UFSM

CIÊNCIA e NATURA

ISSN 2179-460X
OPEN ACCESS

Ci. e Nat., Santa Maria, v. 46, e84868, 2024 • https://doi.org/10.5902/2179460X84868
Submitted: 22/08/2023 • Approved: 20/05/2024 • Published: 06/09/2024

Environment

Use of *pseudomonas fluorecens* isolated from the Serra de Ouro Branco State Park/Minas Gerais - Brazil in the biodegradation of residual automotive lubricating oils

Emprego de *pseudomonas fluorecens* isoladas do parque estadual Serra de Ouro Branco/Minas Gerais – Brasil na biodegradação de óleos lubrificantes automotivos residuais

Bruno Rodrigues Vieira[I], **Boutros Sarrouh**[I], **Renata Carolina Zanetti Lofrano**[I]

[I] Universidade Federal de São João del-Rei, MG, Brasil

ABSTRACT

About 2% of the oil consumed worldwide is related to the production of automotive and industrial lubricating oils. The pollution derived from these oils in aquatic and terrestrial environments is responsible for several ecological and social problems due to their toxic, mutagenic, and carcinogenic properties. Thus, studies related to bioremediation are of importance, and the use of biological treatments, such as biodegradation, is a viable and effective alternative for the treatment of these compounds. The present study aimed to evaluate the biodegradation performance of residual automotive lubricating oils using the lipases Pseudomonas fluorecens obtained in bioprospecting carried out in the Serra do Ouro Branco State Park, Minas Gerais. Through the colorimetric method using the redox indicator 2.6-dichlorophenol-indophenol (DCPIP), the biodegradability of residual oils was monitored. The results obtained demonstrated the potential of the selected bacteria, since they degraded approximately 61.74 to 83.8 % of the waste studied.

Keywords: Waste oils; Lipases; Indicator 2.6-dichlorophenol-indophenol (DCPIP)

RESUMO

Cerca de 2% do petróleo consumido mundialmente está relacionado à produção de óleos lubrificantes automotivos e industriais. A poluição derivada destes óleos, em ambientes aquático e terrestre, é responsável por diversos problemas ecológicos e sociais, em função de suas propriedades tóxicas, mutagênicas e carcinogênicas. Dessa forma, estudos relacionados à biorremediação são de sua importância e o emprego de tratamentos biológicos, como a biodegradação, é uma alternativa viável e

eficaz para o tratamento destes compostos. O presente estudo teve como objetivo avaliar o desempenho da biodegradação de óleos lubrificantes automotivos residuais, empregando-se lipases Pseudomonas fluorecens obtidas na bioprospecção realizada no Parque Estadual Serra do Ouro Branco, Minas Gerais. Através do método colorimétrico empregando-se o indicador redox 2.6-diclorofenol-indofenol (DCPIP) foi monitorada a biodegradabilidade dos óleos residuais. Os resultados obtidos demonstraram a potencialidade das bactérias selecionadas, uma vez que degradaram aproximadamente de 61,74 a 83,8 % do resíduo estudado.

Palavras-chave: Óleos residuais; Lipases; Indicador 2.6-diclorofenol-indofenol (DCPIP)

1 INTRODUCTION

According to CONAMA Resolutions no. 362 of 2005 and no. 450 of 2012, the mandatory targets set by the Ministries of the Environment and Mines and Energy for the collection of used or contaminated lubricating oil must be met by producers and importers of finished lubricating oil (Conama Resolution, 2005).

In 2019, Interministerial Ordinance MMA/MME no. 475 set targets in terms of volumes (m^3) of lubricating oils sold and collected in 2021. A total of 1.188.517.61 m^3 were sold and only 537.293.76 m^3 were recovered, corresponding to a recovery rate of only 45.21 % (Ministério de Minas e Energia, 2019).

These figures indicate that less than half of the total volume of lubricating oils are not collected or returned to their producers, in accordance with current legislation.

