Potential of *Aspergillus niger* Tiegh 8285 in the bioremediation of water contaminated with benzonitrile

Potencial de *Aspergillus niger* Tiegh 8285 na biorremediação de água contaminada com benzonitrila

Potencial de *Aspergillus niger* Tiegh 8285 en biorremediación de agua contaminada con benzonitrila

Abstract

Benzonitrile is a compound found in pesticides. The use of these pesticides can cause environmental contamination, and the search for non-aggressive methods to eliminate these residues is necessary. In this study, fungi *Aspergillus* isolated from cocoa were investigated for their benzonitrile bioremediation potential. The fungi were cultured in a solid medium supplemented with nitrile and glucose (a), nitrile (b), and glucose (c). Independent variables: time, inoculum, and nitrile were optimized using a central composite design to determine the best microbial growth and wet biomass (dependent variable) as a response in the bioremediation process. *A. niger* Tiegh 8285 showed good adaptation, especially in situation b in nitrile 5 days, 3 mycelial inoculums and 54 μL of benzonitrile for microbial growth, resulting in 1.83 ± 0.03 g of wet biomass, confirming the efficiency of the selected mathematical model. *A. niger* Tiegh 8285 proved to be a promising bioremediation agent for benzonitrile.

Keywords: Biocatalysis; Central composite design; Microorganism; Optimization.
Resumo
Benzonitrila é um composto encontrado em pesticidas. O uso desses agrotóxicos pode causar contaminação ambiental, exigindo a busca de métodos não agressivos para eliminação desses resíduos. Neste estudo, fungos Aspergillus isolados do cacau foram investigados quanto ao seu potencial de biorremediação de benzonitrila. Os fungos foram cultivados em meio sólido suplementado com nitrila e glicose (a), nitrila (b) e glicose (c). Variáveis independentes: tempo, inóculo e nitrila foram otimizados usando um planejamento Composto Central para determinar o melhor crescimento microbiano e biomassa úmida (variável dependente) como resposta no processo de biorremediação. A. niger Tiegh 8285 apresentou boa adaptação, principalmente na situação b, com 5 dias, 3 inóculo micelial e 54 μL de benzonitrila para crescimento microbiano, resultando em 1,83 ± 0,03 g de biomassa úmida, confirmando a eficiência do modelo matemático selecionado. A. niger Tiegh 8285 provou ser um promissor agente de biorremediação para benzonitrila.

Palavras-chave: Biocatalise; Microrganismos; Otimização; Planejamento composto central.

Resumen
El benzonitrilo es un compuesto que se encuentra en los pesticidas. El uso de estos pesticidas puede causar contaminación ambiental, siendo necesaria la búsqueda de métodos no agresivos para la eliminación de estos residuos. En este estudio, los hongos Aspergillus aislados del cacau fueron investigados por su potencial para la biorremediación del benzonitrilo. Los hongos se cultivaron en medio sólido suplementado con nitrilo y glucosa (a), nitrilo (b) y glucosa (c). Variables independientes: tiempo, inóculo y nitrilo fueron optimizados mediante un diseño de Central Composite para determinar el mejor crecimiento microbiano y biomasa húmeda (variable dependiente) como respuesta en el proceso de biorremediación. A. niger Tiegh 8285 mostró buena adaptación, principalmente en situación b, con 5 días, 3 inóculo micelial y 54 μL de benzonitrilo para crecimiento microbiano, resultando 1,83 ± 0,03 g de biomasa húmeda, confirmando la eficiencia del modelo matemático seleccionado. A. niger Tiegh 8285 demostró ser un agente de biorremediación prometedor para el benzonitrilo.

Palabras clave: Biocatálisis; Microorganismos; Mejoramiento; Planificación compuesta central.

1. Introduction
Nitriles are organic compounds with toxic, mutagenic, and carcinogenic properties (Graham et al., 2020). They also cause adverse effects on human health, such as respiratory system inactivation (Heidari & Asoodeh, 2019), loss of hair cells (responsible for balance), neurobehavioral abnormalities, decreased hearing, altered serotonin levels, and corneal opacity (Saldanha-Ruiz, et al., 2012). However, nitriles are widely used as intermediates in organic synthesis reactions (Chmura, et al., 2008; Santos, et al., 2021) such as hydrolysis (Mehtra, et al., 2017; Zhan, et al., 2018) and cycloaddition reactions (Umemoto, et al., 2020). They are also used as ingredients for obtaining plastics (An, et al., 2020), synthetic rubbers (Xiao, et al., 2019), and polymers (Lai, et al., 2020), in addition to herbicides and pesticides (An, et al., 2018).

