ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 12, Issue, 04, pp. 55571-55574, April, 2022
https://doi.org/10.37118/ijdr.24409.04.2022

RESEARCH ARTICLE OPEN ACCESS

BIODEGRADATION OF PHENOL BY *PSEUDOMONAS AERUGINOSA*, *ACINETOBACTER* SP. AND *STENOTROPHOMONAS MALTOPHILIA* ISOLATED OF THE SLUDGE ACTIVATED OF A STEEL INDUSTRY

Paulo Ricardo. Pacheco Dias*[1], Tatiana Oliveira Paiva[1], Ana Maria de Oliveira[2], José Carlos de Magalhães[2] and Enio Nazaré de Oliveira Junior*[2]

[1]Postgraduate Program in Technologies for Sustainable Development, Federal University of São João Del-Rei, Ouro Branco, Minas Gerais, Brazil; [2]Department of Chemistry, Biotechnology and Bioprocess Engineering, Federal University of São João Del-Rei, Ouro Branco, Minas Gerais, Brazil

ARTICLE INFO

Article History:
Received 20th January, 2022
Received in revised form
22nd February, 2022
Accepted 14th March, 2022
Published online 30th April, 2022

Key Words:

Biodegradation,
Sustainability,
Biorremediation.

Corresponding author:
Enio Nazaré de Oliveira Junior

ABSTRACT

The purpose of this work was to study the degradation capacity of phenol by strains of *Pseudomonas aeruginosa*, *Acinetobacter* sp. and *Stenotrophomonas maltophilia* isolated from activated sludge from a steel industry. For this, a study of the kinetics of microbial growth and degradation of phenol, besides a factorial planning was carried out, varying the initial concentration of phenol and the pH. *Pseudomonas aeruginosa* removed 100% of the phenol in 72 h, when the initial concentration of the compound was 250 mg L^{-1} and the pH was controlled at 6.4. The bacterium *Acinetobacter* sp. removed 68.71 (± 0.51) % of the phenol and the *S. maltophilia* strain removed 68.55 (± 0.58) % of the phenol in 96 hours under the same conditions as the assay performed with the *P.aeuruginosa* strain. It was found that the optimum pH for the removal of phenol by *P. aeruginosa* was 6.4, for *Acinetobacter* sp. was 7.0 and *S. maltophilia* was 7.3. The removal of phenol by the three *bacteria* was better when the initial phenol content was lower, with the *P. aeruginosa* bacterium having the best performance and showing promising results even when there were higher initial concentrations of phenol. This work brings the possibility of a process of great environmental interest, since phenol is a recalcitrant compound of great environmental impact and the *bacteria* isolated from the activated sludge of a steel industry are able to biodegrade phenolic compounds and can be used in treatment effluents or in bioremediation techniques.

Copyright © 2022, Paulo Ricardo. Pacheco Dias et al. *This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.*

Citation: ***Paulo Ricardo. Pacheco Dias, Tatiana Oliveira Paiva, Ana Maria de Oliveira, José Carlos de Magalhães and Enio Nazaré de Oliveira Junior.*** "Biodegradation of phenol by *pseudomonas aeruginosa*, *acinetobacter* sp. and *stenotrophomonas maltophilia* isolated of the sludge activated of a steel industry*", International Journal of Development Research*, 12, (04), 55571-55574.

INTRODUCTION

Phenols are recalcitrant compounds of high toxicity, which can have serious environmental impacts if they are inappropriately disposed of in ecosystems. Phenolic compounds are an important class of environmental contaminants, as they are present in many industrial effluents, such as the pharmaceutical and textile industries, refineries, petrochemicals and steel mills, agricultural pesticides, resins and paper, among others (MIRANDA et al., 2013; PASSOS *et al*., 2008). Pseudomonas aeruginosa is a Gram-negative bacillus that can be isolated from different habitats, including water, soil and plants (JAY, 2005). *Bacteria* of the genus Pseudomonas have developed the ability to use substrates toxic to their growth such as phenolic compounds and benzene.