This data indicates the existence of significant accumulated liabilities in numerous generating establishments scattered throughout the country and/or inadequate disposal. In both cases, the toxic waste causes environmental damage and harm to human health.

The inadequate and indiscriminate handling and disposal of used automotive lubricating oils is a worrying source of pollution of both water bodies and soils, which poses a significant challenge in terms of monitoring, control and mitigation, since mechanic workshops are springing up indefinitely in various and different locations without any planning or compliance with current management policies aimed at protecting the environment (Lale et al. 2014).

Environmental contamination by this waste comes from hydrocarbon spills that occur for various reasons, including leaks from wells, tanks and improper disposal of this waste, causing irreversible damage to health and the environment.

The bioprospecting of microorganisms in certain areas, such as that carried out in the Serra do Ouro Branco State Park in the state of Minas Gerais/Brazil, is a valuable study for detecting new biological species that can be used in biotechnological processes, such as the biodegradation of pollutants.

Recognized as one of the regions with the greatest floristic diversity in South America, the Espinhaço Chain has more than 30% endemic species, most of which are associated with rocky outcrop environments. This, among other peculiarities, has granted it the status of Biosphere Reserve by the United Nations Educational, Scientific and Cultural Organization. The high diversity contrasts with the severe soil and climate conditions typical of outcrops in general, such as the high intensity of ultraviolet (UV) rays, daily thermal variations of the substrate that can reach 45ºC, accelerated water loss, poorly developed soil cover and soil with a high heavy metal content due to the presence of iron stones (Jacobi & Carmo, 2008).

In the bioprospecting of microorganisms carried out in the studies by Luz (2021), several microorganisms from different species were selected and the ones that showed the greatest lipolytic activities were the *Pseudomonas fluorecens* lipases, which are common Gram negative bacilli that have an extremely versatile metabolism and can be found in soil, water and different plant species such as bryophytes and bromeliads from which the *Pseudomonas fluorecens* studied were collected. They have optimum temperatures for growth of between 25 and 30^{0}C (Costa, 2012; Paulino Neto, 2016).

These characteristics make these bacteria promising for the biodegradation of residual automotive lubricant oils in both soil and water. And the fact that they show high growth at room temperature makes them viable for application in biodegradation studies under natural environmental conditions.

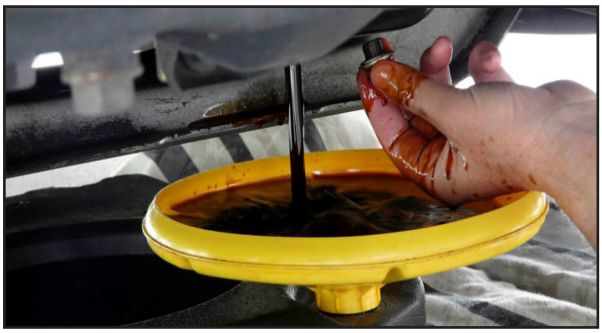
In this context, the aim of this study was to monitor and evaluate the biodegradation of waste automotive lubricant oils using *Pseudomonas fluorecens* microorganisms obtained from bioprospecting carried out in the Serra do Ouro Branco State Park, Minas Gerais/Brazil by Luz (2021) with a view to developing an unprecedented biotechnological process for the biodegradation of waste automotive lubricant oils.

2 MATERIALS AND METHODS

2.1 Collecting and pre-treating waste oil

Waste automotive lubricating oil is removed from engines as shown in Figure 1.

Figure 1 – Photo illustrating the collection of waste automotive lubricating oils



Source: Adapted from Resiclean Environmental (2023)

The waste automotive lubricating oil studied was obtained from a mechanic's workshop in the municipality of Belo Horizonte, Minas Gerais/Brazil. The oil drained directly from the vehicle was collected. It was then filtered through commercial filter paper to remove possible solid impurities and homogenize the material to be studied.