Benzonitrile (C₆H₅CN) is an aromatic nitrile found in the composition of herbicides such as dichlobenil (2,6-dichlorobenzonitrile), bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) and ioxynil (3,5-diiodo-4-hydroxybenzonitrile) (Pei, et al., 2017). Dichlobenil is commonly used in plant and garden nurseries, while bromoxynil and ioxynil are generally used for weed pest control (Pei, et al., 2017). The metabolites from benzonitrile herbicides are hazardous to human health due to their toxicity and they are considered a risk for soil and groundwater contamination (Pei, et al., 2017).

Any chemical contamination in a natural environment, such as rivers and groundwater, is undesirable in any circumstance. Therefore, the environmental pollution problems associated with social and technological development have spurred the search for different methods of water treatment, the most promising of which is bioremediation (Fu, et al., 2020). Bioremediation is a branch of biotechnology in which microorganisms such as bacteria and fungi (Quintella, et al., 2019) are used to transform contaminating and toxic compounds, such as dioxins (Dao, et al., 2019), agricultural effluents (Neoh, et al., 2016), soil contaminated with oil (Chaudhary, et al., 2019; Li, et al., 2020) or heavy metals and pesticides (Zhang, et al., 2020), and wastewater from the pharmaceutical industry (Shah, et al., 2020) into compounds of low or zero toxicity, while obtaining water and carbon dioxide during the process (Liu, et al., 2017). Moreover, bioremediation has been extensively explored in wastewater treatment (Lu, et al., 2014; Catania, et al., 2020; Hubabillah, et al., 2020; Zhou, et al., 2020) and can be
characterized as a renewable process since it uses living organisms. Another advantage is that this process does not require the use of chemical catalysts and the reactions can be carried out at approximate room temperature (Zhang et al., 2013).

Studies have been carried out involving the bioremediation of several compounds such as Myceliophthora thermophila (Salami et al., 2018) and Coriolopsis gallica (Vidal-Limon et al., 2012) in the bioremediation of environmental contaminants, Sinanaonta woodiana, a freshwater mollusk, in the bioremediation of aquaculture effluents (reservoirs for breeding marine species, such as fish and shellfish) (Securo et al., 2020), and Bacillus velezensis in the bioremediation of textile dyeing residues (Gowri et al., 2020).

In bioremediation, each microorganism reacts differently depending on the conditions. Therefore, it is important to provide ideal conditions for the microorganism to increase its bioremediation potential. For this purpose, chemometric techniques are considered useful alternatives. Thus, the aim of this study was to investigate the bioremediation potential of fungi of the genus Aspergillus, isolated from the stem and leaves of cocoa in southern Bahia, in the presence of benzonitrile and to optimize the conditions of bioremediation using central composite design (CCD) using the variables time, inoculum, and volume of nitrile.

2. Materials and Methods

2.1 Microorganisms

Aspergillus niger Tiegh. 8068, A. parasiticus Speare 7967 and A. niger Tiegh. 8285 were isolated from the exterior part of conventional post-harvest and post-fermentation cocoa beans, respectively, in the city of Arataca (Bahia, Brazil), and the endophytic fungi, Aspergillus Tiegh. 8066 and A. niger Tiegh. 8067 were isolated from stems and leaves at the Matinha Municipal Park (Itapetinga, Bahia, Brazil). The fungal strains are stored in mineral oil, PDA, except A. parasiticus Speare 7967, which was stored in mineral oil, Malt, in the URM library of the Mycology Center, Biological Sciences Department at the Federal University of Pernambuco (UFPE, Brazil).

2.2 Screening in a minimal solid mineral medium in the presence of benzonitrile

Screening was carried out in a minimal solid mineral medium [Na2HPO4 (1 g/L); MgCl2.7H2O (0.5 g/L); KCl (0.5 g/L); FeSO4.7H2O (0.01 g/L); CoCl2.6H2O (0.001 g/L); ZnSO4.7H2O (0.0067 g/L); agar (15 g/L) supplemented with glucose (15 g/L) and nitrile (200 µL/L) (De Oliveira et al., 2014) in Petri dishes (10x1 cm) and pH 7 in three different situations: glucose and nitrile (a), nitrile without glucose (b), and glucose without nitrile (c). After microbial growth in a BOD incubator (TE-371, Tecnal, Piracicaba, Brazil) at 30°C for 192 hours, the number of spores was counted using a Neubauer chamber and binocular microscope (BIOVAL L1000).