Therefore, these microorganisms play a very important role in the degradation of pollutants from contaminated areas and can be used in bioremediation techniques (KURBATOV *et al.,* 2006). The genus Acinetobacter is a group of gram-negative *bacteria*, belonging to the Moraxellaceae family. They have cells in the form of cocobacilli and are important soil microorganisms, as they contribute to the degradation of aromatic compounds. The biological or ecological knowledge of *bacteria* of the genus *Acinetobacter* at the species level is still limited. This is due to the fact that the identification of these *bacteria* at the species level is difficult due to the high proteomic similarity that prevents phenotypic identification methods from being applied to closely related species (GERISCHER, 2008). *Stenotrophomonas maltophilia* is a gram-negative bacillus that can be found in a wide variety of environments and geographic regions, occupying distinct ecological niches and multiple sources in both

water and soil. (ALMEIDA *et al*., 2005). In the research by URSZULA et al. (2009), it was found that this strain uses aromatic compounds as the only source of carbon and energy, and the activity of key enzymes in the degradation of phenolic compounds has also been identified. The *bacteria*l strains used in this research were isolated from a sample of activated sludge from a steel industry and, for this reason, were already adapted to the presence of phenol, since the steel effluents present high concentrations of this compound. Thus, these strains have great potential in the treatment of phenolic effluents and in the recovery of areas contaminated by phenolic waste. The objective of this work was to study the phenol degradation capacity of *P. aeruginosa, Acinetobacter sp., and S. maltophilia* strains isolated from activated sludge in a steel industry, identifying the best conditions for removing phenol by the studied *bacteria*l strains.

MATERIALS AND METHODS

Kinetics of microbial growth and phenol degradation: Absorbance is an indirect measure of cell growth, as the medium becomes cloudy as cell growth occurs. Thus, for the study of microbial growth kinetics, a curve was constructed relating absorbance (600 nm) and time. By determining the dry mass, absorbance and cell concentration were related. The pH of the medium was monitored at 6.4 during the assay using phosphate buffer. The composition of the medium employed in this study is in mg L^{-1}: glucose (1000), $MgSO_4.7H_2O$ (2.5), $CaCl_2.2H_2O$ (3.8), $MnSO_4.7H_2O$ (0.14), Fe_2 (SO_4) $.6H_2O$ (0.60), (NH_4) $2SO_4$ (100) and phenol (250). The determination of phenol was also carried out during the kinetics test to evaluate the degradation of this compound over time, using the spectrophotometric technique.

Study of the degradation of phenol by strains of *Pseudomonas aeruginosa*, *Acinetobacter* sp. and *Stenotrophomonas maltophilia*: In order to evaluate the best conditions of phenol biodegradation, a statistical planning was used presenting four axial points and triplicate at the central point. With the objective of verifying if the phenol underwent degradation during the fermentation period, an additional test (abiotic control) was done, whose composition of the medium is the same as the central points of the experimental planning. The independent variables studied were phenol concentration and pH. Table 1 shows the experimental planning where the phenol concentration and pH were varied. The composition of the medium is the same as that used in the kinetics assay, except for the phenol having varied concentrations. The pH of the medium was controlled using phosphate buffer.

Table 1. Experimental planning by varying the phenol concentration and pH

Test	Variable code	Phenol (mg L^{-1})	pH
1	-1, -1	108	5.8
2	-1, +1	108	7.0
3	+1, -1	392	5.8
4	+1, +1	392	7.0
5	-1,41, 0	50	6.4
6	+1,41, 0	450	6.4
7	0, -1,41	250	5.5
8	0, +1,41	250	7.3
9*	0, 0	250	6.4
10*	0, 0	250	6.4
11*	0, 0	250	6.4
12**	0, 0	250	6.4

* Central points ** Abiotic control

RESULTS AND DISCUSSION

Kinetics of microbial growth and phenol degradation: Figure 1 shows the result obtained in the kinetic assay of the bacterium Pseudomonas aeruginosa and, as can be seen, the strain removed all the phenol at an initial dose of 250 mg L-1 in 72 h, degrading from the beginning of the fermentation process, indicating that this bacterium is able to degradation of phenol presenting a great potential in the removal of the compound. AGARRY *et al.* (2008) used a strain of P. aeruginosa in the treatment of effluent from a Nigerian oil refinery, and the results showed that the strain degraded 94.5% of the phenol at a concentration of 100 mg L^{-1} in 72 hours. Through the analysis on the Q-TOF mass spectrometer, the formation of benzoic acid and 4-hydroxybenzoic acid was verified, which indicates that this bacterium used the anaerobic phenol degradation route, possibly because the agitation of the medium was not enough to guarantee a good oxygenation of the medium. *Bacteria* of the genus Pseudomonas are well adapted to anaerobic metabolism and even without adequate aeration; it had excellent ability to remove the compound. This is a very interesting aspect, since this bacterium can be used in anaerobic processes to remove phenol, which has a lower energy expenditure when compared to the equipment and aeration needs of the aerobic process.