2.2 Selection and characterization of microorganisms

The bacteria lipase *Pseudomonas fluorecens* (1) and (2) were obtained in the State Park of Serra do Ouro Branco, in the state of Minas Gerais/Brazil, isolated

and morphologically characterized according to studies reported by Luz (2021). Phylogenetic identification, using 16S rDNA gene sequencing, of the microorganisms was held at the Laboratory of Biotechnology and Biodiversity Environment, Federal University of Viçosa. They were collected from georeferenced samples of bryophyte (figure 2) (20°28'49.4" S and 43°43'04.3" W) and bromeliad (figure 3) (520°28'52" S and 43°43'00.3" W) respectively.

Pseudomonas fluorecens (1) and (2) presented orange color colonies with a shiny appearance, a *gram*-negative cocci cell morphology and enzyme lipolytic activity value, tested in Tween®80 medium, of 1.59 ± 0.19 and 1.89 ± 0.49, respectively.

Figure 2 – Illustrative photo of bryophytes

Source: Adapted from Castilho (2023)

Figure 3 – Illustrative photo of a bromeliad

Source: Adapted from Dextro (2023)

2.3 Preparation of pre-inocula

The biodegradation tests were preceded by the preparation of pre-inocula.

Each pre-inoculum was prepared by transferring a colony of the bacteria being studied from the Petri dish to a 250 mL erlenmeyer flask containing 100 mL of liquid medium (500 mL of distilled water; 1.50 g of malt extract; 1.50 g of yeast extract; 2.50 g of peptone; 1.50 g of glucose). This mixture was kept at 30 ºC under agitation (130 rpm) for 24 h in an orbital shaker (TECNAL/Model 430).

2.4 Preparation of waste automotive lubricating oil samples

The samples for the biodegradation tests were prepared in a 250 mL erlenmeyer flask by adding 150 mL of Bushell-Hass (BH) culture medium (1L of distilled water; 0.20 g of $MgSO_4$, 0.02 g of $CaCl_2$; 1.00 g of KH_2PO_4; 1.00 g of K_2HPO_4; 1.00 g of NH_4NO_3; 0.05 g of $FeCl_3$), 1.50 mL of waste lubricating oil and 30 mL of an aqueous solution of 1.00 $g.L^{-1}$ of 2,6-dichlorophenol indophenol (DCPIP) (VETEC). These were dark blue in color.

The mixtures were then inoculated with 10% (v/v) of the pre-inoculum for Pseudomonas fluorecens lipases (1) and (2) and kept at 30 ºC, under agitation (130 rpm) for 24 h in an orbital shaker (MULTITEC/Model 430). This procedure was carried out in triplicate for both bacteria studied.

For comparative purposes regarding microbiological activity, a sample was prepared as detailed above, excluding the addition of the microorganism (Control sample).

2.5 Biodegradation tests

The biodegradation of the waste oil was monitored over the course of 72 h, with 25 mL aliquots being taken every 12 h, transferred to 50 mL Falcon tubes and subjected to centrifugation (HETTICH ZENTRIFUGEN / Routine 38R) for 10 min at 3000 rpm. Then 5.0 mL of the supernatant liquid was added to a glass cuvette. These were analyzed by molecular absorption spectroscopy in the UV/Vis region at 234 nm (BIOSPECTRO Spectrophotometer / Model SP 220).

The absorbance values obtained were compared with those presented by a calibration curve drawn up from the absorbance values obtained at 234 nm for the concentrations 0.001, 0.020, 0.040, 0.060, 0.080 and 0.1000 mol.L^{-1} obtained by diluting a 0.10 mol. L^{-1} aqueous solution of DCPIP.

