Proceedings of the 5th World Congress on New Technologies (NewTech'19)
Lisbon, Portugal – August 18-20, 2019
Paper No. ICEPR 133
DOI: 10.11159/icepr19.133

Degradation of Selected PAHs by Laccase-Mediator System in Soil

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Extended Abstract

This study investigated the degradation of selected PAHs using laccase produced through cocultivation of *Trichoderma viride* (EXF8977) and *Penicillium chrysogenum* (EXF1818) on solid state fermentation and its mediator system.

The main objective of this work was to evaluate the ability of the laccase-mediator systems to degrade PAHs in soil, as well as the effects of natural and synthetic mediators on the laccase activity during the process of PAHs degradation.

The laccase production was optimized in packed-bed bioreactor, by testing different airflow (0.05 L min^{-1}, 0.1 L min^{-1} and 0.2 L min^{-1}), temperature (RT, 30 and 40 °C) and time (2 and 3 weeks) on kiwi peels as substrate using single fungi species and cocultivation. Extracellular laccase activity was measured spectrophotometrically using 0.5 mM 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) as substrate on 420 nm and enzyme activity was expressed in units per millilitre (U/mL).

Batch degradation experiments were carried out in 100 mL Erlenmeyer flask on soil pH 5, containing PAH mixture (fluorene, anthracene, pyrene, chrysene and benzo(a)pyrene) from 50 till 600 ppm in dark at 20, 30 and 40 °C. In order to obtain optimum laccase degradation conditions different concentration (0.1 mM, 0.5 mM and 1 mM) of synthetic (ABTS) and natural mediators (p-coumaric acid and ferulic acid) were studied. Samples were collected after 0, 3 and 5 incubation days and the compounds were measured by UHPLC. All the assays including controls were performed in triplicate. Thereafter column packed bed reactor was set up to evaluate the degradation with continue flow (0.015 mL/min) of crude laccase enzyme with 1 mM ABTS, p-coumaric acid and ferulic acid for 25 days, by taking samples each 5 days and evaluating degradation ratio and the remaining concentration of laccase in samples.

The selected fungi were able to produce 2.95 U/mL of laccase during SSF in Erlenmeyer flask, however this amount increased with continuous airflow. Highest values were obtained at aeration rates 0.2 L min^{-1}, with a production of 6.32 U/mL, followed by 0.1 and 0.05 L min^{-1} with productions of 5.45 and 3.79 U/mL, respectively.

Data showed laccase extracted from cocultivation of *Penicillium chrysogenum* and *Trichoderma viride* on kiwi peels with an appropriate mediator promoted the degradation of fluorene, anthracene, pyrene, chrysene and benzo(a)pyrene in concentration from 50 ppm till 600 ppm. Reaction system reached highest degradation at 3 days in case of fluorene, chrysene and benzo(a)pyrene, but with anthracene and pyrene maximum degradation rate was observed between 3 and 5 days. The optimizing mediator for fluorene and pyrene was p-coumaric acid, with removal of 81% and 98%, respectively. For anthracene, the best mediator was ABTS with 42% degradation, and laccase with ferulic acid achieved highest degradation rate with chrysene, 61%, and benzo(a)pyrene, 5.4%.

However, in packet bed reactor with continuous flow of laccase with mediator at 0.015 mL/min, for 25 days at RT (26-28 °C), ABTS was found to be the optimum mediator for fluorene, anthracene, chrysene and benzo(a)pyrene, with degradation reaching 74.8 %, 71.9%, 81.8% and 96%, respectively. Around 72.2 % of phenanthrene was degraded with ferulic acid as mediator, and 99.9% degradation of pyrene was reached with p-coumaric acid. Less than 60% degradation rate in 25 days was observed for phenanthrene and anthracene with p-coumaric acid as mediator.

The results achieved in the present research demonstrated the utility of laccase from SSF production and its use in bioremediation in soil, especially to reduce the concentration of PAH. However, more research must be done in real conditions.