2.3 Central composite design for optimization of bioremediation of water contaminated with benzonitrile

Aspergillus niger Tiegh 8285, previously grown in a minimum solid mineral medium a, was inoculated in a minimum liquid mineral medium (De Oliveira et al., 2013), situation a. An Erlenmeyer flask (250 mL) containing 100 mL of minimal liquid mineral medium supplemented with glucose (1.5 g) was autoclaved (CS Prismatec, Itu, Brazil) at 121°C for 15 min. When it reached room temperature, benzonitrile was added. In parallel, small slices of the minimum solid medium containing the fungus mycelia were cut from the stock culture and inoculated. Then, the experiment was incubated in an orbital shaker (Tecnal, Piracicaba, São Paulo, Brazil) at 30°C at 120 rpm. Finally, the reaction was filtered using a vacuum pump (Prismatec, Itu, Brazil), and the wet biomass was weighed.

The procedure was optimized using a central composite design (CCD) (Dos Santos et al., 2016; Marques et al., 2018) with the independent variables reaction time (t), quantity of inoculum (In), and volume of nitrile (Nit) (Table 1) and the dependent variable wet biomass.
Table 1. Experimental factors and variable levels used in the central composite design (CCD) for optimizing bioremediation of benzonitrile by *Aspergillus niger* Tiegh 8285.

| Variables       | Unity | Codification | Variable level |
|-----------------|-------|--------------|----------------|
| Time            | day   | t            | -α -1 0 +1 +α  |
| Inoculum        | mm    | In           | 1.3 2.0 3.0 4.0 4.7 |
| Nitrile         | μL/100 mL | Nit | 16.5 30.0 50.0 70.0 83.5 |

Source: Authors.

The experimental project was modeled and analyzed using Statistica v.12.0 software (Statsoft, USA). The CCD obtained for the maximum biomass production was quadratic, as suggested by the software, and presented in equation 1. In the experimental matrix, the coded values of all parameters vary in five levels (-α, -1, 0, +1, +α) (Table 1), totaling 17 experiments (Table 2). At the beginning and end of each reaction, pH was measured only as a reaction indicator.

\[
Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3
\]  

(1)

Where Y is the predicted value, \(\beta_0\) is constant, \(\beta_1, \beta_2, \beta_3\) are linear coefficients, \(\beta_{12}, \beta_{13}, \beta_{23}\) are coefficients between products, and \(\beta_{11}, \beta_{22}, \beta_{33}\) are quadratic coefficients. All experiments were performed in triplicate and expressed as average values.

Table 2. Results obtained in the central composite design (CCD), pH measured in the experiments and experimental and predicted results for optimizing bioremediation of benzonitrile by *Aspergillus niger* Tiegh 8285.

| Essay | t   | In | Nit | pH  | Experimental values | Damp mass (g) | Predicted values |
|-------|-----|----|-----|-----|---------------------|--------------|-----------------|
| 1     | 4.0 | 2.0| 30.0| 6.84| 1.75                | 1.70         |
| 2     | 4.0 | 4.0| 70.0| 6.91| 1.87                | 1.87         |
| 3     | 8.0 | 2.0| 70.0| 6.83| 1.73                | 1.71         |
| 4     | 8.0 | 4.0| 30.0| 6.98| 1.64                | 1.66         |
| 5     | 6.0 | 3.0| 50.0| 6.52| 1.80                | 1.81         |
| 6     | 4.0 | 2.0| 70.0| 6.91| 1.82                | 1.86         |
| 7     | 4.0 | 4.0| 30.0| 6.80| 1.71                | 1.78         |
| 8     | 8.0 | 2.0| 30.0| 6.88| 1.47                | 1.53         |
| 9     | 8.0 | 4.0| 70.0| 7.12| 1.65                | 1.76         |
| 10    | 6.0 | 3.0| 50.0| 6.90| 1.83                | 1.81         |
| 11    | 2.6 | 3.0| 50.0| 6.83| 1.52                | 1.52         |
| 12    | 9.3 | 3.0| 50.0| 7.82| 1.37                | 1.29         |
| 13    | 6.0 | 1.3| 50.0| 6.52| 1.56                | 1.57         |
| 14    | 6.0 | 4.7| 50.0| 6.53| 1.78                | 1.68         |
| 15    | 6.0 | 3.0| 16.5| 6.50| 2.11                | 2.08         |
| 16    | 6.0 | 3.0| 83.5| 6.50| 2.35                | 2.30         |
| 17    | 6.0 | 3.0| 50.0| 6.52| 1.79                | 1.81         |

Source: Authors.
2.4 Model adequacy and validation

The adjustment quality of the statistical model was verified by the coefficient of determination ($R^2$), and the significance of the model was tested by Fisher's test (F-value) through analysis of variance (ANOVA), according to Ferreira et al 2007. Model adequacy was analyzed using the observed vs predicted graph. The interactions between the variables and their influence on the response obtained were analyzed using a Pareto chart, and the optimal point of maximum bioremediation was obtained using equation two and the response surface graph. The selected statistical model was validated by running the experiment under optimized conditions and comparing it with the expected response (Ferreira, et al., 2007; Bezerra, et al., 2020).

