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Development and characterisation of functionalised ceramic microtubes for bacteria filtration

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ABSTRACT

A very challenging objective of today's research in the membrane technology for microfiltration applications is the development and processing of ceramic microtubes which are especially attractive due to their excellent chemical, thermal and mechanical properties compared to polymeric membranes. Another challenge is the fabrication of self-cleaning membranes to avoid blockage of filtering pores and to ensure a long service life. In this study we present the fabrication of zirconia microtubes featuring a specific surface functionalisation for bacteria filtration and digestion, using a sequence of slurry preparation, extrusion process, final sintering and subsequent surface modification. Tubular zirconia membranes with 1.6 and 1.0 mm outer and inner diameters, respectively, were extruded and sintered at temperatures between 950 °C and 1250 °C after a debinding step. The ceramic microtubes were characterised by microstructural analysis including Hg intrusion porosimetry, BET analysis, strength tests and profilometer measurements. A sintering temperature of 1050 °C was found to provide membrane properties featuring an open porosity of 51.3% with pore sizes ≤0.2 μm, a BET surface area of 7.85 m^2/g and an average bending strength of 57.0 MPa, being suitable for bacteria filtration. In addition, a straightforward procedure for heterogeneous membrane functionalisation using an acid hydroxylation pre-treatment, followed by utilisation of 3-aminopropyltriethoxysilane (APTES) and finally immobilisation of the antibacterial model enzyme lysozyme was successfully realised.

1. Introduction

The attractiveness of membrane processes for wastewater treatment is attributed to the improved performance and due to technological advantages [1]. The membrane separates the contaminated feed water in a clean water flow (permeate) and a concentrated waste stream. Even high quality water can be obtained by microfiltration membranes that remove microorganisms including bacteria. Ideally, the permeate is of reproducible quality and the process can be used for various purposes, e.g. decontamination and recycling of process water for industry and removal of pathogenic viruses for drinking water supply [2–7].

With respect to established industrial microfiltration applications for a successful removal of bacteria from process water the filtration efficiency and tool life are still restricted due to a limited functionality of the membranes. Both limitations, the undesired growth of bacteria cells through the membrane pores during long filtration times and the bacterial contamination of the permeate caused by high pressures can be counteracted by using convenient antibacterial components immobilised on the membrane surface [8–10].

Potential antibacterial components are proteins (e.g. lactoferrin) including enzymes (e.g. lysozyme), antimicrobial peptides (e.g. magainin, protegrin) and metal ions (e.g. silver ions) [11–20].

In contrast to polymeric membranes ceramic membranes have a stronger application potential because of their higher chemical, thermal and mechanical stability. Based on these excellent membrane characteristics back-flushing methods can be used to regenerate the fouled membrane without affecting the membrane surface. In addition, ceramic materials do not show any swelling behaviour as some polymers. The main drawback of ceramic membranes is related to their low toughness whereas zirconia is favoured instead of other ceramic materials. As fare as the membrane geometry is concerned tubular membranes are preferred due to their high surface-area-to-volume-ratio, the easy scale up and low operating pressure as compared to flat-sheet membranes. Thus, a large volume throughput and an increased immobilisation capacity are the main advantages [21–23].

In order to functionalise oxide surfaces, a surface activation step is necessary. Introduction of terminal hydroxy groups on the oxide surface can be achieved chemically by an either acidic or alkaline pre-treatment or by incorporation of an acid or basic oxide

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coating [3,4]. Such activated surfaces can consecutively be treated with desired silanisation reagents to obtain specific functional end groups on the membrane surface for the subsequent immobilisation of, e.g. antibacterial components [24,25].

The aim of this work is to prepare ZrO_2 microtube membranes for bacteria filtration by extrusion that feature ideally pore sizes in the range of 0.2 μm for bacterial retention, large pore volumes and open porosities for realising high permeate flux and sufficient mechanical strength. Therefore, the effects of variation of the sintering temperature on the surface structure and porosities were studied in detail.

In contrast to conventional ceramic membranes for microfiltration applications the prepared ceramic microtubes were additionally functionalised by antibacterial components to improve the antifouling behaviour. Therefore, the challenge is the fabrication of self-cleaning membranes to avoid blockage of filtering pores and to ensure a long service life. A straightforward procedure for the heterogeneous membrane functionalisation using the model enzyme lysozyme (E.C. 3.2.1.17, peptidoglycan N-acetylmuramoylhydrolase) as an antibacterial agent is developed. This lytic enzyme can degrade the structure of bacteria cell walls by catalysing the hydrolysis of β-(1–4) linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in peptidoglycan. Gram-positive cells (e.g. *Micrococcus luteus*) are susceptible to this hydrolysis as their cell walls have a high proportion of peptidoglycan. Gram-negative bacteria are less susceptible due to the presence of an outer membrane and a lower proportion of peptidoglycan.

