Biochemical Engineering Journal 93 (2015) 115–118

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Short communication

Stabilization of enzymes in micro-emulsions for ultrasound processes

Madalena Martins, Nuno Azoia, Carla Silva [1], Artur Cavaco-Paulo *

Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

ARTICLE INFO

Article history:
Received 28 July 2014
Accepted 21 September 2014
Available online 5 October 2014

Keywords:
Micro-emulsions
Enzymes
Homogenization
Stabilization
Ultrasound
Bleaching

ABSTRACT

Oil-in-water proteinaceous micro-emulsions are described as novel methodology for the stabilization of enzymes. Proteins are tightly packed at the oil–water interface of micro-emulsions and it was found that micro-emulsions of laccases enzymes have enhanced stability under high temperatures and ultrasound fields (*see graphical abstract scheme and data*). This stabilization technique seems to be a promising methodology to apply on other enzyme-based processes where the operational conditions required high levels of mass transport.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Laccase is a polyphenol oxidase, which belongs to the family of multicopper oxidases. It catalyzes the oxidation of a range of inorganic and aromatic compounds with the concomitant reduction of molecular oxygen to water [1–3]. The combination of ultrasonic energy with enzymatic treatments has becoming a promising approach to improve enzyme efficiency, accelerating mass transfer during some textile processing steps such as desizing, scouring, bleaching and dyeing, preserving however the integrity of the fabrics [4–6]. In literature, studies put forward a dependence of the enzymatic enhancement with the intensity or long sonication time [7–9]. Enzymatic activity of laccases is strongly affected upon ultrasound treatment, the formation of aggregates leads to the inactivation of the enzyme caused by the radicals resulting from the cavitation phenomenon [4]. It has been reported that laccase in the presence of ultrasounds could be stabilized using specific stabilizers such as polyvinyl alcohol (PVA) or polyethylene glycol (PEG) [10]. However, they can hinder the oxidation of the textile substrate and in the presence of ultrasound, might contribute to non-enzymatic bleaching effects.

The stabilization of proteins has been an important driving force for development of protein formulations [11]. Often the increased stability, selectivity and activity of the proteins are obtained by combining techniques from immobilization to formulation as well as emulsifying properties [12,13]. The structural organization of proteins adsorbed at fluid interfaces has been recognized as main influence to control the stability of emulsion-based products [13].

The main focus of this work is to find new stabilization methods to improve the stability of laccase enzymes under ultrasonic fields. Previously we found that oil micro-droplets (50–500 nm) can be stabilized with proteins under high shear forces of ultrasound [14]. Globular proteins like Serum Albumin didn't show any change of the relative amounts of secondary structure and several layers of protein were present at the water oil interface [15]. At the interface of micro-emulsions, it can be assumed that external layers are made of fully folded active proteins. By a suitable selection of the system components and preparation method, thermodynamically and kinetically stable systems can be obtained [16,17]. The micro-emulsions were produced by high pressure homogenization method [16] and their physical and morphologic characterization was carried out. Proteins are tightly packed at the oil–water interface of micro-emulsions [17] and it can be expected a considerable stabilization in operational conditions that require high shear forces. An application example is given for ultrasound-assisted pre-treatment of cotton fabrics with laccase stabilized on these micro-emulsions.

* Corresponding author. Tel.: +351 253 604 400; fax: +351 253 604 429.
E-mail address: artur@deb.uminho.pt (A. Cavaco-Paulo).
[1] Present address: 3B's Research Group—Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, 4806-909 Taipas, Guimarães, Portugal.

http://dx.doi.org/10.1016/j.bej.2014.09.011
1369-703X/© 2014 Elsevier B.V. All rights reserved.

2. Materials and methods

2.1. Materials

Laccase (EC.1.10.3.2) from ascomycete *Myceliophthora thermophila* was provided by Novozymes, (Denmark). The 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and BSA were obtained from Sigma, Spain. The bleaching reagents and desized cotton were kindly supplied by ACATEL, Portugal.

2.2. Micro-emulsions preparation

The laccase micro-emulsions were prepared by contact of an organic (vegetable oil: Oleo Fula–food grade from Sovena, SA) and an aqueous phase (protein, bovine serum albumin, in acetate buffer pH5). Micro-emulsions in a ratio of 0.5 oil/99.5 H_2O were prepared using different concentrations of BSA (2.5; 5 and 10 g/L) and of laccase (1.5; 2.5; 5 and 10 g/L) to form an oil-in-water (O/W) emulsion. The two phases once mixed were homogenized (39 cycles—13 min) using a homogenizer (EmulsiFlex-C3—Avestin, Canada) under high pressure (1000 bar).

2.3. Physical and morphologic characterization of BSA/laccase micro-emulsions

The zeta-potential and size distribution of micro-emulsions were determined at 25 ± 0.1 °C using a Malvern zeta-sizer NS (Malvern Instruments) by electrophoretic laser Doppler anemometry and DLS, respectively. The morphologic characterization was evaluated using the transmission electron microscopy (TEM) technique.

