Efficiency of an immobilized cell bioreactor bioaugmented with a fludioxonil-degrading bacterium

Z. Mavriou[1], I. Alexandropoulou[1], P. Melidis[1], D.G. Karpouzas[2] and S. Ntougias[1],*
[1] Laboratory of Wastewater Management and Treatment Technologies, Department of Environmental Engineering, Democritus University of Thrace, Vas. Sofias 12, 67132 Xanthi, Greece
[2] Laboratory of Plant and Environmental Biotechnology, Department of Biochemistry and Biotechnology, University of Thessaly, Viopolis, 41500 Larissa, Greece

*Corresponding author: E-mail: sntougia@env.duth.gr, Tel/fax: +30 25410 79313

Abstract
Postharvest fungicides prevent fungal infections in fruits and vegetables, extending their shelf life. Fludioxonil is a phenylpyrrole fungicide, which is commonly applied for the postharvest protection of various fruits, including citrus and pomes. However, the application of this fungicide results in the generation of fludioxonil-rich wastewater. Due to adverse effects of fludioxonil to aquatic life, effective treatment of fludioxonil-rich wastewater produced by the fruit processing industry is required. In this work, an immobilized cell bioreactor was inoculated with the fludioxonil-degrading bacterium FLX-12, which was isolated from activated sludge. The bioaugmented system was capable of reducing fludioxonil concentration by 99%. In addition, COD (Chemical Oxygen demand) removal efficiency also reached 80%, resulting in effluent COD concentration of less than 65 mg/L. Moreover, significantly greater electrical conductivity values were determined in the effluent compared to the influent, as a consequence of the metabolic activity of the immobilized biomass. Indeed, the bioaugmentation of the immobilized cell bioreactor with strain FLX-12 enhanced system's ability to depurate this fungicide-rich wastewater.

Keywords: *fludioxonil; postharvest treatment; immobilized cell bioreactor; fungicide-degrading bacterium*

.

1. INTRODUCTION

Postharvest treatment of fruits and vegetables by the fruit packing industry prevents infections and reduces production losses [1]. Especially, the application of postharvest fungicides contributes to the protection of fruits from fungal species, inclunding *Botrytis cinerea*, *Penicillium digitatum* and *P. italicum*/*P. expansum* [2]. Among them, fludioxonil is used as a postharvest fungicide to extend the fruit's shelf life during storage [3]. Fludioxonil (4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile-) is a phenylpyrrole fungicide, which can protect various fruits, like apples, pears and citrus, from green and blue mold infection [4].

Fludioxonil can be applied in fruit packing industry by using drenchers or spraying systems, resulting thus in the generation of wastewaters of high fludioxonil concentration [5]. Wastewaters containing high concentrations of fungicides are toxic to the environment because of their high persistence to soils and water [6]. Especially, fludioxonil has been found to affect

ISBN: 978-618-5271-XX-X

various aquatic organisms [7]. It is worth noticed that conventional biological systems cannot effectively treat these kinds of wastewaters, due to their low biodegradability [8].

A few studies have referred to the degradation of fludioxonil. Apart from the photolytic degradation of fludioxonil within a short period of time [9], Alexandrino et al. (2020) identified microbial consortia capable of biodegrading fludioxonil [10]. A 95% reduction in fungicide concentration was achieved during treatment of fludioxonil-rich wastewater in an immobilized cell bioreactor inoculated with activated sludge and operated under aerobic conditions [11]. In this work, the performance of an immobilized cell bioreactor bioaugmented with strain FLX -12, a bacterial isolate that was isolated from activated sludge and was capable of degrading the postharvest fungicide fludioxonil, was evaluated during treatment of fludioxonil-rich wastewater.

2. MATERIALS AND METHODS

An immobilized cell bioreactor was inoculated with the bacterial strain FLX- 12, a fludioxonil degrader isolated from activated sludge at the Laboratory of Wastewater Management and Treatment Technologies, Democritus University of Thrace, Greece, and fed with wastewater containing 200 mg/L fludioxonil. The total volume of the bioreactor was 550 ml, whereas a column of 150 mL was filled with porous beads. The bioreactor was operated under aerobic conditions using an air pump for constant aeration. Recirculation of the effluent was achieved by a peristaltic pump and the hydraulic retention time was set at 10 days.

Based on "Standard Methods for the Examination of Water and Wastewater", the chemical oxygen demand (COD) in the influent and the effluent of the bioreactor was determined. In particular, samples were digested with 0.1 N $K_2Cr_2O_7$ uder acidified conditions at 148^oC for 2 h. After digestion, COD concentration was estimated after titration with 0.02 N ammonium ferrous sulfate using a 25 mM ferroin solution as redox indicator.

