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Enhancement of Enzymatic Process by Electric Potential Application

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Abstract

The purpose of this study is to investigate a new bio-electrochemical technique based on the utilisation of electric potential to enhance the enzymatic reaction. The efficiency of bio-electrochemical reactor has been achieved by studying the production of reducing sugar by enzymatic hydrolysis of olive mill. The results indicate that the application of a continuous electric potential of about 50 mV allowed a significant increase of the saccharification efficiency by about 25% (compared to an enzymatic process without electric potential). For an electric potential higher than 60 mV, the saccharification efficiency decreased, suggesting that the enzyme, a biological substance, could be damaged at high electric potential. It has been shown that the kinetics of the bio-catalyzed reactions could be controlled by an applied electric potential.

Keywords: Biotechnology; Bioprocess; Biochemical engineering; Electrochemistry; Bio-electrochemical reactor; Enzymatic reactor efficiency

Introduction

The use of an electric field or an electric potential to improve the performance of the processes has been applied through several techniques such as ultrafiltration and electrosorption [1-3]. The electric field has also been used to increase the output capacity of the olive oil and fruits juice extraction [4]. The use of an electric field has not been studied yet in an enzymatic process. Recently, a new analysis technique using a field-effect bio-detector based on the application of a gating voltage to immobilized enzymes on the working electrode of the detector was presented [5,6]. Also, it was reported that the enzyme biospecificity was preserved in the presence of the applied field.

The main purpose of this work was the determination of the ability of the electric potential to increase the enzymatic process efficiency. The effect of the electric field on the enzymatic reactor efficiency was studied using the Trichoderma reesei enzyme and olive mill substrate as a biocatalytic system. This work was aimed to be achieved by adopting optimal conditions obtained from a previously batch mode experiment [7], i.e. pH 5, T=50°C and Enzyme to Substrate ratio of 0.1 g enzymes/g olive mill.

Material and Methods

The batch bio-electrochemical reactor equipped with electrodes is presented in Figure 1. The electric potential has been applied by integrating two electrodes within the enzymatic batch reactor by means of a generator (TACUSSEL-France). The vessel volume of the reactor is of about 0.5 dm3. The enzyme (Trichoderma reesei) and the solid substrate (olive mill) have been submitted to continuous electric potential.

The commercial enzyme Trichoderma reesei (Sigma, France) has been used during the experiments. Its activity is 11.2 units mg-1 (one unit will liberate 1 µmole of glucose from cellulose in 1 hour at pH 5, T=37°C, for an incubation time of 2 hours).

Enzyme solutions have been prepared with an acetic acid/sodium acetate buffer solution (0.05 mol/L, pH 5).

The substrate is solid olive mill residue (SOMR), collected from an olive oil plant (Tadmait Kabylia region: Algeria) and transported to the laboratory at T=4°C.

Although the olive mill residue was already chopped in the oil manufacturing process, it was submitted to a fine size reduction step (granular size of 460 µm) and finally dried at T=70°C. For all experiments, the olive mill residue was finally pre-treated with NaOH to remove the most of the lignin content, as reported in a previous investigation [7].

The main characteristics of this lignocellulosic material are given in Table 1. Abdi et al. [7] detailed the analytical methods [7]. It is important to note that the chemical composition of the crude SOMR indicates that carbohydrates (cellulose and hemicellulose) are the main components confirming that this material would be an interesting substrate.

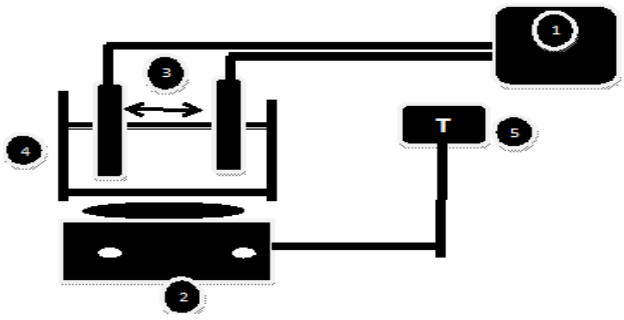

Figure 1: Batch bio-electrochemical reactor.

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Parameter	Crude SOMR (%)	Treated SOMR (%)
Dried matter	88.23	85.12
Organic matter	92.11	96.11
Fat matter	4.02	1.88
Total ashes	4.55	10.45
Total nitrogen matter	7.33	6.26
Crude cellulose	42.54	40.30
Hemicellulose	19.43	7.23
Lignin	21.53	0.45
Potassium	0.11	0.19
Sodium	0.20	10.16
Calcium	0.39	--

[a]Concentrations expressed in percentage of dry matter

Table 1: Chemical composition of the solid olive mill residue (SOMR) before and after pre-treatment with NaOH[a].

