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Filtration behavior of casein glycomacropeptide (CGMP) in an enzymatic membrane reactor: fouling control by membrane selection and threshold flux operation

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ABSTRACT

Sialylated human milk oligosaccharides (HMOs) can be produced by enzymatic trans-sialidation using casein glycomacropeptide (CGMP) as the substrate. By performing the reaction in an enzymatic membrane reactor (EMR), simultaneous separation of the HMOs from CGMP and enzyme reuse can be achieved. In this study, the filtration performance and fouling behavior during ultrafiltration (UF) of CGMP for the enzymatic production of 3′-sialyllactose were investigated. A 5 kDa regenerated cellulose membrane with high anti-fouling performance, could retain CGMP well, permeate 3′-sialyllactose, and was found to be the most suitable membrane for this application. Low pH increased CGMP retention but produced more fouling. Higher agitation and lower CGMP concentration induced larger permeate flux and higher CGMP retention. Adsorption fouling and pore blocking by CGMP in/on membranes could be controlled by selecting a highly hydrophilic membrane with appropriate pore size. Operating under threshold flux could minimize the concentration polarization and cake/gel/scaling layers, but might not avoid irreversible fouling caused by adsorption and pore blocking. The effects of membrane properties, pH, agitation and CGMP concentration on the threshold flux were studied based on the resistance-in-series model. Higher hydrophilicity of the membrane, elevated pH and agitation, and lower CGMP concentration were found to increase the threshold flux and decrease membrane fouling.

1. Introduction

Casein glycomacropeptide (CGMP) is released from κ-casein by chymosin during cheese making, and it constitutes 20–25% of total protein in cheese whey [1–3]. The CGMP whey peptide is relatively small, with an average molecular weight of 7500 Da, however due to glycosylation and aggregation its actual size can range from 23,000 to 28,000 Da [3,4]. CGMP contains varying amounts of sugars including *N*-acetylneuraminic acid (sialic acid), galactose, and *N*-acetylgalactosamine [2]. CGMP is recognized as a bioactive peptide and many of its biological and functional properties are attributed to its carbohydrate chains, especially those containing sialic acids [3]. Recently, Meyer and co-workers reported the biocatalytic production of 3′-sialyllactose and other sialylated human milk oligosaccharides (HMOs) using an engineered sialidase or trans-sialidase and CGMP as the substrate [5–7]. HMOs can stimulate the growth and activity of beneficial intestinal bacteria and are therefore beneficial to the immune systems of infants [8]. The work by Meyer and co-workers opens a gate for the potential valorization of CGMP and large-scale production of HMOs. In order to scale up the enzymatic production of HMOs, the efficient separation of HMOs from CGMP and enzyme reuse are required. An enzymatic membrane reactor (EMR) can satisfy these two requirements simultaneously [9]. Moreover, the HMO products can be continuously removed from the EMR during the reaction, not only reducing product inhibition but also decreasing product hydrolysis by sialidase [7]. In our previous study, it was found that ultrafiltration (UF) could retain CGMP and fully maintain enzyme activity for seven cycles, while the product (i.e. 3′-sialyllactose) freely passed through the membrane [7].

A major limitation in applying membrane technology to industrial fluids is the gradual decline in permeate flux due to concentration polarization (CP) and membrane fouling [10], especially for feeds containing proteins [11,12]. When using an EMR for the continuous

Abbreviations: CGMP, casein glycomacropeptide; EMR, enzymatic membrane reactor; HMOs, human milk oligosaccharides; MWCO, molecular weight cut-off; PS, polysulphone; PES, polyethersulphone; RC, regenerated cellulose; TMP, trans-membrane pressure; UF, ultrafiltration; VRR, volume reduction ratio; CP, concentration polarization; HPAEC-PAD, high-performance anion exchange chromatography with pulsed amperometric detection; HPLC, high performance liquid chromatography

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production of HMOs from CGMP, previous studies have showed that CGMP was rejected by the UF membrane and accumulated in the reactor, resulting in a severe CP layer and membrane fouling [7]. The CP/fouling layer caused a flux decline and largely decreased the production efficiency of the EMR. Furthermore, the dense and charged fouling layer of CGMP may act as an additional selective "film" and retain the target product, as both CGMP and 3′-sialyllactose are negatively charged at neutral pH [3]. In our preliminary pilot-plant work, it was found that the fouled UF membrane (polysulphone, molecular weight cut-off=5 kDa) had almost 100% retention of 3′-sialyllactose (molecular weight=0.63 kDa). Benhabieles et al., also reported that when the permeate flux declined due to fouling formation during the UF of fish protein hydrolysate, the solute rejection was significantly increased [13]. Therefore, in order to optimize the production of HMOs from CGMP by EMR, it is necessary to clarify the effect of the fouling behavior of CGMP during UF and to minimize the formation of a fouling layer, as well as to maximize the permeation of HMOs across the membrane.