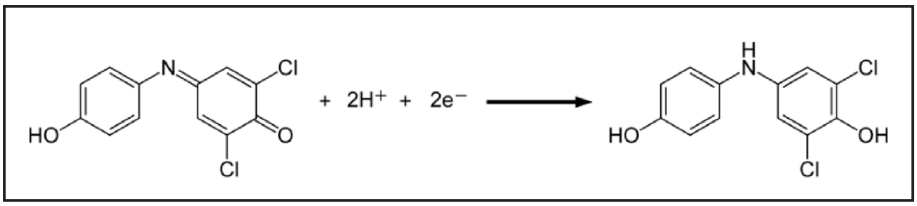
3 RESULTS AND DISCUSSION

Colorimetry is an analysis method based on comparing colors or, more specifically, the length range of one color based on another that is used as a standard. It consists of a simple, efficient and low-cost technique in which, through the variation in color, the occurrence of an oxidation-reduction reaction between the dye, which is an electron acceptor, and a compound that generates electrons can be gauged.

In practice, the process compares the color produced by a chemical reaction with a standard color. Colorimetry applied to biodegradation studies generally uses the redox indicator 2,6-dichlorophenol-indophenol (DCPIP) (COSTA, 2022).

The DCPIP molecule is an aromatic molecule and is therefore very chemically stable due to the resonance (electronic delocalization) involving its electron pairs (figure 4) (Bidoia et al., 2010).

Figure 4 – Oxidation-reduction reaction of the indicator 2,6-dichlorophenol indophenol (DCPIP) as an electron acceptor

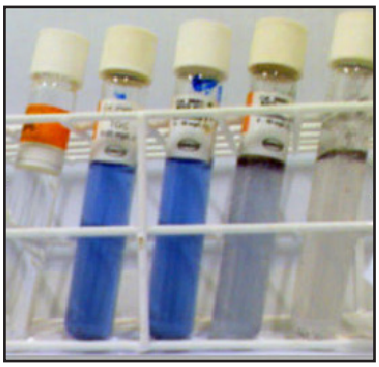


Source: Authors (2023)

Thus, when the DCPIP indicator is oxidized its color is blue, and when reduced it is colorless. The change in color is due to a change in the functional groups present in the molecule's structure with a consequent loss of the molecule's aromaticity (figure 5).

The nitrogen atom that connects the two aromatic rings via a single bond and a double bond is the electron acceptor responsible for breaking the double bond between nitrogen and carbon, resulting in only a single bond. (BRUICE, 2013).

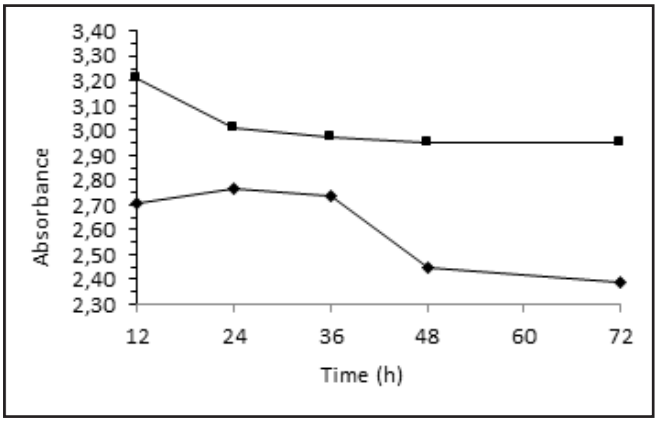
Figure 5 – DCPIP decolonization process through time as observed in lubricating oil biodegradation



Source: Adapted from Castilho (2023)

The breaking of the double bond between nitrogen and carbon alters the molecular structure by breaking the aromaticity and consequently the color. The change in color can be used in a series of reactions in which electron exchange occurs, such as the biodegradation of compounds (Bidoia et al, 2010).

Figure 6 – Behavior of the absorbance of samples with DCPIP indicator inoculated with *Pseudomonas fluorecens* lipases (1) (■) and (2) (♦) by incubation time



Source: Authors (2023)

Figure 6 shows the absorbance behavior curves resulting from the reduction in the concentration of 2,6-dichlorophenol-indophenol (DCPIP) over the course of 72 h, where the absorbance was monitored every 12 h.

