Cometabolic removal of ibuprofen, diclofenac and ciprofloxacin driven by glycerol fermentation in an acidogenic biofilm reactor

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Abstract. The presence of pharmaceuticals in wastewater has increasingly become a public health issue globally due to the potential impacts it can cause on the environment and public health. In this study, we sought to analyze the cometabolic effect of glycerol fermentation on the removal of ibuprofen (IBU), diclofenac (DCF), and ciprofloxacin (CIP). The results indicated a positive effect of the glycerol addition on the removal of these compounds: without GOH – 65%, 35%, and 25%; with GOH – 91%, 48%, and 45%, respectively for IBU, DCF and CIP. A significant increase in the production of volatile fatty acids, especially propionate and valerate, was observed during the glycerol fermentation, which may have favored the prevalence of fermentative bacteria, as well as the production of specific microbial enzymes active in the biotransformation of the microcontaminants. Glycerol proved to be a good alternative supplementation in wastewater treatment systems to optimize the pharmaceutical compounds removal, in addition to the fact that it is a by-product of the biodiesel production with high availability on the market in many parts of the world.

Keywords: Antibiotic, anti-inflammatory drugs, emerging pollutants, pharmaceutical compounds, volatile fatty acids.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) and antibiotics are among the drugs most administered by the population, representing a potential source of release of these compounds into the environment through wastewater. Ibuprofen and diclofenac are two NSAIDs frequently found in sewage, as is ciprofloxacin, a fluoroquinolone antibiotic (Carvalho & Santos, 2016; Rastogi et al., 2021). In many cases, these compounds are not fully removed in the usual biological treatment systems, making it increasingly necessary to search for alternative solutions to optimize their elimination in an economically sustainable way.

Understanding the mechanisms involved in the elimination of the emerging pollutants from waters has become increasingly necessary in view of the multitude of compounds present in the various environmental matrices, and that can cause negative impacts on the environment and human health. One of the mechanisms reported in the literature for the removal of these compounds in biological treatment systems concerns heterotrophic cometabolism, in which their biodegradation is facilitated in the presence of easily biodegradable organic compounds (Kennes-Veiga et al., 2021).

Glycerol is a carbon source easily assimilated by fermentative microorganisms, being able to produce a series of metabolites in acidogenic condition (Dias et al., 2021), which can potentially be recovered or assimilated by methanogenic microorganisms to produce biomethane. It is noteworthy that glycerol is generated in abundance as a by-product of biodiesel production, and its availability in the market is much greater than the market demand to consume it. In addition, the organic load contained in the glycerin of biodiesel is quite high, being able to present considerable yields in the production of soluble products of its fermentation, such as organic acids and solvents (Clomburg & Gonzalez, 2013).

Given the context presented, this study sought to analyze the influence of glycerol fermentation on the biodegradation of the pharmaceutical compounds ibuprofen, diclofenac and ciprofloxacin through heterotrophic cometabolism, applied in an acidogenic biofilm reactor.

2. Material and methods

To assess the effect of glycerol fermentation on the removal of the target compounds, an experimental apparatus consisting of a bench-scale acidogenic biofilm reactor was set – Figure 1. The biofilm was developed on polyurethane strips to facilitate its adherence and growth. The acidogenic inoculum was produced by an acidic pretreatment (HCl 5 mol L^{-1}, pH 3) of a methanogenic sludge from a UASB bioreactor to inhibit methanogenic archaea. The start-up organic loading rate was kept quite high (24 kgCOD m^{-3} d^{-1}) to facilitate the selection of fermentative bacteria in the reactor. The hydraulic retention time was set in 6 h and the temperature was kept in mesophilic range (30 ºC) (Carneiro et al., 2022).

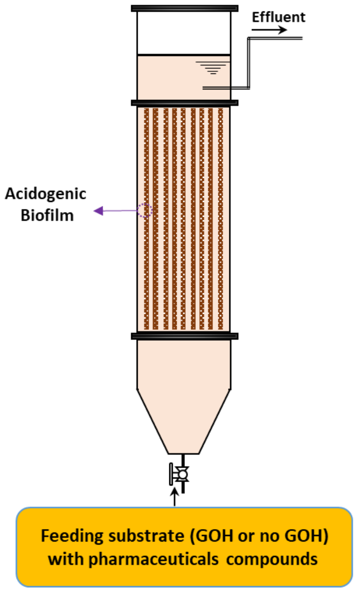

Figure 1. Schematic representation of the up-flow acidogenic biofilm reactor according to Carneiro et al., (2022)

The reactor operation was divided into two distinct phases – the first with the feeding of a substrate that is easily degraded - beef extract (530), sucrose (211), soluble starch (221), KH_2PO_4 (30); NaCl (250), $MgCl_2.6H_2O$ (28), and $CaCl_2.2H_2O$ (18); and a second phase with the glycerol addition to this substrate in order to be able to compare the heterotrophic cometabolism effect of the pharmaceutical compounds. Glycerol was added to a concentration of approximately 1 gCOD L^{-1}. The pharmaceutical compounds were added to the feeding substrate at a target concentration of 10 ug L^{-1} based on values reported in the literature (Park et al., 2020; Tran et al., 2018).

