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Effect of the pH on growth and esterase activity of *Fusarium* culmorum grown on media supplemented with di (2-ethylhexyl) phthalate in submerged fermentation

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Di (2-ethylhexyl) phthalate (DEHP) is a plasticizer widely used in the manufacture of plastics, and it is an environmental contaminant. Fusarium culmorum has shown ability to degrade DEHP due its esterases production. Cultivation conditions are essential in successful enzyme production by the organism, that's why optimization of the pH is crucial in the fermentation process. This fungus was grown at different pHs (5.5, 6.0, 6.6, 7.0, 7.5, 8.0, 8.5 and 9.0) in a medium added with DEHP (initial concentration 1000 mg/L) as sole carbon source at 25 °C for 228 h in submerged fermentation. In this work, the influence of pH on the specific growth rate, maximum biomass, esterase activity (evaluated by biochemical tests and polyacrylamide gel electrophoresis) and enzymatic yield parameters for F. culmorum were determined. It was found that the greatest kinetic growth and enzymatic yield parameters were observed at a pH of 6.5. Four esterase activity bands (isoenzymes) were observed in the DEHP-supplemented media, having a molecular weight of about 20 kDa, 25 kDa, 37 kDa and 50 kDa approximately. In general, the bands were observed between 72 and 228 h. These studies showed that 6.5 was the optimum pH for growth and esterase production of *F. culmorum*.

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P25-13

Enzymatic depolymerization of polyurethanes for biorecycling process

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Polyurethanes (PU) are synthetic polymers intended for long term applications such as isolation panels for construction or furniture. PU are specifically designed to resist against environmental factors such as climate constraints (low and high temperature, moisture), abrasion and microbial attack (biotic and abiotic degradations). These resistances lead to the pervasive spread of this material in the environment. In combination with other plastic materials released, it may exert negative and unpredictable effects on both aquatic and terrestrial ecosystems. Biochemical recycling appears as a promising solution for PU waste management. Specific enzymes are able to depolymerize/deconstruct polymers to release

chemicals. These chemicals can then be used as building blocks to synthesize new macromolecular architectures.

In the frame of our study, a collection of hydrolases was screened on two model urethane substrates leading to the selection of an amidase able to cleave the urethane bond and an esterase able to hydrolyze a polyester PU dispersion. Enzymatic activity was then evaluated on four thermoplastic PU (TPU). The highest activity was measured for the esterase on a polyester PU with 33% weight loss after 51 days of incubation at 37 °C. Deep cracks on the polymer surface and the presence of oligomers in the remaining TPU pieces confirmed the high enzymatic efficiency. The corresponding main degradation products were identified to understand the scission mechanism. Combining the esterase and the amidase also led to a significant hydrolysis of this polyester PU. Specific degradation products were detected revealing the efficiency of the urethane bonds hydrolysis.

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Bioseperations

P26-1

Reactive extraction of 6-aminopenicillanic acid

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6-Aminopenicilanic acid (6APA) is the main component of semi-synthetic Penicillins, antibiotics that are obtained by 6APA acylation and formation of amidic bonds different from natural ones. 6APA is the biosynthetic product of some Penicillin producing fungus grown on nutritive media without precursors. Because the biosynthesis process is economically inefficient, some chemical or enzymatic methods for Penicillin G hydrolysis to 6APA were proposed on industrial scale. The solution obtained by enzymatic hydrolysis of Penicillin G contains about 4-5% 6APA, 1.8-3% phenyl acetic acid (PAA) and 0.8-1% unhydrolized Penicillin G (PG). Whereas the industrial separation of 6APA needs large amounts materials and high energy consumption for extraction and acidification processes, the aim of this work was to establish the conditions for the selective separation of 6APA by reactive extraction from mixture obtained by enzymatic hydrolysis of Penicillin G. In order to select the most efficient extraction system, the individual extractions of 6APA with organophosphoric acids (di-2-(ethylhexyl) phosphoric acid, D2EHPA) and high molecular weight amines type (lauryl-trialkyl-methyl-amine, Amberlite LA-2) extraction agents were studied. The results indicated that by increasing the pH value of the aqueous phase (pH > 6-7) the extraction degree obtained with Amberlite LA-2 was significantly higher compared to those obtained for physical or reactive extraction with D2EHPA. Using the influences of the pH value of the aqueous phase and the concentration of Amberlite LA-2 in 1,2-dichloroethane on selective separation of a mixture of 6APA, PG and PAA, 6APA was selectively separated at pH = 10, the overall extraction degree being 98.8%.

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