Time By Using *Candida Sloofi*

ISSN No:-2456 –2165

a). Effect of initial Phenol Concentration on Percentage Degradation with Sacchromyces Cervasiaec

Figure 4 showing that percentage degradation is strong function of initial phenol concentration the coefficent of this is almost constant wich is due to growth rate of microbes are constant it is indipendet of concentration growth rate may be lagged because of the for the initial phenol concentrations 200, 400, 600 and 800 ppm experiments conducted the percentage degradation is above 95% for the lower concentrations that is for 200 and 400 ppm. Similar tocandita species It is also observed that percentage degradation is show some assymetry of graphs which is because of rate of degradation is also function of amout of bio mass present and which is it selfs function of initial concentration therefore amout of bio mass increses with initial phenol concentration hence they are symmetricall and very close to each other. For 800 ppm percentage degradation is lagged at initial stagesup to 10 hours but later it shows similar behaveior with lower concentrations

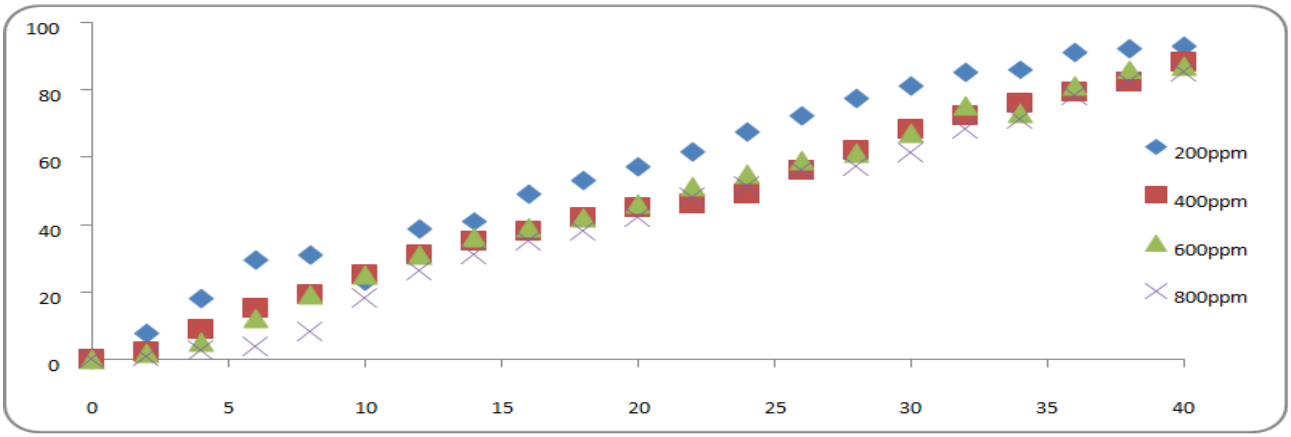

Figure 4:- Study of kinetic Behaviour of Phenol% Degradation Takes Place By Varying Concentration By Using *Sacchromyces Cervasiae*

C. Effect of Initial Phenol Concentration on Phenol Biodegradability With Mixed Culture of Saccharomy cerevisiae Andcandida Sloofi

Figure5 represents the effect of in let concentration on out let phenol concentration during the startup and steady state with *mixed culture* at initial phenol concentration of 200,400, 600 &800ppm. The phenol concentration has lagged at 2hrs during the start up phase for 600 and 800 ppm but it has increased after lag phase, the growth of microorganism is increased exponentially hence phenol concentration in shake flask are reduced drastically during the experimental study period of 40 hr. from Fig 6 the degradation percentage is also shown similar trends like individual Bacterial species the difference has where as in mixed culture % degradation of 200 ppm is almost achived 100% in 40 hours of study period, at 40 hours total degradation of 400, 600 and 800 ppm are also increased compared with individual bacterial culture experiment's

