Biodegradation of fludioxonil in bioreactor systems in the presence of amino acids

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Abstract

Deterioration of fruit quality by fungal infections during post-harvest storage is a major concern of the fruit processing industry. The application of post-harvest fungicides on fruits and vegetables has contributed significantly to the extension of their shelf life during storage. Direct disposal of wastewaters containing high concentrations of fungicides, including fludioxonil, is prohibited since they are toxic to certain aquatic organisms. Biodegradation mechanisms of fludioxonil remain unknown since microorganisms that are specialized in the degradation of fludioxonil are limited. In this work, the growth of a bacterial isolate capable of degrading the fungicide fludioxonil was examined in batch bioreactors under aerated conditions. In particular, various carbon sources were co-metabolized with fludioxonil to investigate the growth aspects of the novel fludioxonil-degrading isolate.

Keywords*: fludioxonil; postharvest fungicide; fungicide-degrading bacterium; co-substrate metabolism*

1. INTRODUCTION

Fungicides are used in fruit processing industries to protect fruits and vegetables from fungal infections. Among the most common fungicides used in the postharvest treatment of fruits are fludioxonil, imazalil and thiabendazole. Fludioxonil (4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile-) is a phenylpyrrole fungicide, which inhibits the transport-associated phosphorylation of glucose, affecting mycelial growth [1]. It is effective against microbes, like *Penicillium*, *Botritis*, *Rhizopus*, *Colletotrichum*, *Monilinia* and *Gloeosporium* spp., and it is applied in various fruits, such as apples, pears and citrus [2,3]. Fludioxonil is characterized as persistent in soils [4].

During the postharvest application of fungicides, large quantities of wastewaters containing high concentrations of fungicides, including fludioxonil, are generated. Direct disposal of such wastewaters in the environment, commonly in surface water, is prohibited since fludioxonil exerts toxicity against certain aquatic organisms [5]. Mixture of fludioxonil with other fungicides has been reported to exhibit toxic effects on the earthworm *Eisenia andrei* and the amphibian *Rhinella*

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arenarum [6]. Yin et al. (2018) have reported the prolonged persistence of fludioxonil in soils and surface water, affecting the sediment-dwelling and pelagic organisms [7]. Moreover, negative impact of fludioxonil on non-target soil invertebrates, particularly in nematodes, has been observed [8]. In addition, high concentrations of fludioxonil may affect amphibian embryos [9].

The European Commission, recognizing the hazardous nature of wastewaters containing high residual concentration of pesticides, has imposed their compulsory management and treatment [10]. Due to the complex structure of fungicides, these wastewaters are not effectively treated by conventional biological systems. Chemical oxidation methods, like Fenton, photo-Fenton, O_3/UV, H_2O_2/UV and TiO_2/UV, are effective in fungicides breakdown, but the cost for installation and operation of such treatment approaches is considered as expensive [2]. Biobeds have also been found to be capable of effectively removing a mixture of fungicides, including fludioxonil [11]. Recently, Alexandrino et al. (2020) reported the defluorination of 10 mg/L fludioxonil by enriched microbial consortia [12]. However, biodegradation mechanisms of fludioxonil still remain unknown since microorganisms that are specialized in the degradation of fludioxonil are limited. In this work, the ability of a novel fludioxonil-degrading isolate to utilize various carbon sources in the presence of fludioxonil in batch bioreactors under aeration was examined in order to elucidate aspects regarding the degradation capability of this strain.

2. MATERIALS AND METHODS

Batch bioreactors of 500 ml working volume each were inoculated with the isolated fludioxonil-bacterium, which has the ability to utilize fludioxonil as the sole carbon and energy source. A solution consisting of minerals and trace elements, i.e. 0.2 mM Na_2HPO_4, 0.8 mM KH_2PO_4, 1 mM KCl, 0.4 mM $MgSO_4.7H_2O$, 0.4 mM $CaCl_2.2H_2O$, 17 μM $MnSO_4.4H_2O$, 0.4 mM $FeSO_4.7H_2O$, 0.8 μM $NiCl_2.6H_2O$, 0.2 μM Na_2SeO_3, 0.7 μM $CoCl_2.6H_2O$, 0.7 μM $NaMoO_4.2H_2O$, 0.4 μM $ZnSO_4.7H_2O$ and 0.2 μM H_3BO_4, was sterilized in a autoclave for 20 min at temperature of 121^oC. After the sterilization process, 250 mg/L of fludioxonil and the carbon source tested (500 mg/L) were added aseptically in the above-described solution and the medium was then inoculated with the isolated bacterium examined. Mixtures of fludioxonil/proline and fludioxonil/histidine were tested in laboratory batch reactors, whereas the growth of the isolated bacterium in medium consisting of fludioxonil as the sole carbon source was served as the control. An oxygen diffuser connected to an air pump was set inside the bioreactor in order to provide adequate aeration, i.e. dissolved oxygen values above 4 mg/L. A magnetic stirrer was also set on the bottom of the bioreactor for constant stirring at 150 rpm.