3. Results and Discussion

3.1 Screening in a minimal solid medium in the presence of benzonitrile

Fungi of the genus Aspergillus from different biomes in the state of Bahia with biocatalytic potential in the presence of aromatic nitriles were selected through screening in a minimal solid mineral medium supplemented with glucose and benzonitrile, according to situations a, b, and c, using quantitative evaluation through the number of spores (Table 3). As observed in Table 3, the fungi showed microbial growth in situations a, b, and c and a better adaptation in media supplemented with benzonitrile (a and b), indicating the inducing role of nitrile since it is the only source of nitrogen, as well as the possible biocatalytic potential of the respective fungi. These results corroborate data from the literature that stress the importance of nitriles as an inducer in microbial growth (Coady, et al., 2013; De Oliveira, et al., 2013; De Oliveira, et al., 2014), in particular, benzonitrile (Agarwal, et al., 2017; Serra, et al., 2019).

| Table 3. Results obtained from screening in minimal solid mineral medium for benzonitrile by counting spores using a Neubauer chamber and binocular microscope. |
|---|---|---|
| Fungi | Glucose and nitrile (spores x mL$^{-1}$) | Nitrile without glucose (spores x mL$^{-1}$) | Glucose without nitrile (spores x mL$^{-1}$) |
| A. niger Tiegh. 8068 | 14.2 x 10$^4$ | 19.1 x 10$^4$ | 14.3 x 10$^4$ |
| A. niger Tiegh. 8285 | 19.4 x 10$^4$ | 63.2 x 10$^4$ | 1.8 x 10$^4$ |
| A. parasiticus Speare 7967 | 12.6 x 10$^4$ | 7.73 x 10$^4$ | 5.8 x 10$^4$ |
| A. niger Tiegh. 8066 | 27.7 x 10$^4$ | 21.5 x 10$^4$ | 13.0 x 10$^4$ |
| A. niger Tiegh. 8067 | 23.6 x 10$^4$ | 13.8 x 10$^4$ | 7.3 x 10$^4$ |

Source: Authors.

According to Table 3, A. niger Tiegh 8285 showed a significant increase in spores in the situation that only contained benzonitrile b (63.2 x 10$^4$ spores x mL$^{-1}$), which represents an increase of approximately 35 times compared to situation c. The good adaptation of A. niger Tiegh 8285 revealed that the addition of benzonitrile alone in a medium with minimal amounts of nutrients was sufficient to supply the needs of the microorganism.

3.2 Optimization of bioremediation

From the results obtained from screening in minimal solid mineral medium (Table 3), A. niger Tiegh 8285 was selected to evaluate its potential in the benzonitrile bioremediation process through the central compound design with 17 experiments (Table 2).
The analysis of variance (ANOVA) calculated model efficiency and adequacy for the experimental design used, as shown in Table 4. The computed F value (22.80) for the model was considerably higher than in the Table 4 (3.67), showing that the model was significant.

Table 4. Analysis of variance to adjust the quadratic model with a 95% confidence level.

| Factor       | SS    | df | MS     | F calc. | F tab. |
|--------------|-------|----|--------|---------|--------|
| Model        | 0.82  | 9  | 0.091  | 22.80   | 3.67   |
| Residue      | 0.028 | 7  | 0.003985 |         |        |
| Lack of fit  | 0.027 | 5  | 0.005405 |         |        |
| Pure error   | 0.000867 | 2 | 0.000433 |         |        |
| Total        | 0.845612 | 16 |         |         |        |
| R²           | 0.97  |    |         |         |        |
| R²-adj       | 0.92  |    |         |         |        |

SS - Sum of squares; DF= Degree of freedom; MS = Medium square

Source: Authors.

The model’s capacity was assessed using the R² determination coefficient, which was calculated to be 1.0. The value found for R² (0.97) indicates that the model reported 97% of the experimental data, and there were only 3% of errors, to which noise can be attributed. The observed vs. predicted graph (Figure 1) corroborates the model's adjustability, with experimental values close to the predicted values, indicating the authenticity of the polynomial model.

Figure 1. Observed vs. predicted graph showing the approximation between the results obtained experimentally from wet biomass in each of the experiments of the central composite design (CCD) and the theoretically indicated values (red line).

According to the analysis of the Pareto chart (Figure 2), the variables that most influence the response (P <0.05) were quadratic effect of time and nitrile. The quadratic variable time was the most significant variable, with a negative correlation,
indicating that an increase in time reacts negatively to the response and results in a decrease in wet biomass, revealing that all the nutrient is bioconsumed in a short time; therefore, A. niger Thieg 8285, in addition to being resistant, needs little time to bioremediate nitrile. The quadratic variable had a positive correlation, indicating that, as the volume of nitrile increased, the expected response (biomass) also increased since the culture medium is deficient in nutrients and the only source of nitrogen is the benzonitrile itself. This result shows that A. niger Thieg 8285 was induced to consume nitrile to survive, thus proving it adapted to the established conditions.