To estimate Total Kjeldahl Nitrogen (TKN), samples were digested for 4 h and subjected to distillation. The ammonia vapor entrapped in a boric acid solution was titrated with 0.01 N H_2SO_4. Ammonium nitrogen concentration ($NH4^+$-N) was also estimated via distillation and titration in the presence of methyl red-methylene blue indicator. Nitrates reduction to nitrites was carried in a column packed with cadmium granules, while nitrite concentration was measured colorimetrically at 453 nm using sulfanilamide/(1-naphthyl) ethylenediamine-dihydrochloride indicator.

During bioreactor operation, pH and electrical conductivity (EC) were determined using a METROHM and a CRISON probe, respectively. Fludioxonil concentration was measured isocratically in an ECOM HPLC-PDA consisting of a 5 Fortis UniverSil C18 250 x 4.6 mm column and operating at flow rate 0.8 mL/min.

3. RESULTS AND DISCUSSION

Regarding bioreactor performance, total and soluble COD in the effluent were 84 ± 3 mg/L and 64 ± 3 mg/L, respectively, while in the influent was 284 ± 4 mg/L. COD removal was high and reached 80%. Influent and effluent pH values were stable and equal to 6.94 ± 0.02 and 7.22 ± 0.02, respectively. EC values in the influent were estimated to be 3.08 ± 0.01 mS/cm, while in the effluent were stabilized to 4.93 ± 0.05 mS/cm after a gradual decrease

ISBN: 978-618-5494-53-7

occurred in the first 30 days. In addition, TKN concentration in the influent was determined to be 20.02 ± 0.49 mg/L, whereas effluent TKN was below 2.65 ± 0.30 mg/L. Concentrations of nitrate, nitrite and ammonia were negligibly detected.

Fludioxonil concentration was significantly reduced from 200 mg/L to 1.53 ± 0.68 mg/L, as showed in Figures 1 and 2. Figure 1 displays the chromatographic peak, which corresponds to the fludioxonil concentration in the influent, while Figure 2 shows the respective peak in the effluent of the bioreactor. The fludioxonil removal efficiency was estimated to be equal to 99.24 ± 0.34%, indicating the effectiveness of depuration process.

Regarding biotreatment of fludioxonil, vineyard effluent containing 9.5 mg/L fludioxonil was treated in an activated sludge system [12]. Also, mixture of fungicides, including fludioxonil, were depurated in bio-organic mixtures [13,14]. Moreover, degradation of fungicides by specialized degraders has been previously reported. The fungicides 1H-1,2,4-triazole and tricyclazole were degraded by using the bacteria *Shinella* sp. NJUST26 and *Sphingomonas* sp. NJUST37 [15]. Moreover, thiabendazole degradation was also acheived by a bacterial consortium, relying on a *Sphingomonas* [16].

