

ORIGINAL ARTICLE

Decolourization of paprika dye effluent with hydrogen peroxide produced by glucose oxidase

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*Department of Textile Engineering, University of Minho, Guimarães, Portugal***Abstract**

Hydrogen peroxide was produced from bran by a two-step process using cellulase/xylanase and glucose oxidase, sequentially. The decolourization efficiency of the produced reagent was tested using paprika oil dye (effluent from industrial source) and high levels of colour removal (96%) were achieved after saponification pre-treatment and hydrogen peroxide application. The method is economically and environmentally advantageous since lower energy and chemical input are needed and wastewater pollution is considerably reduced. At the same time, the utilization of waste materials was successfully achieved.

Keywords: *effluent, paprika oil, dye, glucose oxidase, hydrogen peroxide, decolourization.*

Introduction

Biotechnological systems for exploiting renewable raw materials have been developed for the textile industry in order to discover efficient, environmentally friendly processes that require less energy and water (Gübitz & Cavaco-Paulo 2001; Nierstrasz & Cavaco-Paulo 2010). Several enzymes have been applied in textile treatments, such as amylases for starch-size removal, cellulases and hemicellulases (xylanase) for changing the polymerization degree of desized cotton fabrics and laccases to remove colour from dyeing effluents (Campos et al. 2001; Csiszár et al. 2001; Kumar 2007). Glucose oxidase (β -D-glucose:oxygen 1-oxidoreductase, EC 1.1.3.4) is a flavoenzyme that catalyses the oxidation of β -D-glucose to gluconic acid using molecular oxygen as an electron acceptor with simultaneous production of hydrogen peroxide (Wu et al. 2005; Bankar et al. 2009). The resulting hydrogen peroxide is an environmentally acceptable oxidizing agent that can be used, for example, in textile industry to promote the whiteness of fabrics (López & Cavaco-Paulo 2008; Tzanov et al. 2002) and treat strongly coloured wastewaters. The latter are conventionally treated by application of membranes, physico-chemical and

advanced oxidizing processes (Hung et al. 2005). However, chemo-enzymatic treatments are being studied as an alternative, environmentally friendly approach (Karam & Nicell 1997).

In this work glucose, enzymatically generated from a cellulosic material (bran), was used as the substrate for hydrogen peroxide production by glucose oxidase (GOD). The efficiency of the produced oxidising agent was tested on the decolourization of an industrial effluent, mostly composed by paprika dye, which is frequently used in food, textile, cosmetic and pharmaceutical industries as a natural red dye (Deli et al. 2001).

Materials and methods*Materials*

The bran used for glucose extraction was obtained from an industrial source as well as the dye effluent (paprika oil) used for decolourization. This dye was composed mainly of carotenoids (capsanthin and capsorubin), responsible for the red colour; glycosides (capsianoside II) and glycolipids (monogalactosyldiacylglycerol). The glucose-oxidase GC199

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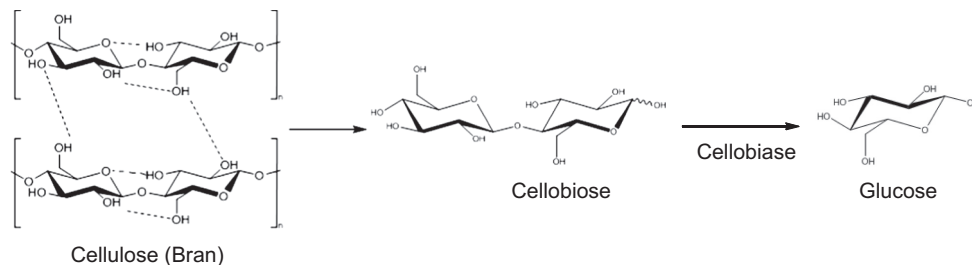


Figure 1. Schematic representation of cellulose hydrolysis and glucose production with Cellulase Multifect CX GC: endoglucanase (1); exoglucanase (2) and cellobiase (Gilbert & Hazlewood 1993).

from *Aspergillus niger* (1.500 U ml^{-1} , pH 5.1–5.4, 40–60°C, with very low catalase activity), cellulase Multifect® CX GC from *Trichoderma reesei* ($3200 \text{ U g}_{\text{protein}}^{-1}$, pH 2.7–5.7, 35–70°C) and xylanase Optimase® CX 255L from *Trichoderma reesei* ($56\,000 \text{ ABX g}_{\text{protein}}^{-1}$, where ABX means Acid Birchwood, pH 4–8, 60–80°C). All other reagents were used as supplied without further purification.