To analyze the performance of the acidogenic reactor, physical-chemical analyzes were carried out, such as pH, Chemical Oxygen Demand (COD), carbohydrates (CH), glycerol (GOH) and volatile fatty acids (VFA). Pharmaceutical compounds were analyzed by liquid chromatography coupled to mass spectrometry (LC-MS/MS - ABSciex QTRAP 5500), and online solid phase extraction.

3. RESULTS AND DISCUSSION

Table 1 presents the performance results of the acidogenic reactor for the operational phases without and with GOH application. It can be observed that the removal of carbohydrates and glycerol was complete in the reactor, which demonstrates its good acidogenesis efficiency. The elimination of organic matter in terms of COD is probably due to the biomass growth in the reactor and the production of a portion of gases dissolved and entrained in the reactor headspace.

Table 1. Acidogenic bioreactor operational performance (average ± standard deviation) for the two operational phases (with and without glycerol).

Parameter	No GOH	GOH
COD Removal (%)	38.6 ± 15.9	30.4 ± 13.1
CH Removal (%)	98.1 ± 1.6	97.7 ± 1.1
GOH Removal (%)	-	100.0 ± 0.1
pH	5.28 ± 0,49	4.19 ± 015

The GOH addition provided a decrease in the pH of the medium, which shows a favoring of the fermentative microbial communities for the production of organic acids. This can be corroborated by Figure 2, which shows the different volatile fatty acids produced in each operational phase. Clearly, a deviation from the metabolic pathway for the production of propionic and valeric acids in the medium occurs during the glycerol fermentation.

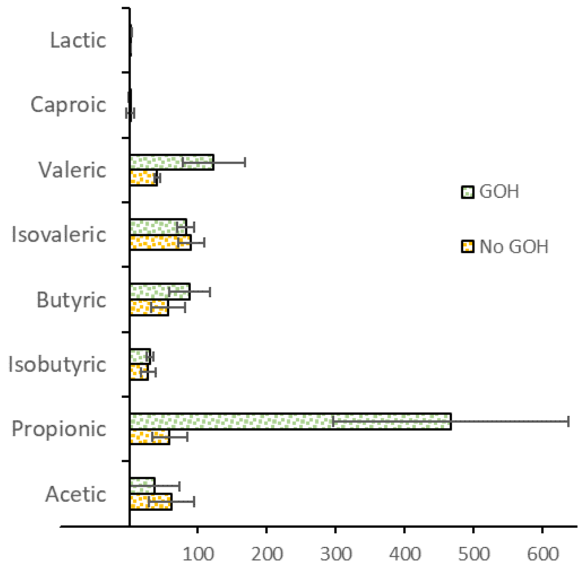

Figure 2. Volatile fatty acids produced in the acidogenic biofilm reactor with and without glycerol in the feeding substrate.

With regard to the removal of pharmaceutical compounds, it is observed in Figure 3 a significant increase in the biodegradation of IBP, DCF and CIP during glycerol fermentation. As previously hypothesized, the removal of these microcontaminants may be linked to heterotrophic cometabolism mechanisms. The enzymes produced in the bioconversion of glycerol can act in the biotransformation of the drugs. Gonzalez-Gil et al. (2019) and Kennes-Veiga et al. (2022)showed in their studies that many organic micropollutants are biotransformed by the action of different microbial enzymes.

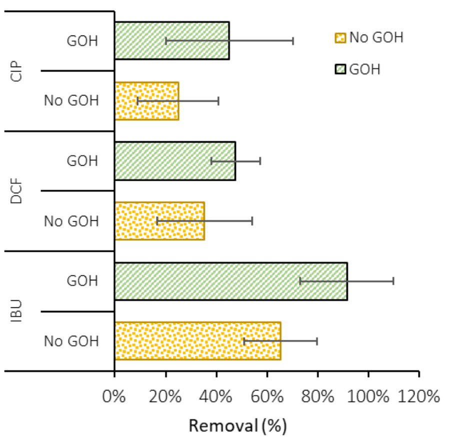

Figure 3. Removal efficiency of IBU, DCF and CIP compounds in the acidogenic biofilm reactor with and without glycerol in the feeding substrate.