The absorbance is directly proportional to the concentration of the sample solution (HARRIS, 1999). Therefore, by analyzing Figure 5, it can be seen that the concentration of DCPIP in the sample decreased, indicating the oil residual biodegradation.

The average decrease in absorbance in relation to the first 12 h measurement and after 72 h of monitoring was also determined. The results are listed in table 1.

Table 1 – Average values obtained for the absorbance decay in percentage obtained in the tests for the two microorganisms studied, after 72 h in relation to the first measurement of 12 h of monitoring

Microorganisms	Average decrease (%)
Pseudomonas fluorecens (1)	61.74
Pseudomonas fluorecens (2)	83.89

Source: Authors (2023)

It was observed that both lipase bacteria of the *Pseudomonas fluorecens* species were able to biodegrade the automotive lubricant automotive oil samples with significant results.

The Pseudomonas fluorecens lipase (2), which came from a black bryophyte sample and had gram-negative cell morphology, showed the greatest average decrease in absorbance of 83.89 % over the term interval evaluated (table 1). This was followed by *Pseudomonas fluorecens* (1) from bromeliads with an orange color, shiny appearance and gram-negative cocci cell morphology, which showed a percentage decrease in measured absorbance of 61.74 %.

The differences obtained in the average values of the absorbance decays and, consequently, in the effectiveness of the biodegradation of residual automotive lubricating oil were possibly due to the differences in the values of the enzymatic

indices characteristic of each of the lipases used. The *Pseudomonas fluorecens* lipase (2) has a higher value of 1.89 ± 0.49 than *Pseudomonas fluorecens* (1), which has a value of 1.59 ± 0.19. These values indicate the lipase's ability to biodegrade lipolytic compounds, i.e. oily ones, such as the waste studied. Thus, the higher percentage of biodegradation obtained in the tests using the *Pseudomonas fluorecens* lipase (2) can be explained by this greater lipolytic capacity.

4 CONCLUSIONS

It can therefore be concluded that the Serra do Ouro Branco State Park located in the city of Ouro Branco in the state of Minas Gerais/Brazil has as yet little explored sources of biodiversity, containing microorganisms capable of biodegrading compounds that are toxic pollutants to the environment and human health.

In this context, the biodegradation behavior of residual automotive lubricating oils was evaluated using the colorimetric method employing the redox indicator 2,6-dichlorophenol-indophenol (DCPIP) redox indicator against the lipases *Pseudomonas fluorecens* (1) and (2), which produced significant biodegradation percentages of 61.74 % and 83.89 %, respectively, demonstrating the feasibility of exploiting the biodiversity of the target region, the effectiveness of the chosen methodology and the biodegradation capacity of the contaminant studied. In this way, an efficient, inexpensive and alternative methodology for treating this toxic waste was developed.

ACKNOWLEDGEMENTS

The authors would like to thank the Municipality of Ouro Branco/Minas Gerais, Brazil for their support in obtaining samples of the microorganisms studied and the financial and infrastructure support of the Federal University of São João Del Rei/UFSJ, the National Council for Scientific and Technological Development (CNPq) and the Coordination for the Improvement of Higher Education Personnel (CAPES).

REFERENCES

Bidoia, E. D., Montagnolli, R. N., Lopes, & P. R. M. (2010). Microbial biodegradation potential of hydrocarbons evaluated by colorimetric technique: a case study. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology. 2010;1277-1288.

Bruice, P.Y. (2013). Fundamentos de Química Orgânica. Londres: Editora Pearson.

Castilho, R. (2023). Briófitas: O que são, características e exemplos. https://www.todamateria.com.br/briofitas/.

