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Effect of operational parameters on methanol biofiltration coupled with Endochitinase 42 production

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ABSTRACT

Biofiltration is a technology for the biological degradation of multiple gas pollutants. It consists of a biological active bed where the contaminant gas is vented through and aerobically degraded. In this study, a novel methanol biofiltration process coupled with the production of the heterologous protein Endochitinase-42, using a genetically modified *Pichia pastoris* strain, was investigated. Three identical biofilters of 3.3 L packed with Perlite were operated under discontinuing addition of nutritive solution. Four important parameters such as methanol inlet loading rate (ILR), pH, nitrogen concentration, and empty bed residence time (EBRT) were studied. Evaluation of ILR resulted in a maximum elimination capacity (EC) of 643 g m^{-3} h^{-1} and heterologous Ech42 activity of 1020 U L^{-1}. In another stage, it was observed that nitrogen is a limiting factor not only for methanol degradation, but also for protein production, being 7.5 g L^{-1} ammonium sulfate (7 gC/gN) the optimal value with a protein activity of 1172 U L^{-1}. Moreover, pH 5.5 and EBRT of 60 s were the most favorable values for enzymatic production. In general, a set of operational conditions, including 7.5 g L^{-1} ammonium sulfate, pH 5, and EBRT of 60 s were optimal to maximize the enzymatic productivity.

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1. Introduction

Methanol is a volatile organic compound highly soluble in water (1000 g L^{-1}) that is commonly released by several industries (e.g., paper, automotive, paint, etc.,). If inhaled, methanol might cause headache and nausea, decrease the gestational length in pregnant women, and alter neurobehavioral development in children [1]. Due to these and other potential health effects, methanol is considered one of the most hazardous air pollutants by the Environmental Protection Agency [2].

Among the different technologies available for treating methanol vapor, biofiltration is probably the most widely implemented [3–8]. This technology is characterized by the use of microorganisms capable of degrading pollutants. Such microorganisms are fixed and growing on the surface of a particulate material packed in a biofilter column. When the polluted air flows throughout the bed, methanol is decomposed as a result of microbial catalytic activity.

In methanol biofiltration, a wide variety of methylotrophic microorganisms have been found, e.g., *Pseudomonas* sp., *Methylobacterium* sp., *Methylococcus* sp. and *Scytalidinum* sp. [9–11]. Recently, *Pichia pastoris* was used in methanol biofiltration in order to simultaneously obtain a valuable product: heterologous proteins [12–14].

P. pastoris is a methylotrophic yeast well known for its efficiency in heterologous protein expression [15]. Among its multiple features, three advantages can be highlighted: highly efficient methanol inducible promoter (pAOX1), low endogenous protein secretion, and the capacity for post-translational modifications [15,16]. All of these features together make *P. pastoris* one of the best options for protein production.

So far, methanol elimination capacities (EC), reached with the novel *P. pastoris* biofiltration system, have been as high as those obtained in conventional methanol biofiltration [12,14]. Also, according to the recovered protein, the system has proven its economic profitability [14]. However, the process is still little known and more research is needed before its industrial implementation.

In this study the effects of operational parameters such as methanol inlet loading rate (ILR), nitrogen concentration, pH, and empty bed retention time (EBRT) on the performance of methanol

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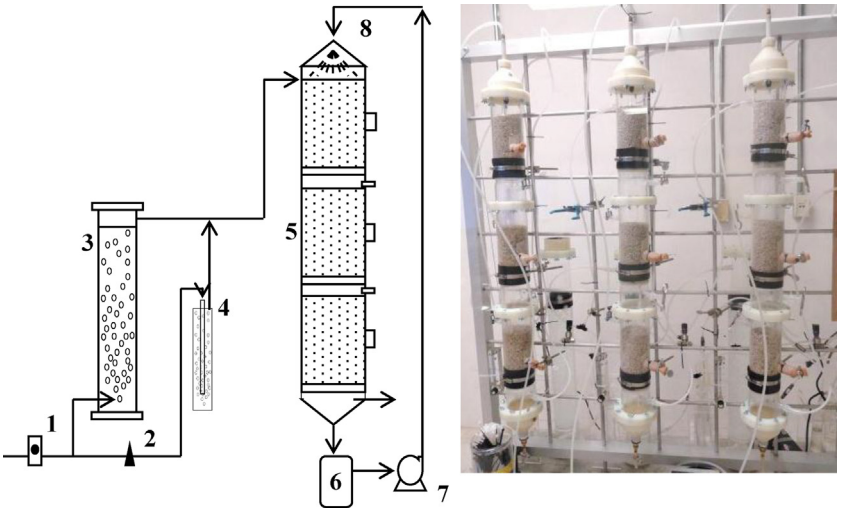

Fig. 1. Diagram of the experimental biofilters. 1 – flowmeter, 2 – needle valve, 3 – humidifier, 4 – methanol reservoir, 5 – biofilter, 6 – mineral solution, 7 – recirculation of leachate, 8 – sprinkler.

treatment coupled with the production of a heterologous protein using *P. pastoris* were evaluated.