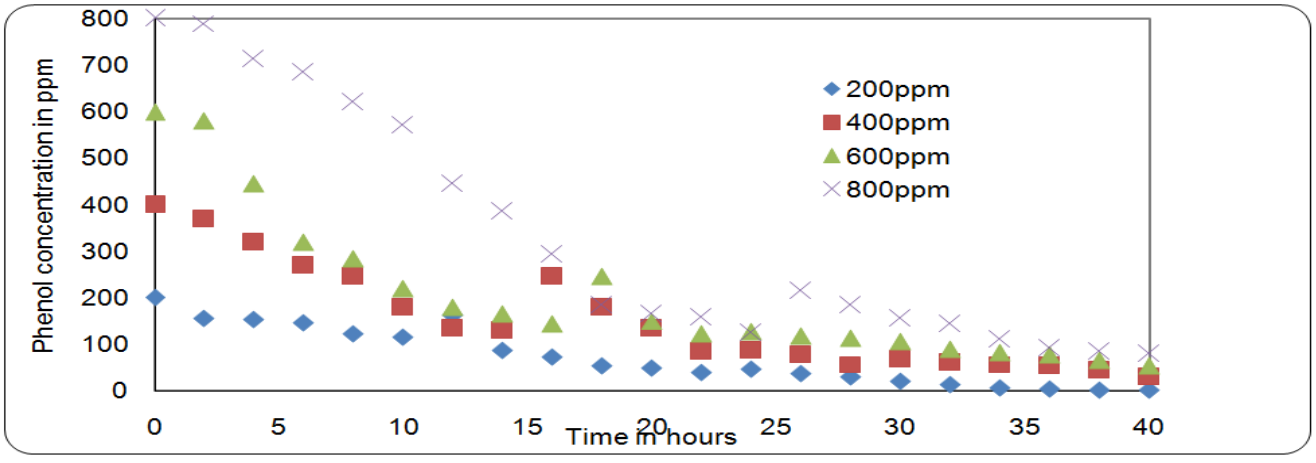

Figure 5:- Study of Kinetic Behaviour of Phenol By Varying Concentrations With Respect To Time By Using Mixed Culture.

