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Sustainable polysaccharide-based biomaterial recovered from waste aerobic granular sludge as a surface coating material

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ABSTRACT

To evaluate the possibility of utilizing polysaccharide-based biomaterial recovered from aerobic granular sludge as a coating material, the morphology, molecular weight distribution and chemical composition of the recovered biomaterial were investigated by atomic force microscopy, size exclusion chromatography and pyrolysis–GC–MS to have a better understanding of the properties of the biomaterial. The biomaterial recovered from aerobic granular sludge demonstrates chain-like structure. The molecular weight of 1/3 of the biomaterial is higher than 70 kDa. It is amphiphilic due to containing polysaccharides as a major fraction and lipids as a minor fraction. The biomaterial easily forms a film on a hydrophilic surface (e.g. paper), and functions as a water resistant barrier. Biomaterial recovery from waste aerobic granular sludge in biological wastewater treatment process provides a new resource of sustainable materials.

1. Introduction

Biological wastewater treatment involves converting dissolved and suspended organic contaminants in water into biomass (sludge) and evolved gases (CO_2, CH_4, N_2 and SO_2) [1]. As a novel biotechnology, sludge granulation emerged in the last decade for a wide range of biological wastewater treatment processes. This biotechnology is to force microorganisms to form granular sludge rather than floccular sludge (Fig. 1). The compact granular form provides better settling property, more effective sludge-effluent separation and higher biomass retention. These advantages allow running a wastewater treatment plant with 30% less energy input, and require 75% less space combined with significant lower investment costs [2].

The most unique and important property of granular sludge is that, microorganisms produce a significant amount of extracellular biomaterials to form a polymeric hydrogel matrix and then are self-immobilized into this matrix without involvement of any carrier materials. The resultant extracellular biomaterials which form into structural gels make granular sludge distinguished from conventional floccular sludge.

In our previous research, it was found that one of the major hydrogel-forming biomaterials extracted from aerobic granular sludge was polysaccharide-based biomaterial. This biomaterial resembled commercial alginate in the reactions with $CaCl_2$ and saturated $(NH_4)_2SO_4$, in gel formation property with divalent ions, and in UV–visible and MALDI-TOF MS spectra. On the other hand, it was dissimilar with commercial alginate in the reactions with acid ferric sulfate, phenol-sulfuric acid and Coomassie brilliant blue G250, which might be attributed to the appearance of O-acetylated substitution groups [4]. This polysaccharide-based biomaterial is more than 10% w/w of the organic matter in aerobic granular sludge.

At present, the sludge produced from wastewater treatment processes, including the granular sludge, is considered as a waste product. The cost of handling/disposal of the waste sludge represents up to 50% of the wastewater treatment costs [5]. If biomaterials can be recovered from the waste sludge and applied, the sustainability and economics of wastewater treatment can be strongly increased. Therefore, there is a great need for techniques of biomaterial recovery from waste granular sludge, methodologies of characterization and applications of the recovered biomaterials.

The purpose of this research is to better understand properties of the polysaccharide-based biomaterial recovered from aerobic granular sludge and find out potential applications of the biomaterial. The morphology, molecular weight distribution, amphiphilic and film-forming property and composition of the polysaccharide-based biomaterial are investigated in this research. Furthermore, the possibility of using this biomaterial as a surface coating material is evaluated.

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Fig. 1. Aerobic granular sludge by SEM (scanning electronic microscope) [3].

2. Material and methods

2.1. Aerobic granular sludge for investigation

Aerobic granular sludge was sampled from the Nereda® pilot plant, operated by Royal Haskoning DHV at the wastewater treatment plant Epe, The Netherlands (www.royalhaskoningdhv.com). The reactor was fed with municipal sewage. The influent consisted of approximately 25% of slaughterhouse wastewater, which was discharged in the sewage system. Average parameters of the influent were: CODtotal 585 mg/L, suspended solids 195 mg/L, NH_4-N 55 mg/L and PO_4-P 6.3 mg/L. The reactor was operated in Sequencing Batch (SBR) mode for biological phosphate and nitrogen removal. Operational details were described in Lin et al. [4]. After start-up, biomass concentration in the reactor was maintained around 8 to 10 g TSS/L. Oxygen in the reactor was controlled between 2 to 3 mg/L during aeration. Temperature and pH were not controlled in this system and depended on the incoming sewage.

