Carbohydrate Polymers 152 (2016) 710–717

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Enhanced starch hydrolysis using α-amylase immobilized on cellulose ultrafiltration affinity membrane

Viktoriia Konovalova[a], Kateryna Guzikevich[a], Anatoliy Burban[a], Wojciech Kujawski[b,*], Karolina Jarzynka[c], Joanna Kujawa[b]

[a] Department of Chemistry, National University of Kyiv-Mohyla Academy, 2 Skovoroda Street, 04070 Kiev, Ukraine
[b] Nicolaus Copernicus University in Toruń, Faculty of Chemistry, 7 Gagarina Street, 87-100 Toruń, Poland
[c] Nicolaus Copernicus University in Toruń, 11 Gagarina Street, 87-100 Toruń, Poland

ARTICLE INFO

Article history:
Received 21 March 2016
Received in revised form 10 July 2016
Accepted 17 July 2016
Available online 18 July 2016

Keywords:
Cellulose affinity membranes
α-Amylase immobilization
Membrane surface modification
Starch hydrolysis
Ultrafiltration
Concentration polarization

ABSTRACT

In order to prepare ultrafiltration membranes possessing biocatalytic properties, α-amylase has been immobilized on cellulose membranes. Enzyme immobilization was based on a covalent bonding between chitosan and a surface of cellulose membrane, followed by an attachment of Cibacron Blue F3G-A dye as affinity ligand. Various factors affecting the immobilization process, such as enzyme concentration, pH of modifying solution, zeta-potential of membrane surface, and stability of immobilized enzyme were studied. The applicability of immobilized α-amylase has been investigated in ultrafiltration processes. The immobilization of α-amylase on membrane surface allows to increase the value of mass transfer coefficient and to decrease the concentration polarization effect during ultrafiltration of starch solutions. The enzyme layer on the membrane surface prevents a rapid increase of starch concentration due to the amylase hydrolysis of starch in the boundary layer. The presented affinity immobilization technique allows also for the regeneration of membranes from inactivated enzyme.

1. Introduction

The enzymatic starch degradation leading to various low molecular mass products is commonly accomplished by the use of α-amylase. The starch hydrolysates are widely exploited in numerous industries (e.g. paper, textile, detergent, food, pharmaceutical, and brewing fermentation (Ju, Chen, & Lee, 1995; Li et al., 2013; Tawil, Viksø-Nielsen, Rolland-Sabaté, Colonna, & Buléon, 2012)). Usually, hydrolysis of liquid fraction of starch is performed using the free enzyme (α-amylase) in a discontinuous (batch) process (Gupta, Gigras, Mohapatra, Goswami, & Chauhan, 2003; Ju et al., 1995). However, the batch process is usually associated with the enzyme recovery stage. The development of the continuous process with α-amylase immobilized on membrane surfaces is an interesting alternative and can result in the reduction of the processing costs (Gangadharan, Madhavan Nampoothiri, Sivaramakrishnan, & Pandey, 2009; Homaei, Sariri, Vianello, & Stevanato, 2013; Jesionowski, Zdarta, & Krajewska, 2014; Shewale & Pandit, 2007).

The immobilization of α-amylase has been carried out on various particulate supports, polymers (Liao & Syu, 2005), nanomaterials (Ansari & Husain, 2012; Ernest, Shiny, Mukherjee, & Chandrasekaran, 2012; Tüzmen, Kalburcu, & Denizli, 2012), and ultrafiltration membranes (Bayramoğlu, Yilmaz, & Arica, 2004; Ju et al., 1995). The most interesting is enzyme immobilization on membranes in a continuous flow mode and/or at larger scale systems (Adamczak, Oberta, & Ceynowa, 2009; Ceynowa & Rauchfleisz, 2001; Jesionowski et al., 2014; Jochems, Satyawali, Diels, & Dejonghe, 2011; Koter & Ceynowa, 2003; Liu et al., 2006). The main advantage of the enzyme-immobilized membrane systems is the small amount of enzyme required and the accomplishment of the enzymatic reaction instantaneously with the products separation (Chen, Huang, & Xu, 2013; Gekas, 1986; Jesionowski et al., 2014; López, Mielgo, Moreira, Feijoo, & Lema, 2002; Rios, Belleville, Paolucci, & Sanchez, 2004). Moreover, porous membranes with immobilized enzyme demonstrate higher throughput, since the diffusional path of the substrate to the enzyme is minimized (Homaei et al., 2013).

* Corresponding author.
E-mail address: kujawski@chem.umk.pl (W. Kujawski).

http://dx.doi.org/10.1016/j.carbpol.2016.07.065
0144-8617/

The direct enzyme immobilization on the membrane surface or within the pores may be accomplished using various methods: covalent attachment, adsorption, electrostatic interactions, and entrapment (Homaei et al., 2013; Jesionowski et al., 2014; Jochems et al., 2011; Labus, Gancarz, & Bryjak, 2012; López et al., 2002). Covalent bonding between the enzyme functional groups and activated membrane functional groups is practically irreversible (Homaei et al., 2013; Koseoglu-Imer, Dizge, & Koyuncu, 2012). This fact significantly decreases operating lifetime of the biocatalytic membrane as the biocatalytic properties of the membrane with immobilized enzyme are lost as soon as enzyme is inactivated. However, an increase in the effective operating lifetime of the biocatalytic membrane can be achieved through the development of effective and simple methods of enzyme replacement on the membrane surface.