Results and Interpretation

Feasibility of the bio-electrochemical reactor

The feasibility of this process has been examined first, by operating a fixed electric potential of 100 mV. To evaluate the electric potential influence on the enzymatic reactor performance, the reducing sugars concentrations were determined: Pre-treated SOMR without electric potential, pre-treated SOMR with E=100 mV and a no pre-treated SOMR with E=100 mV respectively.

The results are presented in Figures 2a and 2b. It was observed that the SOMR must be pre-treated by NaOH to be efficiently hydrolysed by the T. reesei's enzyme. Indeed, after the first hour of hydrolyse, it was noticed that the pre-treated SOMR (with or without application of electric field) revealed better results than the untreated SOMR. After 24 hours the saccharification yield reached about 35% for untreated SOMR, while 70 and 80% were respectively obtained for pre-treated SOMR without and with electric potential application. With a fixed electric potential of 100 mV a slight increase of reducing sugar (compared to an enzymatic process without electric potential of the production) by enzymatic hydrolysis of olive mill, was obtained. Indeed, during the first five hours, the electric potential did not provide significant benefit for the saccharification of SOMR, suggesting that a polarization of the latter onto the electrode must be obtained before an improvement of the enzyme efficiency.

Influence of the electric potential on the efficiency of the bio-electrochemical reactor

The influence of the electric field on the enzymatic saccharification of the olive mill was realised through batch process, under optimal conditions obtained from an earlier study [7], with applications of electric potential varying from 10 to 200 mV

The reducing sugars concentration, produced according to the time, is presented in Figures 3a and 3b. The results obtained for 0.04 V and 0.05 V are practically in the same order and close to the best saccharification efficiency value. These results indicate that the enzyme was most improved at electric potentials ranging from 0.04 to 0.05 V.

The lower results obtained by the enzymatic process for potentials higher than 60 mV could be explained by the fact that the enzyme might be deposited under strong electric field on the electrodes and then destroying the enzyme activity. Similar results were previously reported [8] for phenol degrading bacteria in the presence of high electric current. A bioelectrokinetic test suggested that bacterial inactivation might occur by interaction with the surfaces of the electrodes, resulting in cell wall or membrane degradation through oxidation or reduction. The cell viability may be also affected, at high applied potential or induced field, by irreversible permeabilization of the cell membrane which results in direct oxidation or reduction of the cellular constituents [8,9].

In contrast, a lower electric potential (less than 60 mV) preserved the enzyme's activity and its biospecificity. Furthermore, the results obtained indicate that the application of an optimal electric potential close to 50 mV allows the increase of the saccharification efficiency by a significant percentage of about 25% (as compared to results obtained without application of an electric field [7]). The maximal efficiency values obtained for each electric potential after 24 hours of hydrolyses are plotted in Figure 4. The difference between enzymatic process without and with an application of electric potential of about 50 mV was relatively very high and then allowed to deduce the efficiency of the electric potential to improve the enzymatic reactor performances.

These results may be explained by the fact that during the catalytic cycle, enzyme alternates between two conformational states, E1 and E2. The use of an electric field allows driving oscillation or fluctuation of enzyme conformation between the E1 and the E2 states. Tsong TY [10] reported that the electric field-induced conformational oscillation or fluctuation leads to uphill pumping of the cation, K+ into and Na+ out of a cell, by the enzyme without consumption of ATP. Biochemical specificity of the catalysis was also preserved in their study and data indicated that Na, K-ATPase can harvest energy from the applied electric field to perform chemical work.

In the present investigation, it was demonstrated that the kinetics of the bio-catalyzed reactions can be controlled by an applied potential.

