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Ethanol production from whey in a bioreactor coupled with direct contact membrane distillation

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ABSTRACT

The ethanol production from whey in a bioreactor integrated with direct contact membrane distillation (MDBR) was investigated. Fermentation was studied using concentrated whey and deproteinized whey enriched with lactose or sucrose. Whey lactose was prehydrolyzed in an enzymatic process using β-galactosidase into a mixture of glucose and galactose. The fermentation process was performed using *Saccharomyces cerevisiae* yeasts species.

It was found that MDBR can be successfully applied for ethanol production from whey. A continuous removal of produced ethanol and other volatile compounds from a fermenting broth by membrane distillation resulted in a high efficiency of sugar conversion into ethanol. The efficiency of ethanol production in MDBR in the fermentation of deproteinized whey enriched with sucrose was close to the theoretical value and 1.9× higher than that in the process carried out in a bioreactor without MD. It was found that salt present in the concentrated whey decreased the process efficiency.

1. Introduction

Cheese whey represents a serious environmental problem due to high volumes produced and a high organic matter content (biological oxygen demand, BOD of 30–50 g dm^{-3} and chemical oxygen demand (COD) of 60–80 g dm^{-3}) [1]. Lactose present in whey is largely responsible for high organic load and very strong pollution potential [2]. However, whey lactose (4.5–5% w/v) can be used as a substrate for the production of valuable compounds by fermentation [1–3]. Alcoholic fermentation is an interesting alternative for the bioremediation of the polluting liquor that remains after the separation of whey proteins. Pollution reduction and lactose conversion to ethanol are achieved simultaneously and remain a common practice at present [3–5]. The number of microorganism able to metabolize lactose directly to ethanol is limited. Moreover, they are often inhibited by moderate concentrations of sugar and ethanol [1,5]. The well-known fermentative potential of *Saccharomyces cerevisiae* cannot be exploited directly to produce ethanol from whey, because that yeast lacks in assimilatory mechanisms for lactose [5]. Thus, a potential alternative is the previous enzymatic hydrolysis of lactose by β-galactosidase to a mixture of glucose and galactose and subsequent ethanol fermentation [4,5]. Direct fermentation of whey to ethanol is not economically viable due to low lactose content. The use of concentrated whey for ethanol fermentations yields high ethanol concentrations and reduces distillation costs for ethanol separation [5,6]. Whey ethanol can be used in food, chemical, pharmaceutical and cosmetics industries and as an alternative and environmental fuel [4–7]. Membrane technologies such as highly selective and energy-saving unit operations have a great potential in ethanol production [8]. Pervaporation (PV) is one of the most promising solutions for the recovery of alcohol from fermentation broths [8–11]. The basis of separation in PV is a difference in dissolution and diffusion of separated components across the dense, selective membranes. The studies on ethanol production from various materials such as glucose, lignocellulose were carried out. The concentration of recovered ethanol and the ethanol flux were dependent in a large degree on the ethanol concentration in the broth. PV can be successfully utilized for the dehydration of bioethanol. It has been estimated, that the application of PV in the bioethanol production can reduce the total costs of production by 20 €/ton [9–11].

The separation of ethanol from the fermentation broth may be successfully performed using membrane distillation (MD) [12,13]. MD is an evaporation process of volatile compounds of a hot feed through a porous hydrophobic membrane. In direct contact membrane distillation (DCMD) the feed is in a direct contact with the membrane and the permeate is directly condensed in the cooling stream flowing along the membrane surface. Non-volatile solutes

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contained in the feed are completely retained by the membrane. The driving force for mass transfer is the difference in vapor pressure between the feed and the permeate side of the membrane [12,14]. The ethanol production from lactose solutions in a bioreactor integrated with the direct contact membrane distillation (MDBR) was previously investigated [15,16]. It was found that the use of DCMD for removal of ethanol and other volatile metabolites from a broth during fermentation process resulted in an increase of ethanol productivity.

The major objective of this study was the fermentation of whey in MDBR. The fermentation of concentrated whey and deproteinized whey enriched with sucrose or lactose at different concentrations with the utilization of *S. cerevisiae* yeast was performed. The effect of broth composition on the fermentation efficiency and on a membrane performance was determined.

2. Experimental

2.1. Bioreactor system coupled with DCMD

Experiments were carried out using a bioreactor (BIOTRON LiFlus GX) equipped with a capillary membrane made from polypropylene (Membrana GmbH, Germany). Details of the system performance were presented elsewhere [15]. The membrane module consisted of four capillary membranes with an effective area of 0.0366 m^2.