Methods

Glucose extraction from bran. For glucose extraction from bran, two approaches were followed: enzymatic and acid hydrolysis. For acid hydrolysis, 27 g l^{-1} of bran was incubated with different concentrations of HCl (0.5–5 M) for 24 h at room temperature with stirring. For enzymatic hydrolysis, 27 g l^{-1} of bran was incubated in 0.01 M acetate buffer at pH 5, with a mixture of 0.05% (v/v) cellulase and xylanase for 1 h at 25°C using 100 rpm agitation.

Hydrogen peroxide production. In order to obtain the best conditions for hydrogen peroxide production, several parameters were studied, namely concentration of glucose (2, 4 and 6 g l^{-1}), ionic strength of acetate buffer (0.01–0.5 M), pH (4–8), temperature (20, 35°C), incubation time (30–180 min) and enzyme concentration (0.04, 0.1 and 0.2 U ml^{-1}). The conditions tested were chosen according to the preliminary and published results.

Subsequently, the optimum conditions were followed using 2 g l^{-1} glucose in 0.01 M acetate buffer

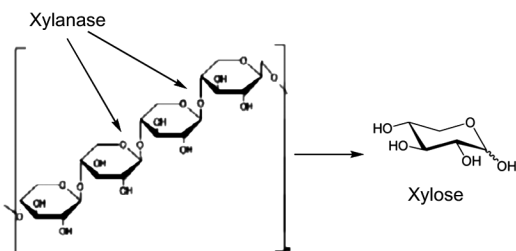


Figure 2. Schematic representation of xylan (β -1,4-D-xilopiranosil) hydrolysis by xylanase (Optimase CX 255L) and one possible constituent sugar (Gilbert & Hazlewood 1993).

pH 5, with GOD (0.1 U ml^{-1}) for 2 h at room temperature with 100 rpm agitation.

Dye effluent decolourization. The decolourization of the dye effluent was assessed by following two steps: preliminary chemical decolourization using 50% (w/v) NaOH for 15 min at room temperature with stirring, followed by enzymatic decolourization using 0.5 g l^{-1} hydrogen peroxide (60:40 – hydrogen peroxide:dye, by vol) produced by glucose oxidase action for 24 h at 90°C, pH 11 using 100 rpm agitation.

Determination of glucose concentration. The concentration of glucose extracted from bran was determined by using the neocuproine method (Cavaco-Paulo & Almeida 1996), which is based on the alkaline reduction of the complex neocuproine copper II (yellow solution) into neocuproine copper I (orange solution). The colour change after boiling the sample was monitored at 475 nm using a Helios Gamma UV-Vis spectrophotometer (Thermo Scientific, Waltham, MA, USA). The calibration curve was plotted using standard glucose samples ($1\text{--}6 \text{ g l}^{-1}$).

Determination of hydrogen peroxide. The concentration of hydrogen peroxide produced by enzymatic action was determined by redox titration using

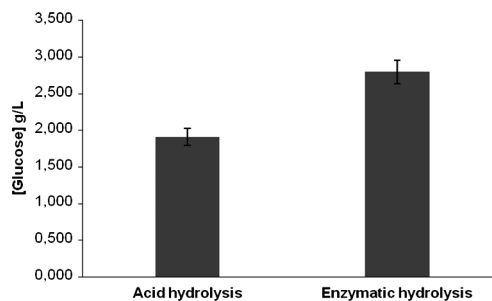


Figure 3. Generation of glucose from bran (27 g l^{-1}) using acid (HCl 5 M) during 24 h of incubation at room temperature with stirring agitation and enzymatic hydrolysis (cellulase/xylanase 0.05% (v/v) during 2 h of incubation at 25°C in 0.01 M sodium acetate buffer pH 5 at 100 rpm).

