Journal of Food Engineering 157 (2015) 1–6

Contents lists available at ScienceDirect

Journal of Food Engineering

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

Removal of bacteria and yeast in water and beer by nylon nanofibrous membranes

Solomon Mengistu Lemma, Alfonso Esposito, Marco Mason, Lorenzo Brusetti, Stefano Cesco, Matteo Scampicchio *

Free University of Bozen-Bolzano, Piazza Università, 1, 39100 Bolzano, Italy

ARTICLE INFO

Article history:
Received 16 October 2014
Received in revised form 20 January 2015
Accepted 8 February 2015
Available online 18 February 2015

Keywords:
Dead-end filtration
Bacteria
Yeast
Electrospinning
Nylon-6

ABSTRACT

This work explores the capability of nylon-6 nanofibrous membranes prepared by electrospinning to remove bacteria and yeast cells. Nanofibrous membranes have been widely used as affinity membranes to selectively capture molecules onto the membrane surface. However, their capacity to remove microbial cells in food beverages was not yet reported. Here, dead-end filtration experiments working under constant flow-rate were tested with beer samples fortified with yeasts (*Saccharomyces cerevisiae*) and bacteria (*Flavobacterium johnsoniae* and *Iodobacter fluviatilis*) ranging from 1.0×10^4 to 5.1×10^8 CFU/mL. The filtration experiments resulted in resistance to flow proportional to the cells dimensions. Yeasts formed soft cakes with the lowest resistance. Conversely, it could be assumed that bacteria formed a close-pack arrangements resulting in cakes with higher density, smaller interstitial space and, thus, higher resistance to flow. Microcalorimetric experiments and plate counts demonstrated that NFM were able to completely remove *S. cerevisiae* from water slurries. Instead, NFM reduced the concentration of *F. johnsoniae* and *I. fluviatilis* of only 5 and 3-log cycles, respectively. However, when the two bacteria strains were mixed together, the filtration resulted in the complete removal of the cells. These results were confirmed during the filtration of beer samples inoculated with *S. cerevisiae*, *I. fluviatilis* and *F. johnsoniae*.

1. Introduction

Filtration is a unit operation commonly used in food processing aimed to remove suspended matter from food fluids. Generally, when a beverage is filtered through a porous membrane, solid particles accumulates on the filter as a cake, whereas the fluid being filtered with a flow rate inversely proportional to the filter resistance (Foley, 2006; Mahdi and Holdich, 2013). This filter resistance increases with the formation of cells cake on the filter media with different morphologies in the filtration process (Ben Hassan et al., 2014; Mahdi and Holdich, 2013). Membrane filtrations have been used extensively for complete or partial removal of microbes in beer, wine and juice to achieve the highest standards of food quality and safety (Lipnizki, 2010).

Recently, there is a trend to prepare membranes having smaller porous size, higher surface availability and working with higher flow rates. Among others, these characteristics are desirable to speed up filtration operation, reduce pressure drops and enhance selective adsorption toward specific molecules or biological matter. Membranes prepared by electrospinning have received a great attention for this purpose as their inherent nanostructure, simple and fast processing, low cost, show a promising potential for filtration applications (Fuenmayor et al., 2014; Daels et al., 2011; Li and Xia, 2004)

The working principle of electrospinning is straightforward. Briefly, a polymer solution is continuously pumped through a metal syringe needle. When a high voltage is applied, an electrostatic repulsion between the polymer and the metal needle causes the instantaneous ejection of the polymer, which forms nanofibers collected as nonwoven membrane. The resulting morphology exhibits various useful characteristics for filtration applications (Barhate et al., 2006; Li and Xia, 2004; Fuenmayor et al., 2014; Gopal et al., 2007) .

Electrospun nanofibrous membranes (NFMs) have been widely used as affinity membranes to selectively capture molecules by binding their specific functional groups onto the membrane surface. For instance, NFM functionalized with amidino diethylenediamine were successfully applied for chelating metal ions (Kampalanonwat and Supaphol, 2010). Also, NFM functionalized with laccase were used as bioreactor for the removal of chlorophenols in water (Dai et al., 2013). Then, NFM were used as pre-filters for the removal of micro-particles from waste-water (Bjorge et al.,

* Corresponding author. Tel.: +39 04710 17210; fax: +39 04710 17009.
E-mail address: matteo.scampicchio@unibz.it (M. Scampicchio).

http://dx.doi.org/10.1016/j.jfoodeng.2015.02.005
0260-8774/

2009; Gopal et al., 2007; Kaur et al., 2012). In addition, NFM were used to selectively adsorb hydrophobic molecules dissolved in water, such as quercetin (Scampicchio et al., 2008), tannins (Fuenmayor et al., 2014), endocrine disrupters (Li et al., 2011) and others persistent organic pollutants (Yue et al., 2012). Such applications are of great potential as NFM have the advantage over conventional particle-based column-bed of reduced pressure drop, higher flow rate and higher surface availability.

However, NFM have not yet been applied for the removal of microbial cells in food beverages. Indeed, such application is of great importance as it may serve to remove specific pathogen bacteria responsible for safety concerns, remove bacteria responsible for changes in the food flavor, taste and appearance and, finally, reduce the overall food microbial concentration to enhance the stability of the final product. Furthermore, NFM could be used to remove yeast cells in fermented broth or must (such as wine, beer and cider processing) for their recovery and later reutilization (Chupakhina and Kottke, 2008; Fillaudeau and Carrère, 2002).