The membranes had pore sizes with a nominal and maximum diameter of 0.2 μm and 0.6 μm, respectively, and the porosity of 70%. The shell side and lumen side diameter of the capillary membranes were 2.6 and 1.8 mm, respectively. The membranes (without housing) were directly immersed in a fermentation broth in the bioreactor tank. The bioreactor was equipped with a high speed agitator which stirred the broth. The membranes were located around the agitator in the way protecting them against mechanical damage. The circulation of distillate from the distillate tank through a cooling system and MD module (the lumen side of capillary membranes) was achieved using a peristaltic pump.

2.2. The operating conditions

The fermentation experiments were carried out at the broth temperature of 310 K. The bioreactor was equipped with a thermostat and a temperature sensor which measured broth temperature continuously. The inlet temperature of the cold distillate was kept at 293 K for all the experiments. The agitator speed was fixed at 400 rpm.

2.3. Material and methods

2.3.1. Pretreatment of whey

Raw acidic whey for experiments was obtained from a local dairy. The whey was characterized by the following parameters: protein concentration in the range of 11–12 g dm^{-3}, reducing sugars (lactose) 32.6–34.6 g dm^{-3}, pH 3.6–4.2. A preliminary treatment of whey consisted of deproteinization by thermal (92 °C) and chemical coagulation (NaOH addition to adjust the pH at 6.2). Precipitated proteins were separated from whey by centrifugation at 6850 × *g* for 10 min at 20 °C. The whey after deproteinization was subjected to the separation of the remained proteins from lactose by ultrafiltration (UF). Direct fermentation of whey to ethanol yields low ethanol concentration due to low lactose content in raw whey. Therefore, the pretreated whey, after UF separation (permeate) [17], was concentrated in the DCMD process. The concentrated whey contained 196 g dm^{-3} of lactose and about 20 g dm^{-3} of proteins. The raw, the deproteinized and the concentrated whey were kept in a freezer until their use.

2.3.2. Fermentation of whey

The influence of broth composition on the fermentation process was studied. The experiments were carried out using the concentrated whey at sugar concentrations amounting to 60 and 113 g dm^{-3}. Moreover, a broth containing deproteinized whey with lactose concentration of 34.6 g dm^{-3}, enriched additionally with lactose or sucrose to the concentration of sugar equal to 50 or 100 g dm^{-3} was applied.

Before fermentation, lactose present in whey was hydrolyzed with *Aspergillus oryzae* β-galactosidase enzyme to glucose and galactose. Previous studies have shown that the ratio of enzyme to lactose equal to 4 mg of enzyme per 1.0 g of lactose in whey was favorable. The hydrolysis process proceeded for 24 h at 283 K. Solutions of prehydrolyzed lactose were fermented in a bioreactor integrated with DCMD. Subsequently, lyophilized yeast after rehydratation was introduced into the solutions. The ratio of sugar to yeast was 15 g g^{-1}. A commercially available dry Gamma Hefe yeast (*S. cerevisiae*, AB Enzymes, Germany) was used. Yeast rehydration was performed for 30 min using small amounts of the whey lactose solution or its mixture with other sugars present in the broth. The mixture was periodically agitated.

The initial mass of fermentation broth used as the feed in DCMD was 2800 g. The cold system was initially filled with 600 g of distilled water. The fermenting broth was agitated in the bioreactor. The yeast consumed the mixture of glucose and galactose (formed from whey lactose) producing ethanol. Ethanol and water vapor diffused through the air filling the membranes' pores and then condensed directly in the cold distillate stream, whereas all nonvolatile components remained in the broth. The solution containing prehydrolyzed whey lactose (or its mixture with other sugars present in the broth) was dosed every 24 h to continue the production in the bioreactor. The amount of the added solution (about 600 g) was equal to the mass of permeate transferred through the membrane from the broth to the distillate within every 24 h of fermentation in the MDBR. As a result, a constant volume of the broth was maintained during the fermentation. The fermentation process combined with DCMD was carried out for 72 h with a continuous separation of ethanol by the capillary membranes. The broth was replaced with distilled water after each fermentation experiment and the MD membranes were rinsed for 1 h. The method of fermentation in MDBR was described elsewhere [15].

Fermentation experiments in a bioreactor without MD were carried out under the same conditions. The initial mass of the broth was equal to 260 g.

2.4. Measurements

In order to determine the concentration of ethanol and sugar in the broth, the samples (25 cm^3) were collected every 24 h. At the same time the ethanol concentration in the distillate and its mass were determined. In the MDBR the distillate constitutes a sum of water present initially in the MD system in a cool loop and a permeate. The permeate is a sum of vapor of water and ethanol transferred through the membrane pores and then condensed directly in a cold distillate.

These measurements permitted to calculate the parameters characteristic for the MDBR system such as permeate flux, ethanol flux, ethanol concentration in the permeate and efficiency of sugar conversion into ethanol.