Figure 2. Pareto chart for optimization of bioremediation of water contaminated with nitrile according to independent variables time, inoculum, and volume of nitrile.

The analysis of the Pareto chart allowed selection of statistically significant terms at 95% significance and removed the non-significant terms ($P > 0.50$) from the mathematical model. After being the adequacy of the model approved using ANOVA, response surfaces (Figure 3a, b and c) were obtained, making it possible to observe the influence of combinations between the independent variables.

Polynomial Eq. 2 was used to express the relationship between the coded independent variables and to predict a response:

$$Y = 1.81 - 0.067X_1 + 0.034X_2 + 0.066X_3 - 0.146X_1^2 - 0.066X_2^2 + 0.134X_3^2 + 0.01X_1X_2 + 0.005X_1X_3 - 0.02X_2X_3$$  

(2)

Based on equation 2 and the response surface, the maximum biomass production point of reaction was 5 days and 12 hours, inoculum 3.38 mm and 54.31 μL of benzonitrile with theoretical production of 1.82 g of wet biomass. The reaction time obtained (5 days and 12 hours) is within the average time interval for several species of A. niger, which is between 5 (Sattar, et al., 2019; Papadaki, et al., 2020; Putri, et al., 2020) and 7 days (Khan, et al., 2019; Aboyeji, et al., 2020). The volume of nitrile obtained from the statistical design used (CCD) revealed that the microorganism studied has resistance to nitrile since microorganisms generally have limited tolerance to high concentrations of nitriles due to their toxicity (Sattar, et al., 2019).
3.3 Statistical validation of bioremediation

For model validation, experiments at the optimum point were performed in triplicate using the ideal conditions provided by equation 2 and the response surface (Figure 3). The wet biomass value predicted by the model was 1.82 g. By performing the optimal point experiment in triplicate, it was possible to obtain an average of 1.83 ± 0.03 g and a recovery of approximately 100% between the theoretical and the experimental value. These results corroborate those obtained in the central composite planning and validate the use of the proposed method for the bioremediation of water contaminated with benzonitrile by A. niger Tiegh 8285.

4. Conclusion

The role of benzonitrile as an inducer was confirmed with fungi of the genus Aspergillus of the state of Bahia, particularly Aspergillus niger Tiegh 8285, isolated from cocoa beans. This study has a high scientific value since a chemometric tool (central compound design) was used for the statistical optimization of bioremediation of water contaminated with benzonitrile by A. niger Thiegh 8285. Moreover, this study is relevant in terms of environmental preservation concerns by presenting a promising method for the treatment of water contaminated with residual nitriles. The result is encouraging for future studies on the bioremediation of aromatic nitriles.

Acknowledgments

We thank the Coordination for the Improvement of Higher Education Personnel (CAPES) and the National Council for Scientific and Technological Development (CNPq) for funding this research and the State University of Santa Cruz (UESC) for providing administrative and technical support.
References

Aboyeji, O. O., Oloke, J. K., Arinkoola, A. O., Oke, M. A., & Ishola, M. M. (2020). Optimization of media components and fermentation conditions for citric acid production from sweet potato peel starch hydrolysate by Aspergillus niger. *Scientific African*, 10, e00554. https://doi.org/10.1016/j.sciaf.2020.e00554

Agarwal, A., & Nirgam, V. K. (2017). Enhanced Production of Nitrilase from *Streptomyces* sp. MTCC 7546 by Response Surface Method. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 87, 603-609. https://doi.org/10.1007/s40011-015-0638-2

An, X., Cheng, Y., Huang, M., Sun, Y., Wang, H., Chen, X., Wang, J., Li, D., & Li, C. (2018) Treating organic cyanide-containing groundwater by immobilization of a nitrile-degrading bacterium with a biofilm-forming bacterium utilizing fluidized bed reactors. *Environmental Pollution*, 237, 908-916. https://doi.org/10.1016/j.envpol.2018.01.087

An, X., Cheng, Y., Miao, L., Chen, X., Zang, H., & Li, C. (2020). Characterization and genome functional analysis of an efficient nitrile degrading bacterium, *Rhodococcus rhodochrous* BX2, to lay the foundation for potential bioaugmentation for remediation of nitrile-contaminated environments. *Journal of Hazardous Materials*, 389, 121906. https://doi.org/10.1016/j.jhazmat.2019.121906