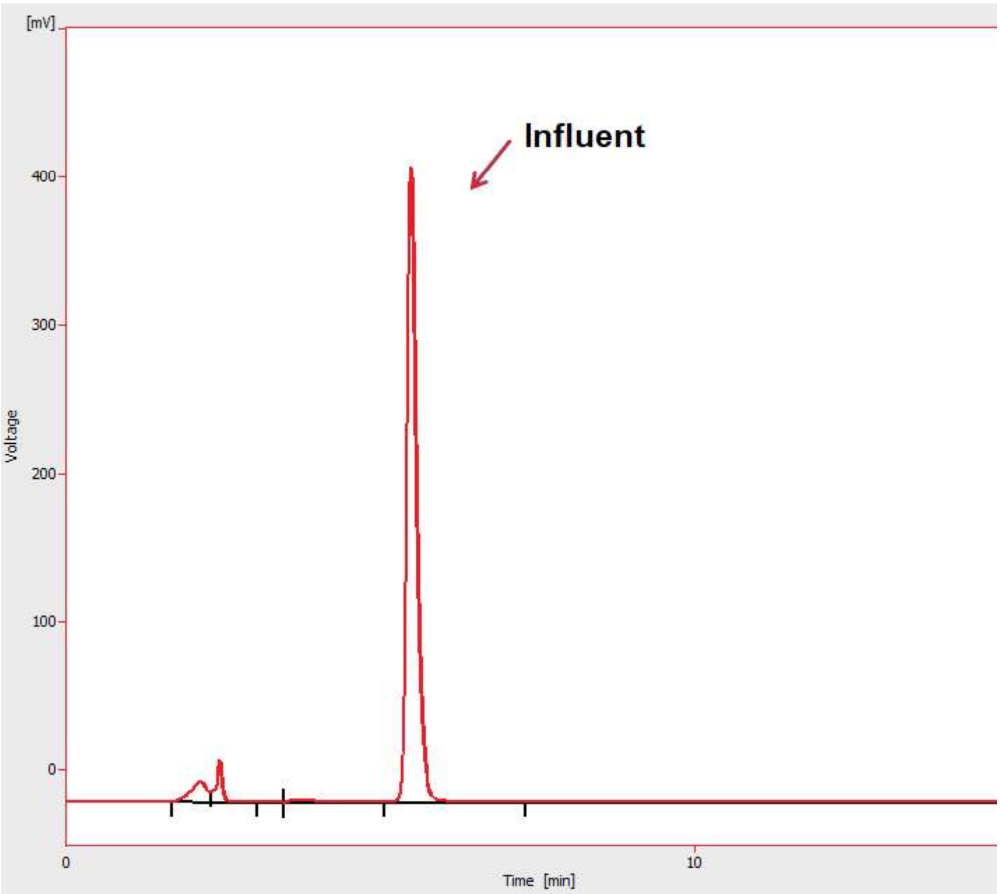

Figure 1. *Fludioxonil concentration in the influent of the immobilized cell bioreactor bioaugmented with the bacterium FLX-12.*

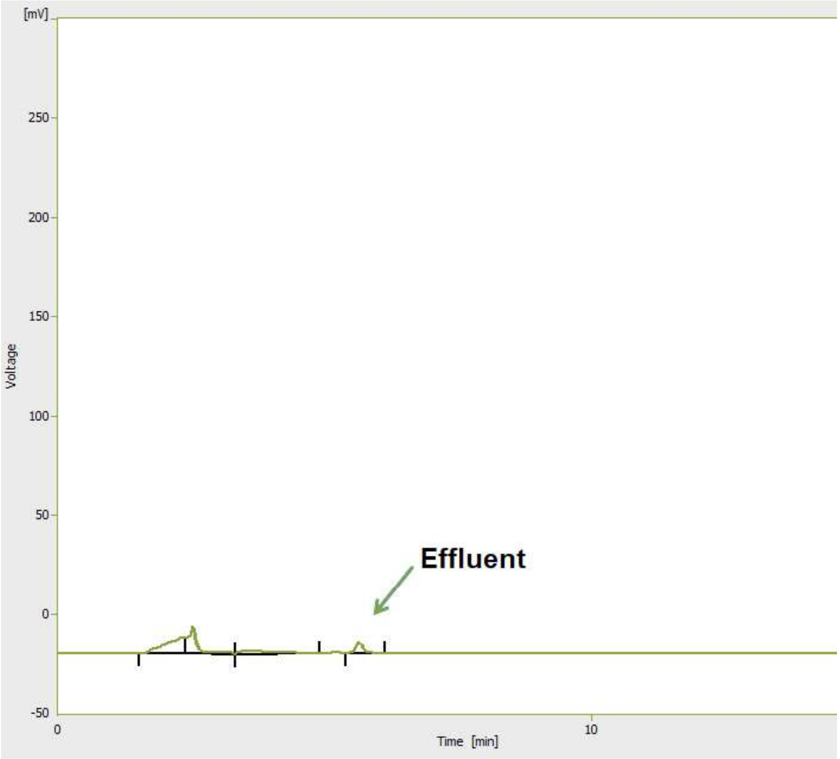

Figure 2. *Fludioxonil concentration in the effluent of the immobilized cell bioreactor bioaugmented with the bacterium FLX-12.*

4. CONCLUSIONS

In this study, an immobilized cell bioreactor, which was bioaugmented with bacterial isolate FLX-12, was evaluated in terms of fludioxonil removal. The bioaugmented biosystem resulted in the reduction of fludioxonil concentration from 200 mg/L in the influent to 1.5 mg/L in the effluent, recording removal efficiencies above 99%. Moreover, a reduction in COD concentration by 80% was also determined. It is concluded that the immobilized cell bioreactor bioaugmented with bacterial isolate FLX-12 was capable of effectively depurating wastewaters containing fludioxonil, providing an effective solution for the treatment of fungicide-rich wastewaters generated by the fruit packing industry.

References

[1] Brandhorst, T.T, Klein, B.S., 2019. Uncertainty surrounding the mechanism and safety of the post-harvest fungicide. *Food and Chemical Toxicology* **123**, 561-565.

[2] Förster, H., Driever, G.F., Thompson, D.C., Adaskaveg, J.E., 2007. Postharvest decay management for stone fruit crops in California using the "reduced-risk" fungicides fludioxonil and fenhexamid. *Plant Disease* **91** (2), 209-215.