Dye ligand affinity chromatography has been extensively investigated since beginning of 1980s at laboratories as well as at larger scales (Kim et al., 2012; Li, Dong, & Zhuang, 2009; Subramanian & Ross, 1984; Tüzmen et al., 2012). Dye ligands are commercially available, generally inexpensive and highly selective adsorbents for enzyme separation. The dye ligands interact with biomolecules as a result of combination of electrostatic, hydrophobic, and hydrogen bonding (Subramanian & Ross, 1984; Tüzmen et al., 2012).

Cibacron Blue F3GA is an anthraquinone textile dye interacting specifically and reversibly with various biomolecules (Tüzmen et al., 2012). Cibacron Blue F3GA dye was used as an affinity ligand for α-amylase immobilization on cellulose membranes (Denizli & Pişkin, 2001). Cellulose membranes possess sufficient strength and high hydrophilicity, they also demonstrate good desalting ability, high flux, and have reactive hydroxyl groups on their surface (Li et al., 2015; Tanyolaç, Işık Yürüksoy, & Özdural, 1998). However, the enzyme molecules immobilized on the membrane surface are highly affected by surface sorption forces which damage their structure and catalytic properties. Therefore, to avoid the negative influence of the surface, a method using an additional spacer is often applied. As it was demonstrated earlier (Butterfield, Bhattacharyya, Daunert, & Bachas, 2001), the use of spacer leads to the increase in activity of immobilized enzymes. Furthermore, the presence of flexible spacer should enhance the activity retention of the immobilized enzymes by ensuring much greater freedom of movement and simultaneously minimizing negative steric hindrances posed by solid supports (Chen et al., 2011). To achieve that purpose, cellulose membranes are previously modified by chitosan. Chitosan is usually selected as a spacer molecule because of a wide variety of reagent molecular lengths and various reactive amino groups available in their structure. Presence of multiple amino groups also implies the enhancement of surface area for immobilization.

The continuously stirred-tank reactors (CSTR) coupled with ultrafiltration for starch hydrolysis using dissolved α-amylase are described elsewhere (Butterfield et al., 2001; Grzeskowiak-Przywecka & Slomińska, 2005). However, this type of membrane reactor has the significant limitation of very low productivity due to the serious concentration polarization of starch and enzyme occurring at the membrane surface (Ju et al., 1995). Utilization of intensive agitation (Grzeskowiak-Przywecka & Slomińska, 2005) and elevated temperatures (Lubiewski, Thanh, & Lewandowicz, 2011) did not result in significant enhancement.

The aim of this study was to explore the starch hydrolysis by ultrafiltration through polymeric membranes with immobilized α-amylase and determination of influence of immobilized enzyme on mass transfer coefficient of starch. A method of the enzyme immobilization, which is based on the affinity chromatography techniques of protein separation, is presented and discussed. It is expected that enzyme layer on a membrane surface should prevent rapid increase in starch concentration near the surface and should decrease concentration polarization effects.

2. Materials and methods

2.1. Materials

Cellulose ultrafiltration flat sheet membrane C030 F samples with molecular weight cut off equal to 30 kDa were supplied by Microdyn Nadir (Germany). According to the manufacturer specification, C030 F is a composite membrane with a thin selective layer of regenerated cellulose with asymmetric pores supported by highly porous non-woven polymeric material. Prior to use, membranes were washed three times using deionized water.

2.2. Modifying agents

Chitosan with a molecular weight of 400 kDa and 85% degree of deacetylation and Cibacron Blue F3G-A dye were purchased from Fluka (Germany). Polyethylene glycol (PEG), sodium periodate and sodium borohydride were provided by Sigma-Aldrich (USA). An α-amylase with an enzymatic activity of 1000 U/mL *Bacillus licheniformis* was supplied by Termamyl SC (Novozymes, Australia). All chemicals were of analytical grade and were used without further purification.

2.3. Chemical modification of cellulose membranes

The modification of the cellulose membrane samples was performed according to the procedure described in the detail elsewhere (Nigmatullin, Konovalova, & Pobigay, 2009) – Fig. 1. This necessary procedure consisted of the following stages:

- oxidative cellulose activation using periodate resulting in the formation of dialdehyde units. The membranes were oxidized in 0.1 M $NaIO_4$ at 50 °C for 1 h. Afterwards, membranes were rinsed in Millipore (Milli-Q Plus) ultrapure water.
- covalent chitosan binding through the formation of imine groups with the subsequent reduction to the carbon/nitrogen bonds

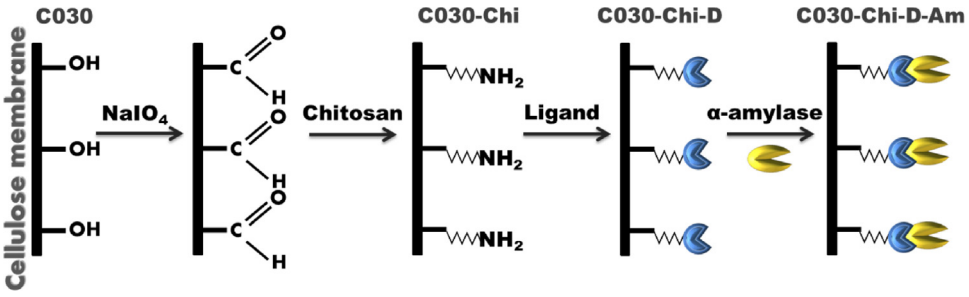

Fig. 1. Scheme of the modification procedure of cellulose C030 membrane.