Bezerra, M. A., Lemos, V. A., Novaes, C. G., de Jesus, R. M., Souza Filho, H. R., Araújo, S. A., & Alves, J. P. S. (2020). Application of mixture design in analytical chemistry. *Microchemical Journal*, 152, 104334. https://doi.org/10.1016/j.microc.2019.104336

Catania, V., Lopresti, F., Cappello, S., Scaffaro, R., & Quatrini, P. (2020). Innovative, ecofriendly biosorbent-biodegrading biofilms for bioremediation of oil-contaminated water. *New Biotechnology*, 58, 25-31. https://doi.org/10.1016/j.nbt.2020.04.001

Chaudhary, D. K., & Kim, J. (2019). New insights into bioremediation strategies for oil-contaminated soil in cold environments. *International Biodeterioration & Biodegradation*, 142, 58-72. https://doi.org/10.1016/j.ibiod.2019.05.001

Chmura, A., Shapovalova, A. A., van Pelt, S., van Rantwijk, F., Tourova, T. P., Muyzer, G., & Sorokin, D. Y. (2008). Utilization of arylicaliphatic nitriles by halotolerant *Halomonas nitrilicus* sp. nov. isolated from soda soils. *Applied Microbiology and Biotechnology*, 81, 371–378. https://doi.org/10.1007/s00253-007-0815-5

Coady, T. M., Coffey, L. V., O’Reilly, C., Owens, E. B., & Lennon, C. M. (2013). A high throughput screening strategy for the assessment of nitrile-hydrolyzing activity towards the production of enantiotropic β-hydroxy acids. *Journal of Molecular Catalysis B: Enzymatic*, 97, 150-155. https://doi.org/10.1016/j.molcatb.2013.08.001

Diao, A. T. N., Voanck, J., Janssens, T. K. S., Dang, H. T. C., Brouwer, A., & de Boer, T. E. (2019). Screening white-rot fungi for bioremediation potential of 2.3,7,8-tetrachlorodibenzop-dioxin. *Industrial Crops and Products*, 128, 153-161. https://doi.org/10.1016/j.indcrop.2018.10.059

De Oliveira, J. R., Mizuno, C. M., Seleghim, M. H. R., Javaroti, D. C. D., Rezende, M. O. O., Landgraf, M. D., Sette, L. D., & Porto, A. L. M. (2013). Biotransformation of Phenylacetoniitrile to 2-Hydroxyphenylactic Acid by Marine Fungi. *Marine Biotechnology*, 15, 97-103. https://doi.org/10.1007/s10126-012-9464-1

De Oliveira, J. R., Seleghim, M. H. R., & Porto, A. L. M. (2014). Biotransformation of Methylphenylacetonitriles by Brazilian Marine Fungal Strain *Aspergillus sydowii* CBMAI 934: Eco-friendly Reactions. *Marine Biotechnology*, 16, 156–160. https://doi.org/10.1007/s10126-013-9534-z

Dos Santos, T. C., Reis, N. S., Silva, T. P., Machado, F. P. P., Bonomo, R. C. F., & Franco, M. (2016). Prickly Palm Cactus Husk as a Raw Material for Production of Ligninolytic Enzymes by *Aspergillus niger*. *Food Science and Biotechnology*, 25, 205-211. https://doi.org/10.1007/s10068-016-0031-9

Ferreira, S. L. C., Bruns, R. E., da Silva, E. G. P., dos Santos, W. N. L., Quintella, C. M., David, J. M., de Andrade, J. B., Breitschutz, M. C., Jardim, I. C. S. F., & Barros Neto, B. (2020). Statistical designs and response surface techniques for the optimization of chromatographic systems. *Journal of Chromatography A*, 1158, 2-14. https://doi.org/10.1016/j.chroma.2007.03.051

Fu, D., Yan, Y., Yang, X., Rene, E. R., & Singh, R. P. (2020). Bioremediation of contaminated river sediment and overlying water using biologically activated beads: A case study from Shedu river, China. *Biocatalysis and Agricultural Biotechnology*, 23, 101492. https://doi.org/10.1016/j.bcab.2019.101492

Gowri, A. K., Karunakaran, M. J., Muthunarayanan, V., Ravindran, B., Nguyen-Tri, P., Ngo, H. H., Bui, X. T., Nguyen, X. H., Nguyen, D. D., Chang, S. W., & Chandran, T. (2020). Evaluation of bioremediation competence of indigenous bacterial strains isolated from fabric dyeing effluent. *Bioresource Technology Reports*, 11, 100536. https://doi.org/10.1016/j.btrep.2020.100536