2. Materials and methods

2.1. Inoculum and growth medium

P. pastoris GS115 transformed with the plasmid pPIC-*ech42* [17], which contains the 42 kDa endochitinase gene from *T. atroviride*, was used as a model of study. The strain was grown in 5 mL of YPD growth medium (10 g L^{-1} yeast extract, 20 g L^{-1} dextrose, 20 g L^{-1} peptone, and 10 g L^{-1} bacto-agar) plus 250 μg mL^{-1} geneticin (G418 sulfate, Phyto Technologies Laboratories, USA) at 28 °C overnight [13]. Cultures were centrifuged for 5 min at 13000 rpm and the resulting pellets were re-suspended in 500 mL of mineral medium YNB w/o amino acids and ammonium sulphate (1.7 g L^{-1}); 2.5 g L^{-1} $(NH_4)_2SO_4$; 100 mM citrate–phosphate Buffer, pH 5) and methanol (1%, v/v). The flasks were incubated for 48 h at 28 °C. The resulting cultures were recirculated through the biofilters for 2 h.

2.2. Biofilters

Three identical glass biofilters were designed and packed with granular perlite (Perlita de la Laguna, Mexico) previously sterilized. Particles of perlite were of ~3.3 mm in diameter, dry weight density of 134 g L^{-1}, and an initial void fraction of 0.43. Each reactor was comprised of three sections with gas and biomass sampling ports. The internal diameter and efficient height in all sections were 8.9 cm and 18 cm, respectively. Therefore, the total volume for each reactor was approximately, 3.3 L. The downflow arrangement of the systems is presented in Fig. 1. All the biofilters were operated under room temperature around 25 °C.

2.3. Experimental setup

In order to evaluate the effects of methanol ILR, nitrogen concentration, pH, and EBRT, the experimental operation was comprised of 6 different stages (Table 1). In Stages 1–3, biofilters were identically operated under three different inlet methanol concentrations. Furthermore, in Stages 4–6, biofilters were used to evaluate nitrogen concentration, pH, and EBRT effects.

The methanol loading rate and EBRT were adjusted by changing the airflow through the methanol reservoir as well as the total air entering the system. Nitrogen supply and pH regulation were accomplished by the addition of 500 mL of mineral solution (1.7 g/L YNB w/o amino acids, ammonium sulfate; 2.5–7.5 g/L $(NH_4)_2SO_4$; 100 mM, citrate–phosphate Buffer pH 3.5–6.5), which was sprinkled on the top of the column and recirculated at 100 mL/min during one hour every two days. Moreover, in Biofilters I and II, Stage 4 is divided into three sub-phases: 4-a, 4-b, and 4c, where sprinkling was every two, four and two days, respectively. In Biofilter III, there were only two sub-phases, 4-a and 4-b, were sprinkling took place every two and four days, respectively.

2.4. Performance evaluation

Methanol and CO_2 concentrations were tested every other day at inlet and outlet of biofilters. Thereafter, biofilter performance was evaluated through the elimination capacity (EC), and mineralization, according to the following equations:

$$EC = (C_{MetOH(in)} - C_{MetOH(out)}) \times \frac{Q}{V} \quad (1)$$

$$Mineralization = \frac{(C_{CO_2(out)} - C_{CO_2(in)})}{(C_{MetOH(in)} - C_{MetOH(out)}) \times Y_{CO_2}/MetOH} \times 100\% \quad (2)$$

Where $C_{MetOH(in)}$ (g m^{-3}) and $C_{MetOH(out)}$ (g m^{-3}) are gas methanol concentrations at inlet and outlet, respectively. Q is airflow (m^3 h) and V (m^3) is the biofilter volume. Similarly, $C_{CO_2(in)}$ (g m^{-3}) and $C_{CO_2(out)}$ (g m^{-3}) are CO_2 concentrations at inlet and outlet of control volume, respectively. Finally, $Y_{CO_2/MetOH}$ (gCO_2/gMetOH) is the stoichiometric mass ratio and equals 1.375 gCO_2/gMetOH. Due to the relatively short time that water was sprinkled into the systems (1 h every 48 h equal to 2% of time), loss of methanol in the leachate was not considered in Eqs. (1) and (2).

The mineralization percentage was calculated as the ratio between methanol biodegraded to CO_2 and total methanol removed from inflow. The biochemical mineralization reaction for methanol was taken as follows:

$$CH_3OH + \frac{3}{2}O_2 \xrightarrow{Microorganisms} CO_2 + 2H_2O \quad (3)$$

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