To test the suitability of NFM for the removal of microbial cells in food beverages, NFM made by nylon-6 were prepared and applied for the removal of bacteria and yeast from drinking water and beer beverages.

2. Materials and methods

2.1. Chemicals and samples

Nylon-6 polymer and LB broth were purchased from Sigma–Aldrich (St. Louis, MO). Formic acid (98%) was purchased from Fluka, Sigma–Aldrich (Steinheim, Germany). Beer samples were purchased from local supermarket of Bozen-Bolzano, Italy.

2.2. Microbial cultures and suspensions preparation

Bacterial strains were selected from the strain library of the Faculty of Science and Technology of the Free University of Bozen-Bolzano, Italy. The strains of bacteria (*Flavobacterium johnsoniae* and *Iodobacter fluviatilis* were isolated from the Alpine environment and stored as glycerol stocks. These microbes are known as ubiquitary in freshwater and known as common contaminants in beer production (Bokulich and Bamforth, 2013). The yeasts (*Saccharomyces cerevisiae)* were obtained from Lallemand Inc. Glycerol stocks of the bacterial strains *F. johnsoniae*, *I. fluviatilis* were thawed and an aliquot of 500 μL was inoculated into 200 mL liquid Luria–Bertani (LB) media and incubated at 37 °C in a shaker (120 rpm) for 72 h. Similarly, 3 mg of lyophilized *S. cerevisiae* was incubated for 24 h. For each bacterial strain and for the yeast, 50 mL of liquid media were centrifuged at 4600 rpm for 5 min. (Thermo Science SL 16R centrifuge, German). Then, the obtained pelleted bacteria (wet mass 387 ± 28 mg) were re-suspended in either 100 mL of physiological solution (NaCl in UV distilled deionized Milli-Q water (Millipore, Bedford, MA), 0.9% w/v) or the same volume of beer for filtration. 0.1 mL of the suspensions before and after filtration were streaked on LB-agar plates for bacterial counts. The densities of suspensions were measured with densometer (DA-130 N, KEM, Japan) at 25 °C. At the end, the suspensions in beer or in LB (depending on the experiment type) had the concentration of 5.1×10^8 CFU/mL for *F. johnsoniae*; 1.0×10^4 CFU/mL for *I. fluviatilis*; 8.0×10^8 CFU/mL for the bacterial mixture of *F. johnsoniae* plus *I. fluviatilis*; 2.9×10^8 CFU/mL for *S. cerevisiae*. Microbial counts for yeast and bacteria were performed as explained in Section 2.4.4.

2.3. Membrane production

Nylon-6 membranes were prepared by electrospinning as described by Scampicchio et al. (2010) with modifications. The Nylon-6 solution of 23% (w/w) was prepared in formic acid. This solution was placed in 5 mL plastic syringes fitted with a metallic needle (Hamilton). The solution in the syringe was pumped at 200 μL/h horizontally by KDS100 syringe pump (KD-Scientific, New Hope, PA). A 22 kV positive potential difference was generated by a Spellman SL150 high voltage power supply. Then, the electrospun nanofibers were deposited on grounded stationary metal collector covered with aluminum foil at 11 cm from the syringe nozzle to the tip. The electrospinning experiments were carried out at room temperature in an enclosed lab hood box.

2.4. Physical characterization

2.4.1. Morphology

The surface morphology of electrospun nanofibrous membranes was observed using a field emission scanning electron microscope (ZEISS SUPRA 40 VP SEM, German). The membranes samples were coated with 15 nm of Au (EMITECH K975X) prior to SEM imaging. The average fiber diameters (AFD) of the samples were determined by analysis of 100 fibers from SEM image.

2.4.2. Thickness and density

Thickness was measured by a micrometer (Mitutoyo 293-815, Japan, with a 0.001 mm accuracy) at different part of the membrane to test homogeneity. Apparent density was calculated by weighting a unit area (cm^2) and by measuring average thickness of each membrane. Porosity (ϕ) was calculated from the density of the nylon-6 polymer (1.084 g/cm^3) at 25 °C and the filter media using the following equation (Ma et al., 2005):

$$\phi = (1 - \rho_m/\rho_b) \quad (1)$$

where ρ_m is the apparent density of the NFM and ρ_b is the nylon-6 bulk density.

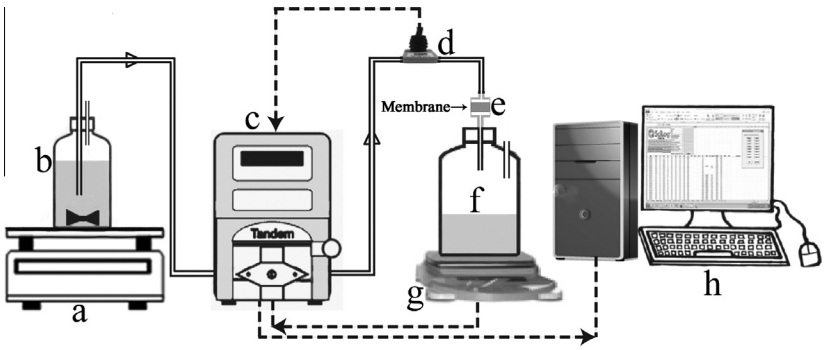

Fig. 1. Schematic diagram of experimental dead-end filtration. Legend: (a) stirrer; (b) sample container; (c) peristaltic pump; (d) pressure sensor; (e) filter holder and membrane; (f) filtrate sample; (g) electronic balance; (h) PC connected to the system.