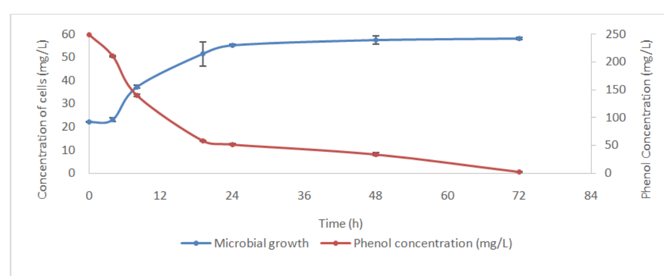

Figure 1. Kinetics of microbial growth and degradation of phenol in the period of 72 hours, temperature of 30 ° C, pH of 6.4 and agitation of 150 RPM for the bacterium Pseudomonas aeruginosa

Figure 2 shows the result obtained in the kinetics test with the bacterium Acinetobacter sp., in which it can be observed that this strain removed a greater amount of phenol in the first twenty-four hours, however, after that time, the degradation process was slower and at the end of 96 hours, the microbial population degraded 68.71 (± 0.51) % of the initial phenol. It is believed that this bacterium has a metabolism well adapted to phenol degradation, since phenol degradation has occurred since the initial incubation moments. However, after the *bacteria* entered the stationary growth phase, the rate of phenol degradation decreased significantly. This may have happened due to its inhibition by the formation of some metabolite, since the test was carried out in batch and in the mass spectrometry analysis there was an enormous amount of signals that were not identified. Through the analysis in the mass spectrometer Q-TOF the formation of benzoic acid and 4-hydroxybenzoic acid was verified, which indicates that this bacterium used the anaerobic route of phenol degradation. The *bacteria* of the genus Acinetobacter found in nature are strict aerobic, however this strain came from an activated sludge station in which one of the tanks is anoxic in order to favor denitrification, which may have contributed for this strain to adapt to anaerobic metabolism. This can also justify its worse results when compared to the *P.aeruginosa* strain, because, although the *Acinetobacter* sp. isolated in this research was able to perform the anaerobic route, it was not as efficient as the bacterium of the genus *Pseudomonas*, which already has an optional anaerobic metabolism. In the study carried out by Abd-El-Haleem et al., 2003, the strain *Acinetobacter* sp. W-17 was able to degrade all phenol at an initial concentration of 500 mg L-1 in 120 hours in minimal mineral medium and with dispersed growth. Superior results were obtained when the Acinetobacter sp. W-17 was immobilized on Ca-alginate gel, where the degradation occurred in 24 hours with minimal mineral medium and 15 hours with modified medium containing waste water. A possibility to improve the performance of the strain of *Acinetobacter* sp. isolated from the activated sludge of the steel industry is to work with the same immobilized, since that way it will have a larger surface area and access of the phenol by the *bacteria*l strain. In addition, efficient aeration is essential for the degradation of the compound by this strain. Figure 3 shows that the bacterium Stenotrophomonas maltophilia removed, in 96 hours, 68.55 (± 0.58)% of the initial phenol, a final performance very similar to the

Acinetobacter sp. However, the microbial growth curve was quite different.

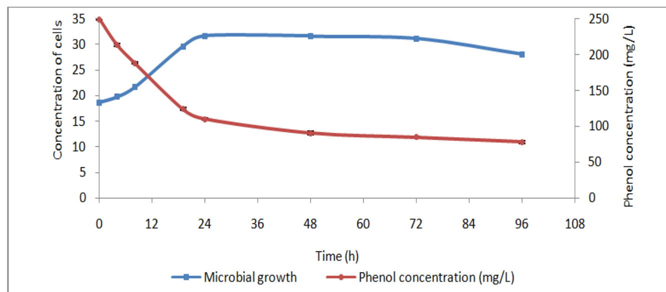

Figure 2. Kinetics of microbial growth and degradation of phenol in the period of 72 hours, temperature of 30 ° C, pH of 6.4 and agitation of 150 RPM for the bacterium Acinetobacter sp

The lag phase of the *S. maltophilia* strain lasted 4 hours and the exponential phase was very long, comprising the period between 4 and 48 hours. After two days of incubation, microbial growth entered the stationary phase. Phenol removal occurred from the initial instants, occurring at a higher rate during the first twenty-four hours. As with the bacterium of the genus Acinetobacter, in the stationary phase of the growth of the *S. maltophilia* strain, the phenol degradation was slower and this may have been caused by some change in the composition of the medium, such as the generation of some metabolite, and in the mass spectrometry analysis there was an enormous amount of signals that were not identified. Through the analysis of a sample after the biodegradation process, the formation of benzoic acid and 4-hydroxybenzoic acid was verified in the Q-TOF mass spectrometer, which indicates that this bacterium used the anaerobic phenol degradation route. In the literature, this bacterium has been described as strict aerobic, however, it was isolated from an activated sludge process with the presence of an anoxic tank for the occurrence of denitrification. This bacterium may have adapted to this condition, performing anaerobic degradation, even if in a more limited way.