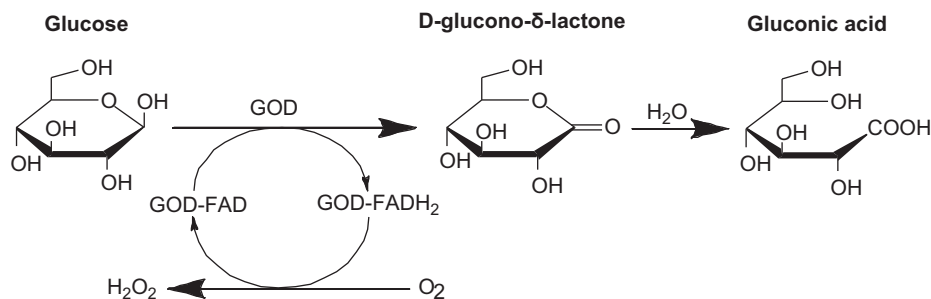


Figure 4. Schematic representation of hydrogen peroxide production with glucose oxidase (Simpson et al. 2007).

potassium permanganate (0.001 M) as titrating solution (Tzanov et al. 2002).

Decolourization quantification. The percentage of dye effluent decolourization was determined using a Helios Gamma UV-Vis spectrophotometer (Thermo Scientific, Waltham, MA, USA) at 450 nm and calculated as follows:

$$\% \text{ decolorization} = \left[\frac{(Abs_{initial} - Abs_{final})}{Abs_{initial}} \right] \times 100$$

Results

Glucose extraction from bran

Cellulases and xylanases encompass a collection of enzymes whose primary function is to hydrolyse β -1,4-glycosidic linkages in the structural polysaccharides, cellulose and hemicellulose (where the major component is xylan), converting them into their constituent sugars (Figures 1 and 2) (Gilbert & Hazlewood 1993). The results demonstrated that the cellulase/xylanase mixture was more efficient than acid hydrolysis. After acid hydrolysis of bran (27 g l⁻¹) with 5 M HCl for 24 h at 20°C, the highest glucose concentration achieved was approximately 2 g l⁻¹, representing 7% of raw product conversion into glucose. The best conditions for enzymatic hydrolysis (0.05% v/v enzyme mixture of

cellulase and xylanase at 25°C for 2 h) produced approximately 3 g l⁻¹ of glucose, corresponding to 11% of bran conversion (Figure 3). Dilute acids can lead to a limited hydrolysis called pre-hydrolysis. This consists in the hydrolysis of the accessible hemicellulosic fraction, leaving the cellulose and lignin fractions almost unaltered (Herrera et al. 2004). The temperature chosen for acid hydrolysis was much lower (20°C) than reported in the literature (100°C), since low energy consumption was required. The enzymatic conversion might have been limited by inhibition of the cellulase by the end-product glucose, hindering further degradation of the bran.

Hydrogen peroxide production by GOD

The GOD reaction involves a reductive and an oxidative step. In the first step, it catalyses the oxidation of β -D-glucose to D-glucono- δ -lactone (which is non-enzymatically hydrolysed to gluconic acid) reducing the flavin adenine dinucleotide (FAD) ring of GOD to FADH₂. In the second step, reduced GOD is reoxidised with oxygen to yield hydrogen peroxide (Figure 4) (Bankar et al. 2009). The production of hydrogen peroxide by GOD was optimized for pH, enzyme concentration, temperature, incubation time, glucose concentration and buffer ionic strength. The highest level of hydrogen peroxide (0.5 g/L) corresponded to 25% of glucose conversion. This limited conversion may be due to

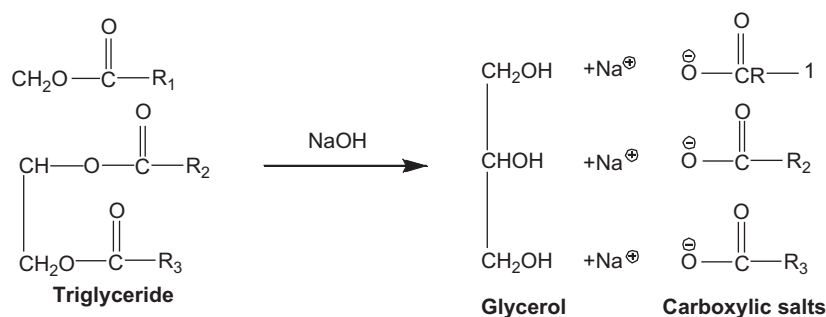


Figure 5. Schematic representation of saponification of paprika oil dye by sodium hydroxide (sludge formation) (Farinha 2009).

