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Enzymatic degradation of biopolyesters

Introduction

This project aims to develop a method for testing the enzymatic degradation of biopolyester films based on quartz crystal microbalance (QCM) measurements. QCM is a balance based on the inverse piezoelectric effect. The Curies discovered this effect at the end of the 19th century. The alternating voltage application on crystalline materials with symmetry properties leads to a cyclic deformation and therefore causes an oscillatory motion

The project report should address and provide a detailed description of the developed method's theoretical basis and practical standard procedure. In addition, an independent method should be used to support method evaluation. Finally, bacterial cultivation of *Pseudomonas lemoignei* should be carried out to produce PHA depolymerases used to characterize the enzymatic degradation of a biopolyester with the prior developed method. The polyester-degrading bacteria *P. lemoignei* has at least five different *phaZ* structural genes which encode for five extracellularly localized depolymerases, and its catabolic abilities are restricted to the utilization of certain scl-PHA such as PHB, PHV, or poly(hydroxybutyrate-co-hydroxyvalerate) (PHB-co-PHV) and few organic acids, which are 3-hydroxybutyric acid, valeric acid, acetic acid, butyric acid, succinic acid and pyruvic acid

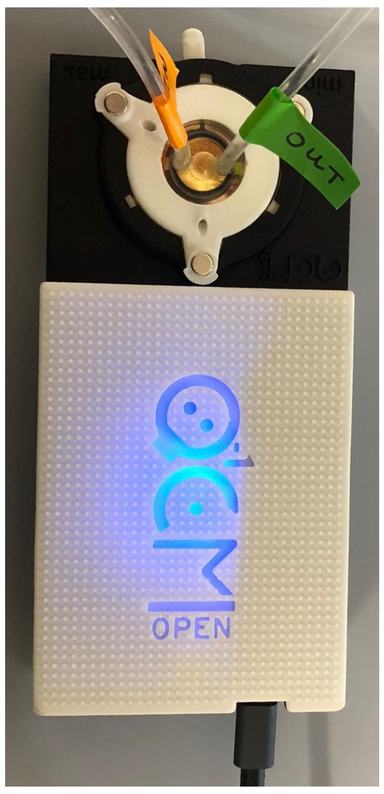

***Figure 1** : Quartz crystal microbalance from openQCM*

Results and Discussion

The cultivation was done in a bioreactor and a pH shift was applied to separate the bacterial growth and the secretion of extracellular depolymerases. The pH shift has increased the enzyme activity concentration by 18% in comparison of a cultivation without pH variation and a sufficient volume of the enzyme solution was produced allowing a concentration step by ultrafiltration. The concentration process has increased more than twice the enzyme activity with a final value of 23.76 U L^{-1} and a protein concentration of 2.50 mg mL^{-1}. The concentrated enzyme solution was used to test the enzymatic degradation of thin poly(3-hydroxybutyrate) (PHB) films under an enzyme solution flow at different concentrations according to the QCM measurements. An interactive MATLAB script was created to process the raw data and extract degradation characteristics as the mean degradation rate (V_{mean}) or the maximum degradation rate (V_{max}). Four different kinetics model from the literature were applied to characterize the degradation process. The Scandola-Figueroa heterogenous kinetic model shows the best goodness of the fit with an R-squared of 0.96 and a RMSE of 0.40 ng cm^{-2} s^{-1}. The model was used to fit V_{mean} values over the protein concentration. Finally, the comparison between the method developed and a spectrophotometric enzyme assay with para-nitrophenyl butyrate (pNPB) reveals that the two methods have different enzymatic kinetics due to the nature of

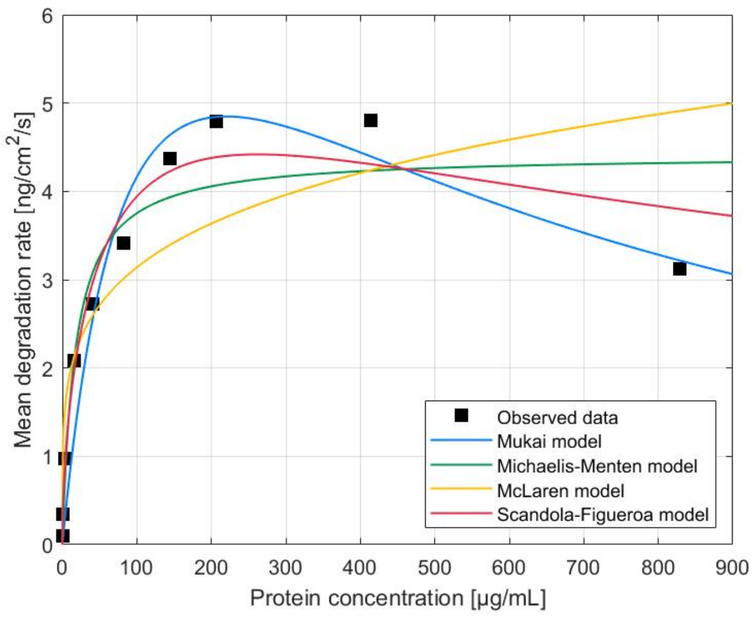

***Figure 2:** Different kinetic models applied to the observed degradation rate by non-linear regression using Levenberg-Marquardt algorithm*

Conclusion

In conclusion, this work has shown some interesting results. The method could be used for further studies on enzymatic degradation with different polymers and enzymes. Furthermore, it could also be used to deepen mechanistic aspects of the enzyme adsorption on the biopolyester surface.