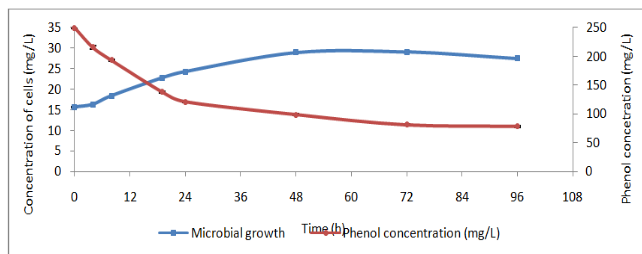

Figure 3. Kinetics of microbial growth and degradation of phenol in the period of 72 hours, temperature of 30 ° C, pH of 6.4 and agitation of 150 RPM for the bacterium Stenotrophomonas maltophilia

Study of the degradation of phenol by strains of Pseudomonas aeruginosa, Acinetobacter sp. e Stenotrophomonas maltophilia: Table 2 presents the results of the statistical planning tests performed for each of the studied *bacteria*l strains. As can be seen in the contour curve of Figure 4, to obtain a better degradation of phenol by the bacterium P. aeruginosa, the pH should be controlled at 6.4 (center point). Observing Table 1, the bacterium P. aeruginosa presented excellent efficiency in the removal of phenol. The mean phenol removal at the central points was 98.9 (± 0.4) %. It can still be seen that the lower the initial concentration of phenol, the better the percentage of removal of the compound. This is easily justified because in the presence of toxic compounds such as phenol, microbial growth is affected, and the higher the concentration of the recalcitrant compound, the greater the inhibitory effect on the microorganism tends to be. The P. aeruginosa strain also shows a good efficiency in the removal of phenol, even when it has higher phenol initial concentrations.

Table 2. Results of statistical planning varying the concentration of phenol and pH for *bacteria* Pseudomonas aeruginosa, Acinetobacter sp. e Stenotrophomonas maltophilia

	% Phenol Removal		
Test	*P.aeruginosa*	*Acinetobacter sp.*	*S. maltophilia*
1	91,6	69,5	64,2
2	100	75,4	70,5
3	79,2	60,5	50,9
4	81	63,0	63,9
5	100	97,0	74,3
6	79,6	64,7	62,5
7	43,9	57,2	60,6
8	69,1	79,2	73,2
9*	99,0	68,9	68,0
10*	98,5	69,1	67,5
11*	99,3	67,8	67,4
12**	0,3	0,5	0,5

*Central points** Abiotic control

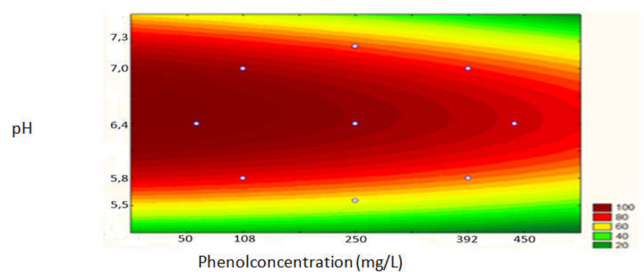

Figure 4. Contour curve of the phenol removal in relation to the initial phenol concentration and pH for the bacterium *Pseudomonas aeruginosa.*

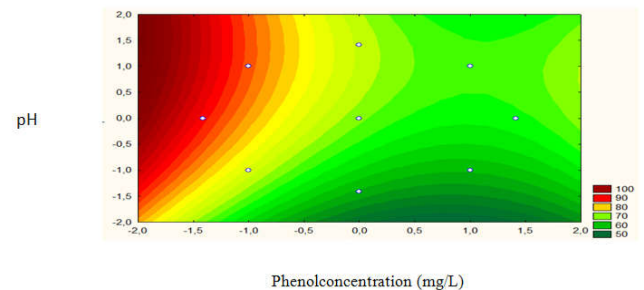

Figure 5. Contour curve of the phenol removal in relation to the initial phenol concentration and pH for the bacterium *Acinetobacter sp.*

Analyzing figure 5, it appears that to obtain a better degradation of phenol by Bacterium Acinetobacter sp., the pH of the medium must be controlled around 7.0. According to figure 6, for the bacterium Stenotrophomonas maltophilia, the pH of the medium must be controlled close to the extreme point of this planning (7.3).