Graham, D., Pereira, R., Barfield, D., & Cowan, D. (2020). Nitrile biotransformations using free and immobilized cells of a thermophilic *Bacillus* spp. *Enzyme and Microbial Technology*, 106, 368-373. https://doi.org/10.1016/j.enmic.2019.06.009

Heidari, A., & Asoodeh, A. (2019). A novel nitrile-degrading enzyme (nitrile hydratase) from *Ralstonia* sp. ZA96 isolated from oil contaminated soils. *Biocatalysis and Agricultural Biotechnology*, 21, 101285. https://doi.org/10.1016/j.bcab.2019.101285

Hubadilah, S., Othman, M. H. D., Gani, P., Sunar, N. M., Tai, Z. S., Koo, K. N., Pauzan, M. A. B., Ismail, N. J., & Zahari, S. M. S. N. S. (2020). Integrated green membrane distillation-microalgae bioremediation for arsenic removal from Pengkor River Kuantan, Malaysia. *Chemical Engineering Process*, 153, 107996. https://doi.org/10.1016/j.cep.2020.107996

Khan, Y. M., Munir, H., & Anwar, Z. (2019). Optimization of process variables for enhanced production of urease by indigenous *Aspergillus niger* strains through response surface methodology. *Biocatalysis and Agricultural Biotechnology*, 20, 101202. https://doi.org/10.1016/j.bcab.2019.101202

Lai, A. N., Wang, Z., Yin, Q., Zhu, R. Y., Hu, P. C., Zheng, J. W., & Zhou, S. F. (2020). Comb-shaped fluorene-based poly (arylene ether sulfone nitrile) as anion exchange membrane. *International Journal of Hydrogen Energy*, 45, 11148-11157. https://doi.org/10.1016/j.ijhydene.2020.02.057

Li, Q., Li, J., Jiang, L., Sun, Y., Luo, C., & Zhang, G. (2020). Diversity and structure of phenanthrene degrading bacterial communities associated with fungal bioremediation in petroleum contaminated soil. *Journal of Hazardous Materials*, 403, 123895. https://doi.org/10.1016/j.jhazmat.2020.123895
Nitriles with a Salt Hydratase in Xylanase and Endoglucanases Produced by Penicillium roqueforti ATCC 10110 Through the Solid-State Fermentation of Rice Husk Residue. Waste and Biomass Valorization, 9, 2061-2069. https://doi.org/10.1007/s12649-017-9994-x

Mehta, A., & Basu, S. (2017). Controlled photocatalytic hydrolysis of nitriles to amides by mesoporous MnO2 nanoparticles fabricated by mixed surfactant mediated approach. Journal of Photochemistry and Photobiology A: Chemistry, 343, 1-6. https://doi.org/10.1016/j.jphotochem.2017.04.013

Neoh, C. H., Lam, C. Y., Ghanii, S. M., Ware, I., Sarip, S. H. M., & Ibrahim, Z. (2016). Bioremediation of high-strength agricultural wastewater using Ochroochromat sp. strain SZ1. Biotech, 6, 1-9. https://doi.org/10.3091/s13205-016-0455-1

Papadaki, E., Kontogiannopoulos, K. N., Assimopoulou, A. N., & Mantzouridou, F. T. (2020). Feasibility of multi-hydrolytic enzymes production from optimized grape pomace residues and wheat bran mixture using Aspergillus niger in an integrated civic acid enzymes production process. Bioresource Technology, 309, 123317. https://doi.org/10.1016/j.biortech.2020.123317

Pei, X., Wang, J., Guo, W., Miao, J., & Wang, A. (2017). Efficient biodegradation of dihalogenated benzonitrile herbicides recombiant Escherichia coli harboring nitrile hydrotase-amidase pathway. Biochemical Engineering journal, 125, 88-96. https://doi.org/10.1016/j.bej.2017.05.021

Putri, D. N., Khoottama, A., Perdani, M. S., Utami, T. S., & Hermansyah, H. (2020). Optimization of Aspergillus niger lipase production by solid state fermentation of agro-industrial waste. Energy Reports, 6, 331-335. https://doi.org/10.1016/j.enerp.2019.08.064

Quintella, C. M., Mata, A. M. T., & Lima, L. C. P. (2019). Overview of bioremediation with technology assessment and emphasis on fungal bioremediation of oil contaminated soils. Journal of Environmental Management, 241, 156-166. https://doi.org/10.1016/j.jenvman.2019.04.019

Salami, F., Habibi, Z., Yousefi, M., & Mohammadi, M. (2018). Covalent immobilization of laccase by one pot three component reaction and its application in the decolorization of textile dyes. International Journal of Biological Macromolecules, 120, 144-151. https://doi.org/10.1016/j.ijbiomac.2018.08.077