Various fouling control strategies may contribute to preventing fouling, such as increasing shear rate on the membrane, modifying pH, or applying an external electric, magnetic or ultrasonic field. However, many fouling control strategies can also exert a negative effect on the enzymes by e.g. accelerating enzyme inactivation. The critical flux concept has been proposed as a mild and facile fouling control method that takes advantage of the transition of a filtration system between non-fouling and particle deposition states by tuning the flux of the system [14]. Field et al. defined the critical flux as the flux below which flux decline with time does not occur. Field et al. also classified the critical flux into 'weak form' and 'strong form', depending on whether adsorption fouling was observed in the system or not [15]. Bacchin et al. further clarified that below the weak form of the critical flux, adsorption fouling was independent of solvent transfer [16]. Because a zero fouling rate was rare in general, Field and Pearce then proposed another concept, threshold flux, which was defined as the flux that divided a low fouling region from a high fouling region [17]. That is, below threshold flux, the fouling evolution was independent of permeate flux, while above it, the membrane fouling increased with permeate flux. To some extent, the weak form of the critical flux can be considered as a special form of the threshold flux. For industrial fluids, the mechanisms of flux decline and membrane fouling are so complicated that a flux concept with a more flexible definition is preferred. The concept of threshold flux only concerns the correlation between resistances caused by fouling and permeate flux, and therefore disregards the type and degree of fouling. Generally, threshold flux represents the highest throughput with the lowest fouling, which is recommended for practical applications [18]. Therefore, more and more researchers have accepted this concept as a substitute for the critical flux, especially in wastewater treatment using membrane filtration [19–23]. To the best of our knowledge, the following work is the first attempt to apply the threshold flux concept to EMRs.

The present work was undertaken to evaluate the filtration performance and fouling behavior of CGMP during UF for the enzymatic production of 3′-sialyllactose by Tr6 mutant sialidase, with a focus on the effects of membrane properties, pH, agitation speed and solute concentration on the threshold flux. A resistance-in-series model was used to analyze the fouling formation at different process parameters. The EMR was equipped with different membranes and their performance was compared in terms of CGMP retention and 3′-sialyllactose permeation. The main objective of this study was to minimize the CP and membrane fouling by CGMP using the threshold flux concept, and to select a suitable membrane and optimal process parameters for pilot-plant testing.

2. Background and theory

2.1. Type of fouling and its relationship with permeate flux

Membrane fouling can be classified in different ways [24–29]. Table 1 shows the different fouling types and their relationships

Table 1
Summarization of fouling types and the relationship with permeate flux [24–29].

Classification standard	Fouling type	Description	Relationship with permeate flux
Fouling mechanism	Concentration polarization	Particle accumulation and deposition on the membrane with good solubility and mobility	++
	Adsorption fouling	Particle adsorption on the membrane surface or pore wall by hydrophobic and electrostatic adsorption	+ *Solute concentration increases with permeate flux, resulting in higher adsorption rate
	Cake, gel or scaling layer	Particle precipitation, aggregation, or gelation on the membrane or on the adsorption fouling layer	++
	Pore blocking	Particle entrapment in the pores	−
	Biofouling	Microorganism adherence or growth on the membrane	+ *Nutrient concentration increases with permeate flux, resulting in higher growth of microorganism
Fouling location	External fouling	Fouling on the membrane	++
	Internal fouling	Fouling in the membrane	−
Fouling reversibility		Reversible fouling	Fouling layer can be removed when pressure is released or by specific physical cleaning.
Irreversible fouling	Fouling layer cannot be removed when pressure is released or by specific physical cleaning.	++	++
Fouling composition		Inorganic fouling	Fouling by inorganic scaling or adsorption of multivalent ions
	Organic fouling	Fouling by organic matters	++
Combined fouling	Fouling by organic-inorganic interactions	++	++

++ indicates a direct correlation; + indicates an indirect correlation; − indicates a negligible correlation.