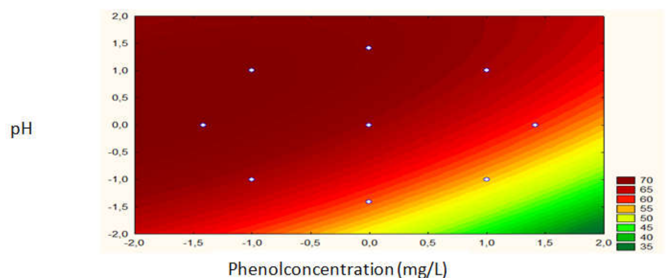

Figure 6. Contour curve of the phenol removal in relation to the initial phenol concentration and pH for the bacterium Stenotrophomonas maltophlia

For both *bacteria*, it is observed that the lower the initial concentration of phenol, the better the percentage of phenol removal,

a similar fact that occurred with the bacterium Pseudomonas aeruginosa and can be explained by the toxicity of the phenol that can cause an inhibitory effect on the microorganism in higher concentrations. Therefore, it is interesting to perform a new kinetics at the optimum pH of each strain and thus obtain the kinetic parameters of inhibition.

CONCLUSION

The *bacteria*l strains *Pseudomonas aeruginosa*, *Acinetobacter* sp. and *Stenotrophomonas maltophilia*, isolated from the activated sludge of a steel industry and used in this work, have a great potential for phenol removal and can be used in the treatment of phenolic effluents or in the recovery of areas contaminated by bioremediation techniques. For this, more research must be done in order to better understand the biodegradation carried out by these *bacteria*, such as, for example, an enzymatic study.

Acknowledgments

The authors are grateful for the financial support of CNPq - National Council for Scientific and Technological Development, FAPEMIG - Fund for Research Support of the State of Minas Gerais, and PPGTDS - UFSJ - Postgraduate Program in Technologies for Sustainable Development of the Federal University of São João Del-Rei.

REFERENCES

Abd-El-Haleem, D., Beshay, U., Abdelhamid, A.O., Moawad, H., Zaki, S. Effects of mixed nitrogen sources on biodegradation of phenol by immobilized Acinetobacter sp. strain (W-17). *African Journal Biotechnology*. v.2(1), p. 8-12, 2003.

Almeida, M.T.G.; Bertelli, E.C.P.; Rossit, A.R.B.; Bertollo, E.M.G.; Martinez, M.B. Infecções Hospitalares por Stenotrophomonas maltophilia: aspectos clínico-epidemiológicos, microbiológicos e de resistência antimicrobiana. *Artigo. Ciência Saúde,* v.12(3), p.141-45. 2005.

Agarry, S.E., Durojaiye, A.O., Yusuf, R.O., Aremu, M.O. Biodegradation of phenol in refinery wastewater by pure cultures of Pseudomonas aeruginosa NCIB 950 and Pseudomonas fluorescence NCIB 3756. *Journal Environ Pollution*. v.32(1), p. 3-11, 2008.

Gerischer, U. Acinetobacter Molecular Biology. University of Ulm, Germany, 2008, 378p.

Jay, J.M. Microbiologia de alimentos. Editora Artmed. 6ªed. 2005.

Kurbatov L., Albrecht, D. Herrmann H., Petruschka L. Analysis of the proteome of Pseudomonas putida KT2440 grown on different sources of carbon an energy. *Environmental Microbiology*. v. 8(3), p. 466-78, 2006.

Miranda, R. C. M.; Gomes, E. B.; Pereira Jr., N.; Marin-Morales, M. A.; Machado, K. M. G.; Gusmão, N. B. Biotreatment of textile effluent in static bioreactor by Curvularia lunata URM 6179 and Phanerochaete chrysosporium URM 6181. Bioresource Technology, v. 142, p. 361-367, 2013.

Passos, C.V.; Burkert, J.F.M.; Kalil, S.J.; Burkert, C.A.V. Biodegradação de fenol por uma nova linhagem de Aspergillus sp. isolada de um solo contaminado do sul do Brasil. Química Nova. v. 32, n.4, p. 950 –954, 2008.

Urszula, G., Izabela, G., Danuta, W., Sylwia, L. Isolation and characterization of a novel strain of Stenotrophomonas maltophilia possessing various dioxygenases for monocyclic hydrocarbon degradation. Brazilian Journal of Microbiology, v.40(2), p. 285–291, 2009.

****