2. Materials and methods

2.1. Materials

The zirconia powder and reagents were obtained from commercial sources and used without further purification: Yttria (3 mol%) stabilised zirconia (TZ-3Y-E, Lot. Z301048P, specific surface area = 15.1 m^2/g) was obtained from Tosoh, Japan. Bees wax (bleached, product number 14367, Lot. 40208217), stearic acid (95%, product number 175366, Lot. U03863-042), 3-aminopropyltriethoxysilane (APTES, 99%, product number 440140, Lot. 07618MH-438), acid orange II sodium salt (product number 75370, Lot. WE304250/1), lysozyme from chicken egg white (lyophilised powder ~95%, MW 14.7 kDa, product number L6876, Lot. 028K0062), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC, commercial grade, product number E7750), sulphuric acid (97%), hydrogen peroxide (35%), decane, hexane and anhydrous toluene were purchased from Sigma–Aldrich Chemie GmbH, Germany. Bradford protein assay kit (product number 500-0006, Lot. 109640) was purchased from Bio-Rad Laboratories, Germany. Double deionised water with an electrical resistance of 18 MΩ (Synergy®, Millipore, Germany) was used for all experiments.

2.2. Preparation of ZrO_2 microtubes by extrusion

After heating 54.79 mL (40 g) decane to 80 °C the binders in the form of bees wax (28 g) and stearic acid (3 g) were dissolved in the organic solvent. Subsequently, 300 g zirconia powder was added to this mixture while stirring constantly with a glass stirrer. Furthermore, 45 mL hexane was added to deagglomerate the suspension with an ultrasonic horn for 5 min at 240 W with a pulse rate of 0.5 s (Branson Sonifier 450, Heinemann, Germany). Further homogenisation and evaporation of the remaining hexane were obtained by using high speed dispermat LC2 equipped with a dissolver disc (40 mm diameter) for 35 min at 80 °C (VMA-Getzmann, Germany). Hereafter, the stable slurry was converted in a temperature controlled double wall vessel of the extruder which was closed with an adequate press ram. Before extrusion, the slurry was tempered at the desired extrusion temperature of 40 °C for 2.5 h. The solids content of the prepared feedstock is 65.1 vol.%.

The extruder was equipped with an extrusion die (1.6 mm diameter) with integrated pin (1.0 mm diameter) to obtain the intended green microtubes. A spindle drive with shaft joint was connected to the press ram of the double wall vessel and the tempered slurry was pressurised controlled by a load cell. For realising defect-free extruded microtube membranes both the press capacity and the speed of the conveyor band were optimally coordinated whereas the linear extrusion speed was 30 cm/min at 40 °C. After extrusion the green microtubes were dried at room temperature (RT) for at least 2 days (relative humidity = 46%) for assuring a complete removal of residual hexane molecules. Sintering process was carried out at temperatures between 950 °C and 1250 °C as shown in Table 1 with preceding dwell times at 280 °C and 500 °C to remove the binder molecules in the form of bees wax and stearic acid (Ceramix, Germany). The sintered ceramic microtubes were then ready for characterisation and surface modification.

2.3. Heterogeneous surface modification of ceramic microtubes for lysozyme immobilisation

ZrO_2 membranes sintered at 1050 °C were selected because the application of this moderate sintering temperature is resulted in both an adequate mechanical strength for sample handling and a sufficient open porosity for the aspired bacteria filtration process. A specific functionalisation strategy to immobilise lysozyme was developed.

In the first step of the modification sequence the ZrO_2 membranes were pre-treated by reacting with freshly prepared Piranha solution (97% H_2SO_4:35% H_2O_2, 3:1 (v/v)) to generate terminal hydroxy groups on the membrane surface. After the incubation process at RT for 30 min the activated membranes were thoroughly rinsed with deionised water until the effluents were pH neutral und dried [24,26].

Each set of pre-treated membranes (293 mg) was placed in individual vials and freshly prepared 3-aminopropyltriethoxysilane (APTES) solutions with concentrations of 0.1%, 1% and 2% APTES by volume (4.27 mM, 42.73 mM and 85.47 mM solutions, respectively) were added. Both deionised water and anhydrous toluene were chosen as solvents whereas aqueous APTES solutions at pH 3.5 were used. Total solution volume was consistently 2 mL for each sample. The sample tubes were placed in a thermomixer and incubated at different reaction conditions (Eppendorf, Germany). Solutions were held at temperatures of either RT or 75 °C. The reaction time was either 15 min, 90 min or 24 h under continuous stirring at 1000 rpm. Upon completion of specified reaction time the ceramic membranes were removed from the APTES solution and washed five times with 2 mL deionised water and toluene, respectively [27,28].

To quantify the amount of residual amino groups acid orange II assay was applied as described previously with slight modifications [29,30]. Both blank sample (hydroxylated membrane material from the pre-treatment step) and test samples (APTES-activated membrane material) were probed with acid orange II reagent (0.5 mM acid orange II in HCl, pH 3) for 22 h at RT and 1000 rpm. After the incubation step acid orange solution was removed and the ceramic membranes were washed with HCl (pH 3). The residual acid orange II content was measured spectro-photometrically by treating the membranes with NaOH solution (pH 12) for 15 min at 1000 rpm and comparing the absorbance of supernatant at 483 nm with that of NaOH solution with acid orange II at various concentrations (duplicate determination).