Saldanha-Ruiz, S., Soler-Martina, C., & Lhorens, J. (2012). Role of CYP2E1-mediated metabolism in the acute and vestibular toxicities of nineteen nitriles in the mouse. Toxicology Letters, 208, 125-132. https://doi.org/10.1016/j.toxlet.2011.10.016

Santos, E. C., de Menezes, L. H. S., Santos, C. L., Santana, P. V. B., Soares, G. A., Tavares, I. M. C., Freitas, J. S., Mota, C. M. S., Bezerra, J. L., da Costa, A. M., Ustanka, A. P. T., Porto, A. L. M., Franco, M., & de Oliveira, J. R. (2021). High-throughput screening for distinguishing nitritases from nitrile hydratases in Aspergillus and application of a Box-Behnken design for the optimization of nitritase. Biotechnology and Applied Biochemistry, 1-10. https://doi.org/10.1002/bab.2269

Sattar, H., Bibi, Z., Kamran, A., Aman, A., & Qader, S. A. A. (2019). Degradation of complex casein polymer: Production and optimization of a novel serine metalloprotease from Aspergillus niger KIBGE-IB36. Biocatalysis and Agricultural Biotechnology, 21, 101256. https://doi.org/10.1016/j.bcab.2019.101256

Serra, I., Capusoni, C., Molinari, F., Musso, L., Pellegrino, L., & Compagno, C. (2019). Marine Microorganisms for Biocatalysis: Selective Hydrolysis of Nitriles with a Salt-Resistant Strain of Myeorezyma guilliermondii. Marine Biotechnology, 21, 229-239. https://doi.org/10.1007/s10021-019-09875-0

Shah, A., & Shah, M. (2020). Characterisation and bioremediation of wastewater: A review exploring bioremediation as a sustainable technique for pharmaceutical wastewater. Groundwater for Sustainable Development, 11, 100383. https://doi.org/10.1016/j.gsd.2020.100383

Sicuro, B., Castelar, B., Mugetti, D., Pastorino, P., Chiarandon, A., Menconi, V., Galloni, M., & Prearo, M. (2020). Bioremediation with freshwater bivalves: A sustainable approach to reducing the environmental impact of inland trout farms. Journal of Environmental Management, 276, 111327. https://doi.org/10.1016/j.jenvman.2020.111327

Umemoto, N., Imayoshi, A., & Tsukabuki, K. (2020). Nitrile oxide cycloaddition reactions of alkenes or alkynes and nitroalkanes substituted with O-alkylxime groups convertible to various functional groups. Tetrahedron Letters, 61, 152213. https://doi.org/10.1016/j.tetlet.2020.152213

Vidal-Limon, A., Suárez, P. C. G., Arrillano-García, E., Contreras, O. E., & Aguila, S. A. (2018). Enhanced Degradation of Pesticide Dichlorophen by Laccase Immobilized on Nanoporous Materials: A Cytotoxic and Molecular Simulation Investigation. Bioconjugate Chemistry, 29, 1073-1080. https://doi.org/10.1021/acs.bioconjchem.7b00739

Xiao, X., Shiqiao, G., Dongmei, Z., Shaohua, N., Lei, J., & Zhaoceng, O. (2019). Mechanical behavior of liquid nitrile rubber-modified epoxy resin: Experiments, constitutive model and application. International Journal of Mechanical Sciences, 151, 46-60. https://doi.org/10.1016/j.ijmecsci.2018.11.003

Zhan, W., Ji, L., Ge, Z., Wang, X., & Li, R. (2018). A continuous-flow synthesis of primary amides from hydrolysis of nitriles using hydrogen peroxide as oxidant. Tetrahedron, 74, 1527-1532. https://doi.org/10.1016/j.tet.2018.02.017

Zhang, H., Yuan, X., Xiong, T., Wang, H., & Jiang, L. (2020). Bioremediation of co-contaminated soil with heavy metals and pesticides: Influence factors, mechanisms and evaluation methods. Chemical Engineering Journal, 398, 125657. https://doi.org/10.1016/j.cej.2020.125657

Zhang, J., Liu, Z. Z., & Zheng, L. (2013). Improvement of nitritase production from a newly isolated Alcaligenes faecalis mutant for biotransformation of iminodiacetonitrile to iminodiacetic acid. Journal of the Taiwan Institute of Chemical Engineers, 44, 169-176. https://doi.org/10.1016/j.jtice.2012.11.010

Zhou, H., Huang, X., Liang, Y., Li, Y., Xie, Q., Zhang, C., & You, S. (2020). Enhanced bioremediation of hydraulic fracturing flowback and produced water using an indigenous biosurfactant-producing bacteria Acinetobacter sp. Y2. Chemical Engineering Journal, 397, 125348. https://doi.org/10.1016/j.cej.2020.125348