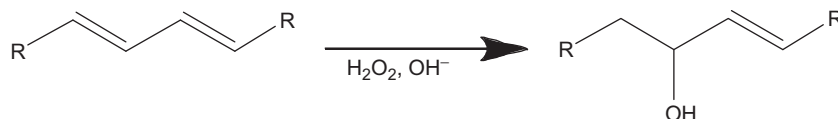


Figure 6. Schematic representation of paprika oil dye decolourization by hydrogen peroxide action (Carey 2003).

the presence of residual compounds (polysaccharides, proteins, fats and mineral salts) arising from glucose release, which could restrict hydrogen peroxide production. As the optimum temperature of glucose oxidase is 50°C, it was expected that a higher level of conversion would be obtained at 35°C than at 20°C. However, the results showed higher hydrogen peroxide production at the lower temperature, which might also reflect the presence of inhibitors.

Decolourization of paprika oil

The enzymatically produced hydrogen peroxide was tested on the decolourization of paprika oil. Since this oily dye has a significant lipid fraction that could hinder colour removal, it was saponified as a pre-treatment to improve decolourization. Paprika oil without any pre-treatment was used as a control.

The colour of paprika oil without any pre-treatment, diluted 80-fold in 0.1 M acetate buffer, was reduced 55% after hydrogen peroxide addition (60% v/v, at 90°C, pH 11 for 24 h, with an agitation of 100 rpm). After saponification using 50% w/v NaOH (dye:NaOH, 1:5, by vol.) for 15 min at 100 rpm, 40% of the initial dye ($Abs_{450\text{ nm}} = 3.159$) was converted into sludge (Figures 5 and 7A) that was removed by filtration; hydrogen peroxide was then applied to the remaining orange solution ($Abs_{450\text{ nm}} = 2.451$) (Figure 7B). Even though the

composition of the sludge needs to be examined, it may have application as an antimicrobial product, due to the high level of carotenoids present, or as soap for specific applications. The results show that after saponification, 96% of the colour was removed by hydrogen peroxide, reaching an almost uncoloured solution ($Abs_{450\text{ nm}} = 0.091$) (Figure 7C).

As paprika oil has a high content of carotenoids with conjugated double bonds, a decrease in colour intensity could be obtained by disruption of these bonds with the formation of unpaired linkages (Carey 2003). Based on this assumption, the hydrogen peroxide was applied at 90°C to generate hydroxyl^{25CF} radicals, which can oxidize and disrupt the double bonds, eliminating the natural colour (Figure 6). However, the resulting decolourization products, like aromatic amines, should be taken into consideration, since their presence at wastewaters will increase toxicity. Polymerization of these aromatic amines would be possible using laccases as specific catalysts (Zille et al. 2005). However, after analysis of the costs of reagents (enzymes, NaOH and others), energy input, time and water consumption, lower costs were found for the process where saponification pre-treatment was included. Based on the current costs for a food company to treat paprika waste, the process described here, incorporating enzymatic production of hydrogen peroxide and saponification as a pre-treatment, is 5-times lower (data not shown). Thus, despite the significant cost of the enzyme, the process is more economic than current industrial practice, involving physico-chemical methods.

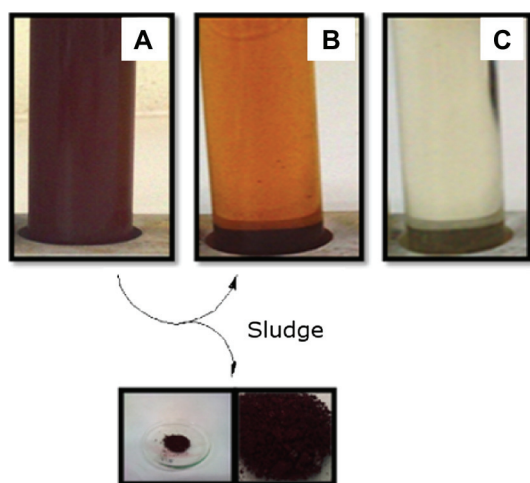


Figure 7. Different stages of paprika oil dye decolourization; (A) Initial dye colour; (B) dye colour after sludge removal; (C) dye colour after decolourization with hydrogen peroxide; the sludge after saponification between stages A and B is also shown.

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