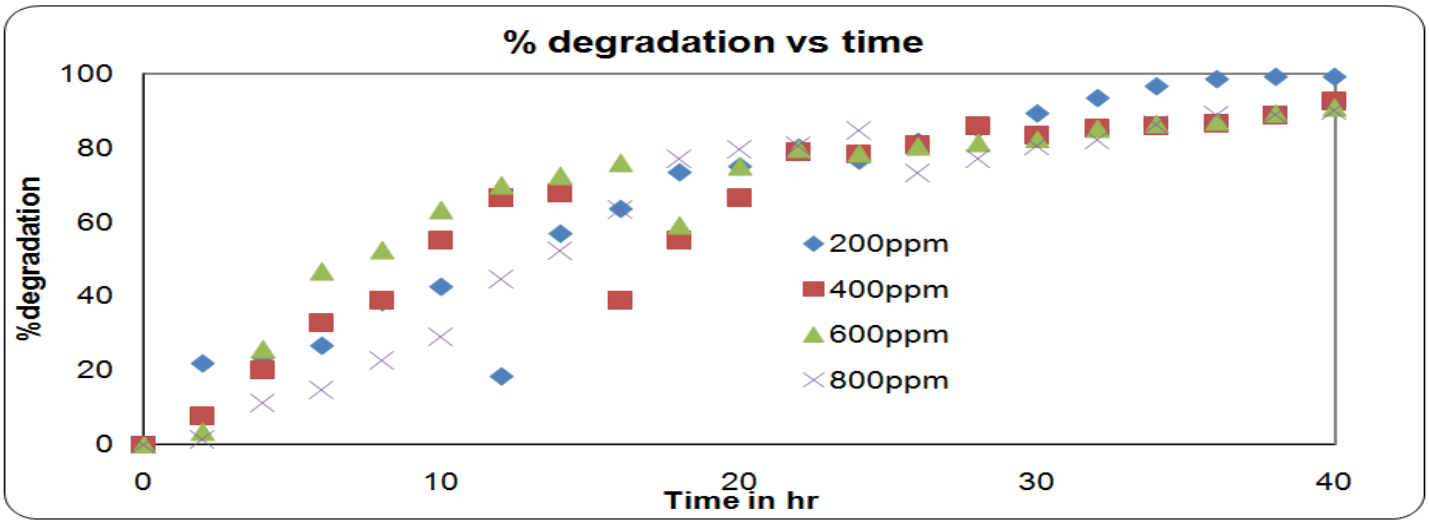

Figure 6:- Study of Kinetic Behaviour of phenol % of Degradation Takes Place By Varying Concentration With Respect To Time By Using Mixed Culture.

IV. CONCLUSIONS

The kinetic and study state degradation of Phenol with solution suspended Candida Slooffi, Sacchromyces cervasiae & mixed culture bacteria for the effect of operating parameters initial concentrations of Phenol and % degradation of Phenol. From the results obtained the following conclusions can be drawn .

- *Candida Sloofi & Sacchromyces Cervasiae* can used for industrially adopted for Phenol degradation
- Candida Sloofi yeast & Sacchromyces Cervasiae having a higher F/M ratios can be adopted and able to degrade efficiently
- It is observed that time taken to reach steady state increased as the initial Phenol concentration increases this may be because of having higher F/M leads to continuous growth of biomass during start up and hence it may take more time for the biomass to reach the steady state
- It is observed that % degradation and rate of degradation is decreased at steady state conditions with increase in initial Phenol concentrations this may be the result of inhibitory characteristics of Phenol.

REFERENCES

[1]. S.N. Dash and P.S. Sagar. ""Adsorption of zinc metal from paper mills wastewater by activated carbon prepared from Sho." 7.1 (2008).

[2]. Ailijiang, Nuerla and Chang, Jiali and Liang, Peng and Li, Peng and Wu, Qing and Zhang, Xiaoyuan and Huang, Xia. Electrical stimulation on biodegradation of phenol and responses of microbial communities in conductive carriers supported biofilms of the bioelectrochemical reactor. Vol. 201. Bioresource technology, 2016.

[3]. Basak, Bikram and Bhunia, Biswanath and Dutta, Subhasish and Chakraborty, Samayita and Dey, Apurba. "Kinetics of phenol biodegradation at high concentration by a metabolically versatile isolated yeast Candida tropicalis PHB5." Environmental Science and Pollution Research 21.2 (2014): 1444-1454.

[4]. Busca, Guido, et al. 2008. ""Technologies for the removal of phenol from fluid streams: a short review of recent developments."." 160.2 (2008).

[5]. Kumar et al 2005. "Biodegradation kinetics of phenol and catechol using Pseudomonas putida MTCC 1194." Biochemical Engineering Journal (2005): 151-159.

[6]. Praveen, Prashant and Loh, Kai-Chee. "Osmotic membrane bioreactor for phenol biodegradation under continuous operation." Journal of hazardous materials (2016): 115-122. Sagar et al 2008. "Effect of Influent Concentration on Biodegradation of Phenol Using Packed Bed Reactor." 7.4 (2008).

[7]. Shetty et al 2007. ""Biological phenol removal using immobilized cells in a pulsed plate bioreactor: Effect of dilution rate and influent phenol concentration." 149.2 (2007). Sundar Kumar et al 2010. "Treatment of Synthetic Wastewater Containing Phenol, Thiocyanate and Nitrate-N in Anoxic Fixed Film Reactor." Nature Environment and Pollution Technology (2010): 355-363.

[8]. Wang, Qing and Li, Yi and Li, Jing and Wang, Yuming and Wang, Chao and Wang, Peifang. "Experimental and kinetic study on the cometabolic biodegradation of phenol and 4- chlorophenol by psychrotrophic Pseudomonas putida LY1." Environmental Science and Pollution Research 22.1 (2015): 567-573.