The growth of the isolated bacterium and the degradation of the fungicide in the simultaneous presence of fludioxonil and the carbon source examined were investigated for a period of ten (10) days in triplicate setups, where subsamples were daily obtained for chromatographic analysis in a High Performance Liquid Chromatography with a Photodiode Array Detector (HPLC-PDA, ECOM, Czech Republic). The concentration of fludioxonil was determined isocratically by separating peaks in a 5 UniverSil C18 250 x 4.6 mm column (Fortis, UK). A volumetric mixture of 75% acetonitrile and 25% H_2O under a flow rate of 0.8 mL/min was used as the mobile phase.

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3. RESULTS AND DISCUSSION

The bioegradation of fludioxonil in the batch bioreactors in the presence of L-histidine as co-substrate was monitored for a period of 10 days. Initially, fludioxonil concentration was gradually decreased for a period of 4 days after inoculation with the isolated fludioxonil-degrading bacterium. Afterwards, fludioxonil concentration was less than 5 mg/L for the rest of the experimental period. In particular, fludioxonil concentration was significantly decreased from 250 mg/L to 3.42 mg/L after 4 days of incubation, corresponding to fludioxonil reduction greater than 99% (Figure 1).

Regarding fludioxonil biodegradation in the presence of proline, lower degradation rate was observed when the mixture of proline and fludioxonil was treated in laboratory batch reactors. Specifically, fludioxonil removal efficiency reached values above 95% after 5 days of inoculation with the effective fludioxonil-degrading bacterium examined.

Efficient removal of fungicides by microbial agents has been rarely recorded. For example, the bacterial strains *Shinella* sp. NJUST26 and *Sphingomonas* sp. NJUST37 were found to be effective degraders of the pesticides 1H-1,2,4-triazole and tricyclazole under the application of a hydraulic retention time of 4 days [13]. Vasileiadis et al. (2020) reported the degradation of fungicide thiabendazole by a bacterial consortium, where predominant role in the detoxification of fungicide-containing wastewater played a strain of the genus *Sphingomonas* [14]. Moreover, the bacterial strain *Pseudomonas aeruginosa* PS-4 was also reported to be specialized in the degradation of the propiconazole [15].

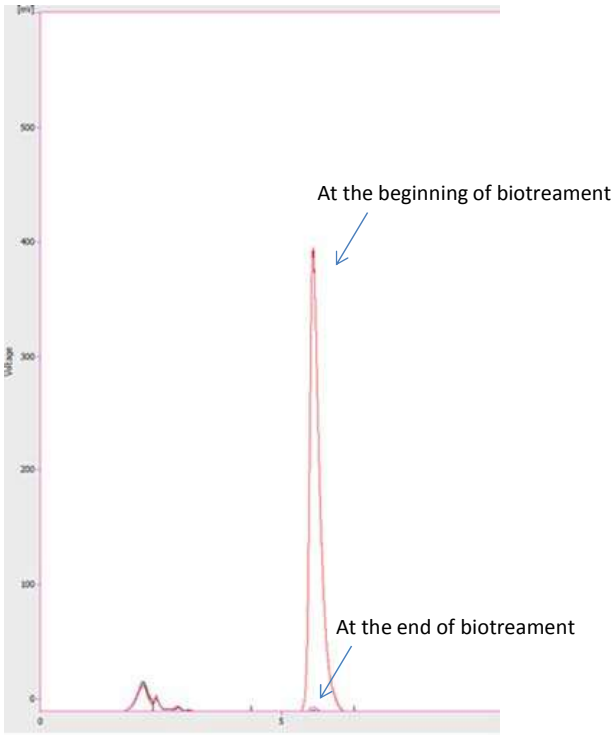

Figure 1. Fludioxonil reduction in a batch bioreactor in the presence of the amino acid L-histidine.

4. CONCLUSIONS

It is concluded that the isolated fludioxonil-degrading bacterium can effectively degrade this pesticide in the presence of amino acids as co-substrates. The high removal rate of fludioxonil in the bioreactor systems is indicative of the effectiveness of this bacterial strain to be used as starting culture in full-scale applications. Such biotechnological solution is important for the fruit packing industry, where reliable biological solutions are necessary for effective management of the huge amounts of fungicide-rich wastewaters.

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