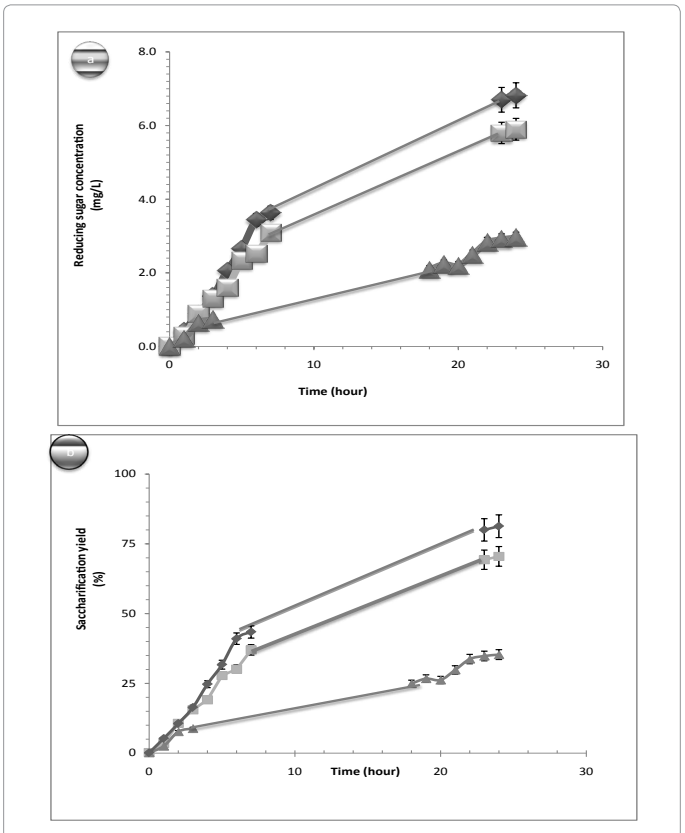

Figure 2: Graph for (a) reducing sugar concentration vs. time. (b) Saccharification yield vs. time.

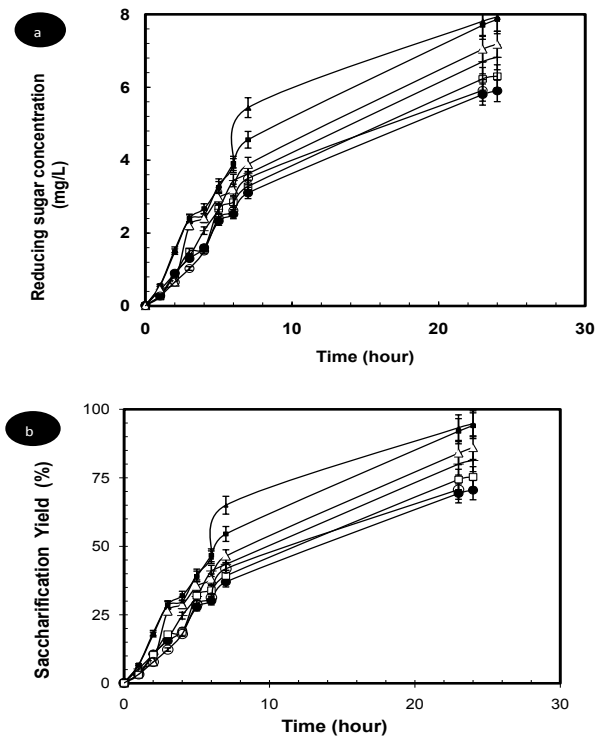

Figure 3: Graph for (a) reducing sugar concentration vs. time. (b) Saccharification yield vs. time.

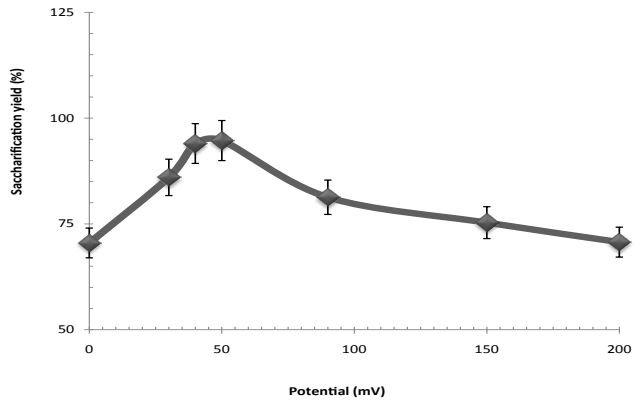

Figure 4: Graph for saccharification yield vs. potential.

An optimal value was determined indicating that oscillation or fluctuation of enzyme conformation between the E1 and the E2 states need a precise energy necessity given by applied potential close to 50 mV. These interesting results obtained after enzymatic experiments confirm the notion of the specific range of the field strength (a so called window) which may be utilized for bacterial manipulation [11].

Conclusion

The ability of the electric potential to increase enzymatic reactor performance was demonstrated in the current work. The kinetics of the bio-catalyzed reactions could be controlled by an applied potential which was an accomplishment in this study. Furthermore, the enzyme's biospecificity was preserved in the presence of electric potential less than 60 mV.

In addition, the effect of the electric potential on the enzymatic activity was highlighted. An improvement of about 25% the olive mill saccharification performance was achieved under an electric potential close to 50 mV which confirms the notion of the specific range of the field strength. The relationship between the increase of the enzyme performance and the changes of the enzyme cell surface and shape, determined by scanning electron microscope analysis, could be stated to understand the bio-catalyzed reactions under electric potential carrying out additional studies.

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