2.4. Measurement of laccase activity

The laccase activity was determined using ABTS as substrate. The oxidation of the substrate was monitored spectrophotometrically by the increase in the absorbance at 420 nm for 2 min [18] at water bath at 50 °C. Enzymatic activity (U) was expressed as U = μmol of ABTS oxidized per min.

2.5. Thermal stability and pH profile of laccase formulated into micro-emulsion and for free form

Thermal stability was estimated from 30 min of incubation (40, 50 and 65 °C) as the residual activity. The residual laccase activity was determined under standard assay (see above Section 2.4) conditions and the final result was displayed by half-life time values. pH profile was measured in the range of 2.0–7.0 using acetate buffer 0.1 M using the fresh enzyme or the formulation. Half-life times of enzyme activity were estimated assuming a first order kinetic decay with data from 30 min incubation.

2.6. Enzyme stability during cotton bleaching under ultrasound

In order to evaluate the stability and efficiency of laccase micro-emulsions, desized fabrics were pre-treated in the ultrasound bath sonicator (VWR Ultrasonic Cleaner) with fixed frequency 45 kHz and power intensity at 120 W. One sample was incubated with 2 U/mL of free laccase and other with 2 U/mL of laccase micro-emulsion, in 0.1 M acetate buffer pH 5, at 50 °C during 30 min. The activity was determined as mentioned in Section 2.4. Cotton samples were further washed with distilled water at 80 °C and dry at room temperature. The pre-treated cotton fabrics were bleached afterwards following the recipe: 1 g/L anti-wrinkle, 0.5% wetting agent, 1.5 g/L sequestrant, 4 g/L NaOH, 8 g/L H_2O_2, 3 g/L equalizer, 1% optical brightener (o.w.f). The bleaching was carried out at 80 °C for 1 h, assisted by ultrasounds equipment (45 kHz; 120 W).

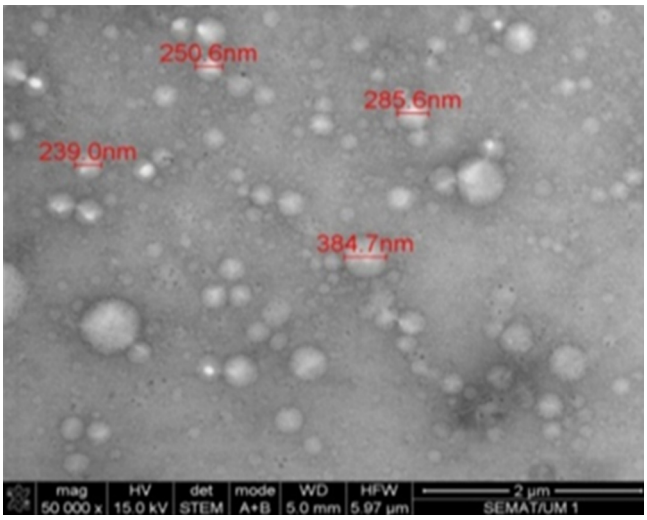

Fig. 1. Morphologic characterization of BSA/laccase (5/10 g/L) micro-emulsions using transmission electron microscopy.

2.7. Measurement of whiteness index

The whiteness index Berger (W^*) of the samples was determined by using a reflectance Data-Colour apparatus at standard illuminant daylight D65.

3. Results and discussion

Taking advantage of BSA emulsifying properties, micro-emulsions made of laccase and BSA were prepared, using different concentrations of both proteins, forming O/W (0.5/99.5) emulsions.

Supplementary Fig. S1 represents the physical appearance of native laccase and the correspondent micro-emulsion where proteins, BSA and laccase, are highly dispersed in the oil as micro-order dispersions. In this process, several factors in the process have been taken into account, namely pressure, temperature, number of passages and flow rate [19,20].

Angel et al. studied the assembly of BSA with other proteins on microspheres production [21]. They suggest that two proteins can form together the microsphere's walls from fluorescence data. TEM showed an average particle size of around 250 nm (Fig. 1), corresponding to the values obtained by DLS measurements (see Supplementary Fig. S2). It illustrates that the proteinaceous micro-emulsions are spherical with a regular surface. This morphology would allow a higher protein stabilization, since the protein it is packed sphere with contained movements and a minimal contact with water [22].

The influence of proteins concentration was analysed in terms of particle size and polydispersity index (PDI). The results presented in Fig. 2 show that the z-average diameter and PDI of micro-emulsions is dependent of the BSA and laccase concentration used for each sample. Thus, best results with lower z-average diameter and PDI were obtained for the formulation 5/10 of BSA/laccase g/L (250 nm; PDI: 0,132). BSA presents a high content of hydrophobic residues and it is know that only high concentrations yield a narrower size distribution and lower particle's size [15,17,19].

Since emulsions can suffer aggregation over time, the stability of produced micro-emulsions was measured in terms of particle size and zeta-potential. These results displayed that laccase micro-emulsions were maintained stable during 10 weeks presenting

Download English Version:

https://daneshyari.com/en/article/3083

Download Persian Version:

https://daneshyari.com/article/3083

Daneshyari.com