Costa, D. P., & Luizi-Ponzo, A. P.(2010). Introdução: As briófitas do Brasil. Catálogo de plantas e fungos do Brasil [online]. Rio de Janeiro: Editora SciELO Books, 1, 61-68.

Costa, E. R. C., De Souza, A. F., Rodrigues, D. M., Mendonça, R. De S., Da Silva, A. K. D., Takaki, G. M. De C., & Andrade, R. F. Da S. (2022). Biodegradation of diesel oil by Penicillium citrinum UCP 1183 isolated from mangrove sediments. Research, Society and Development. 11(10):e573111033071.

Dextro, R. B. Bromélias. https://www.infoescola.com/biologia/bromelias/ .

Harris, D.C. (2011). Análise Química Quantitativa. Rio de Janeiro: Editora LTC. http://dx.doi.org/10.33448/rsd-v11i10.33071.

Jacobi, C. M., & Carmo, F. F. (2008). Diversidade dos campos rupestres ferruginosos no Quadrilátero Ferrífero, MG. Megadiversidade. 4(1-2):43-46.

Lale, O. O., Ezekwe, L. C., & Lale, N. E. S. (2014). Effect of Spent Lubricating Oil Pollution on Some Chemical Parameters and the Growth of Cowpeas (Vigna unguiculata Walpers). Resources and Environment. 4(3):173-179.

Luz, B. D. Da S., Sarrouh, B., Bicas, J. L., & Lofrano, R. C. Z. (2021). Lipase production by microorganisms isolated from the Serra de Ouro Branco State Park. Engineering Sciences An. Acad. Bras. Ciênc.93(1):e20190672. https://doi.org/10.1590/0001-3765202120190672.

Ministério de Minas e energia. (2022) Portaria Interministerial nº 475, de 19 de dezembro de 2019. https://www.gov.br/mme/pt-br/acesso-a-informacao/legislacao/portarias-interministeriais/portaria-interministerial-mme-mma-n-475-2019.pdf.

Paulino-Neto, H. F., Nakano-Oliveira, E., De Assis Jardim, M. M., & Vasconcellos-Neto, J. (2016). Frugivory in Bromelia balansae (Bromeliaceae): The Effect of Seed Passage through the Digestive System of Potential Seed Dispersers on Germination in an Atlantic Rainforest, Brazil. J Ecosyst Ecography. 6(224):2-8.

Resiclean Ambiental. Descarte de óleo lubrificante deve ser tão cuidadoso quanto de outros resíduos.https://www.resiclean.com.br/cuidado-descarte-oleo-lubrificante/.

Resolução Conama nº 362 de 23 de junho de 2005. http://conama.mma.gov.br/?option=com_sisconama&task=arquivo.download&id=457.

Authorship contributions

1 - Bruno Rodrigues Vieira

Universidade Federal de São João del-Rei - Master of Education
https://orcid.org/0000-0001-7957-3247 - brunorgvieira@gmail.com
Contribution: Data curation, Formal Analysis, Investigation, Project administration, Supervision Writing- review & editing

2 - Boutros Sarrouh

Universidade Federal de São João del-Rei - Doctor of Science
https://orcid.org/0000-0003-4476-2309 - bsarrouh@ufsj.edu.br
Contribution: Data curation, Formal Analysis, Writing – original draft, Writing – review editing

3 - Renata Carolina Zanetti Lofrano

Universidade Federal de São João del-Rei - Doctor of Science
https://orcid.org/0000-0002-5688-3936 - renataczlofrano@ufsj.edu.br
Conceptualization, Data curation, Formal Analysis, Investigation, Writing – original draft, Writing – review editing

How to quote this article

Vieira, B. R., Sarrouh, B., & Lofrano, R. C. Z. (2024). Use of pseudomonas fluorecens isolated from the Serra de Ouro Branco State Park/Minas Gerais - Brazil in the biodegradation of residual automotive lubricating oils. *Ciência e Natura*, 46, e84868. https://doi.org/10.5902/2179460X84868.