[3] Palou, L., 2018. Postharvest treatments with gras salts to control fresh fruit decay. *Horticulturae* ***4*** (4), 46.

[4] D'Aquino, S., Palma, A., Angioni, A., Schirra, M., 2013. Residue levels and efficacy of fludioxonil and thiabendazole in controlling postharvest green mold decay in citrus fruit when applied in combination with sodium bicarbonate. *Agriculture and Food Chemistry* **61**, 296-306.

ISBN: 978-618-5494-53-7

[5] Russouw, A., Meitz-Hopkins, J., Den Breeyen, A., Lennox, C., 2021. Postharvest applications of fludioxonil and pyrimethanil to control *Phlyctema vagabunda* on apple in South Africa. *Crop Protection* **141**, 105451.
[6] de Sousa e Silva, A., Sales Morais, N.W., Maciel Holanda Coelho, M., Lopes Pereira, E., Bezerra dos Santos, A., 2020. Potentialities of biotechnological recovery of methane, hydrogen and carboxylic acids from agro-industrial wastewaters. *Bioresource Technology Reports* **10**, 100406.
[7] Brock, T.C.M., Romão, J., Yin, X., Osman, R., Roessink, I., 2020. Sediment toxicity of the fungicide fludioxonil to benthic macroinvertebrates-evaluation of the tiered effect assessment procedure. *Ecotoxicology and Environmental Safety* **195**, 110504.
[8] European Food Safety Authority (EFSA), 2007. Conclusion regarding the peer review of the pesticide risk assessment of the active substance fludioxonil. *European Food Safety Authority (Scientific Report)* **110**, 1-85.
[9] Choi, S.W., Shahbaz, H.M., Kim, J.U., Kim, D.-H., Yoon, S., Jeong, S.H., Park, J., Lee, D.-U., 2020. Photolysis and TiO_2 photocatalytic treatment under UVC/VUV irradiation for simultaneous degradation of pesticides and microorganisms. *Applied Sciences* **10** (13), 4493.
[10] Alexandrino, D.A., Mucha, A.P., Almeida, C.M.R., Carvalho, M.F., 2020. Microbial degradation of two highly persistent fluorinated fungicides-epoxiconazole and fludioxonil. *Journal of Hazardous Materials* **394**, 122545.
[11] Mavriou, Z., Alexandropoulou, I., Melidis, P., Karpouzas, D.G., Ntougias, S., 2021. Biotreatment and bacterial succession in an upflow immobilized cell bioreactor fed with fludioxonil wastewater. *Environmental Science and Pollution Research* **28** (4), 3774-3786.
[12] Esteve, K., Poupot, C., Mietton-Peuchot, M., Milisic, V., 2009. Degradation of pesticide residues in vineyard effluents by activated sludge treatment. *Water Science and Technology* **60**(7),1885-1894.
[13] Coppola, L., Comitini, F., Casucci, C., Milanovic, V., Monaci, E., Marinozzi, M., Taccari, M., Ciani, M., Vischetti, C., 2011. Fungicides degradation in an organic biomixture: impact on microbial diversity. *New Biotechnology* **29**(1), 99-106.
[14] Papazlatani, C.V., Karas, P.A., Tucat, G., Karpouzas, D.G., 2019. Expanding the use of biobeds: Degradation and adsorption of pesticides contained in effluents from seed-coating, bulb disinfestation and fruit-packaging activities. *Journal of Environmental Management* **248**, 109221.
[15] Wu, H., Shen, J., Jiang, X., Liu, X., Sun, X., Li, J., Han, W., Wang, L., 2018. Bioaugmentation strategy for the treatment of fungicide wastewater by two triazole-degrading strains. *Chemical Engineering Journal* **349**, 17–24
[16] Perruchon, C., Chatzinotas, A., Omirou, M., Vasileiadis, S., Menkissoglou-Spiroudi, U., Karpouzas, D.G., 2017. Isolation of a bacterial consortium able to degrade the fungicide thiabendazole: the key role of a *Sphingomonas* phylotype. *Applied Microbiology and Biotechnology* **101 (9)**, 3881-3893.

Acknowledgements
This research, carried out within the frame of the research project entitled "Development and implementation of novel biobased methods for the treatment of pesticide-contaminated wastewaters from agro-industries, MIS 5030360", has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH –CREATE – INNOVATE (project code: T1EDK-02566).

European Union
European Regional Development Fund
EPAnEK 2014-2020
OPERATIONAL PROGRAMME
COMPETITIVENESS • ENTREPRENEURSHIP • INNOVATION
Co-financed by Greece and the European Union
ΕΣΠΑ 2014-2020
Partnership Agreement 2014 - 2020