Another point worth mentioning is the greater removal of IBU compared to DCF and CIP compounds. This greater persistence of these chemicals may be due to the presence of halogenated groups in their molecular structure (F in ciprofloxacin; Cl in diclofenac), corresponding to electron withdrawing groups that could confer greater recalcitrance in treatment processes (Wijekoon et al., 2015). Even so, the greater diversity of microbial enzymes in the glycerol fermentative bioreactor allowed the enhancement of the pharmaceutical compounds removal.

4. CONCLUSIONS

Glycerol fermentation in the acidogenic biofilm reactor was able to enhance the removal of the emerging pollutants ibuprofen, diclofenac and ciprofloxacin through heterotrophic cometabolism. The production of volatile fatty acids in the reactor was considerably increased by the addition of glycerol, altering the metabolic pathways for the prevalence of propionic acid, which may be correlated with the enzymatic biotransformation of microcontaminants in the medium. Further studies are needed to clarify the enzymes involved in glycerol fermentation that can act directly in the elimination of the micropollutants. Glycerol has been shown to be a promising carbon source to drive the removal of contaminants of emerging concern in wastewater.

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References

Carneiro, R. B., Gomes, G. M., Zaiat, M., & Santos-Neto, Á. J. (2022). Two-phase (acidogenic-methanogenic) anaerobic fixed bed biofilm reactor enhances the biological domestic sewage treatment: Perspectives for recovering bioenergy and value-added by-products. *Journal of Environmental Management*, *317*. https://doi.org/10.1016/j.jenvman.2022.115388

Carvalho, I. T., & Santos, L. (2016). Antibiotics in the aquatic environments: A review of the European scenario. In *Environment International* (Vol. 94, pp. 736–757). Elsevier Ltd. https://doi.org/10.1016/j.envint.2016.06.025

Clomburg, J. M., & Gonzalez, R. (2013). Anaerobic fermentation of glycerol: A platform for renewable fuels and chemicals. In *Trends in Biotechnology* (Vol. 31, Issue 1, pp. 20–28). https://doi.org/10.1016/j.tibtech.2012.10.006

Dias, M. E. S., Lopes, J. C., Carneiro, R. B., Damianovic, M. H. R. Z., & Foresti, E. (2021). Using Compounds Derived from the Glycerol Fermentation as a Carbon Source for Denitrification and Biological Phosphorus Removal. *Water, Air, and Soil Pollution*, *232*(8). https://doi.org/10.1007/s11270-021-05296-9

Gonzalez-Gil, L., Krah, D., Ghattas, A. K., Carballa, M., Wick, A., Helmholz, L., Lema, J. M., & Ternes, T. A. (2019). Biotransformation of organic micropollutants by anaerobic sludge enzymes. *Water Research*, *152*, 202–214. https://doi.org/10.1016/j.watres.2018.12.064

Kennes-Veiga, D. M., Gónzalez-Gil, L., Carballa, M., & Lema, J. M. (2022). Enzymatic cometabolic biotransformation of organic micropollutants in wastewater treatment plants: A review. In *Bioresource Technology* (Vol. 344). Elsevier Ltd. https://doi.org/10.1016/j.biortech.2021.126291

Kennes-Veiga, D. M., Vogler, B., Fenner, K., Carballa, M., & Lema, J. M. (2021). Heterotrophic enzymatic biotransformations of organic micropollutants in activated sludge. *Science of the Total Environment*, *780*. https://doi.org/10.1016/j.scitotenv.2021.146564

Park, J., Kim, C., Hong, Y., Lee, W., Chung, H., Jeong, D. H., & Kim, H. (2020). Distribution and removal of pharmaceuticals in liquid and solid phases in the unit processes of sewage treatment plants. *International Journal of Environmental Research and Public Health*, *17*(3). https://doi.org/10.3390/ijerph17030687

Rastogi, A., Tiwari, M. K., & Ghangrekar, M. M. (2021). A review on environmental occurrence, toxicity and microbial degradation of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). In *Journal of Environmental Management* (Vol. 300). Academic Press. https://doi.org/10.1016/j.jenvman.2021.113694

Tran, N. H., Reinhard, M., & Gin, K. Y. H. (2018). Occurrence and fate of emerging contaminants in municipal wastewater treatment plants from different geographical regions-a review. In *Water Research* (Vol. 133, pp. 182–207). Elsevier Ltd. https://doi.org/10.1016/j.watres.2017.12.029

Wijekoon, K. C., McDonald, J. A., Khan, S. J., Hai, F. I., Price, W. E., & Nghiem, L. D. (2015). Development of a predictive framework to assess the removal of trace organic chemicals by anaerobic membrane bioreactor. *Bioresource Technology*, *189*, 391–398. https://doi.org/10.1016/j.biortech.2015.04.034