2.2. The polysaccharide-based biomaterial recovery from aerobic granular sludge

Polysaccharide-based biomaterial was isolated from the biomass according to Lin et al. [4]. Dried aerobic granular sludge (0.5 g) was homogenized for 5 min (LabGEN tissue homogenizer, Cole-Parmer, USA) and extracted in 80 mL 0.2 M Na_2CO_3 at 80 °C for 1 h. After centrifuging at 15,000 rpm for 20 min, the pellet was discarded. The supernatant pH was adjusted to 2 by adding 0.1 M HCl. The precipitate was collected by centrifugation (15,000 rpm, 30 min), washed by di-deionized water until effluent pH reached 7, and dissolved in 0.1 M NaOH. The biomaterial in the supernatant was precipitated by the addition of cold absolute ethanol to a final concentration of 80% (vol/vol). The precipitate was collected by centrifugation (15,000 rpm, 30 min), washed three times in absolute ethanol and lyophilized.

The isolated biomaterial (0.5 g) was first dissolved in 15 mL of NaOH solution (0.05 M) and the pH was then adjusted to 7.0 by 0.5 M HCl. The biomaterial solution was placed inside a dialysis tubing (3500 MWCO) and dialyzed against demineralized water for 48 h to remove loosely bound ions and afterwards lyophilized.

2.3. Morphology of the polysaccharide-based biomaterial by the atomic force microscopy

Imaging of polysaccharide-based biomaterial was carried out in air at ambient temperature and humidity using freshly-cleaved mica pretreated by 3 mM $NiCl_3$. Aliquots (2 μL) of biomaterial (5 mg/L) were deposited onto mica surfaces for 10 s, and then quickly removed by the pipette. Those surfaces were air dried (1 h) in a dust-free enclosure. Samples were scanned with a Digital Instruments Multimode atomic force microscope (Veeco nanoscopy iva dimension 3100, Veeco Inc., Santa Barbara, USA).

2.4. Composition analysis by pyrolysis–gas chromatography–mass spectrometry

Pyrolysis was carried out on a Horizon Instruments Curie-Point pyrolyzer. The lyophilized polysaccharide-based biomaterial was heated for 5 s at 600 °C. The pyrolysis unit was connected to a Carlo Erba GC8060 gas chromatograph and the products were separated by a fused silica column (Varian, 25 m, 0.25 mm i.d.) coated with CP-Sil5 (film thickness 0.40 μm). Helium was used as carrier gas. The oven was initially kept at 40 °C for 1 min, next it was heated at a rate of 7 °C/min to 320 °C and maintained at that temperature for 15 min. The column was coupled to a Fisons MD800 mass spectrometer (mass range m/z 45–650, ionization energy 70 eV, cycle time 0.7 s). Identification of the compounds was carried out by their mass spectra using a NIST library or by interpretation of the spectra, by their retention times and/or by comparison with literature data.

To obtain the lipid content in the recovered biomaterial, the methods proposed by Smolders et al. were followed with modification [6]. Pure fatty acids (Sigma-Aldrich) were used as external standard. Freeze-dried biomaterial samples and fatty acid standards were weighed using an analytical balance and transferred into tubes with screw caps. One milligram of C_{15} fatty acid in 1-propanol was used as internal standard. 1.5 mL of a mixture of concentrated HCl and 1-propanol (1:4), and 1.5 mL of dichloroethane were added into the tubes and heated for 2 h at 100 °C. After cooling, free acids were extracted from the organic phase with 3 mL water. One milliliter of the organic phase was filtered over water-free sodium sulfate into GC vials. The lipids in the organic phase were analyzed by gas chromatography (model 6890N, Agilent, USA) equipped with a FID, on an HP Innowax column.

2.5. Polysaccharide-based biomaterial molecular weight determination

Size exclusion chromatography was performed with a Superdex 75 10/300 GL column (AKTA Purifier System, GE Healthcare). Elution was carried out at room temperature using Phosphate Buffer Saline (PBS) containing 10 mM (HPO_4^-, H_2PO_4) pH 7.4, 2.7 mM KCl and 137 mM NaCl, at a constant 0.4 mL/min flow rate and detection was monitored by following the absorbance of the eluted molecules at 210 nm.

Superdex 75 10/300 GL (GE Healthcare) column separates molecules of 1000 to 70,000 Daltons (Da). Measurement of the elution volume of dextran standards (1000 Da, 5000 Da, 12,000 Da, 25,000 Da and 50,000 Da) led to the calibration equation:

$$\text{Log (MW)} = 6.212 - 0.1861\ \text{Ve}$$

MW molecular weight of the molecule in Dalton (Da)
Ve elution volume in mL (assayed at the top of the peak).

Chromatogram profiles were recorded with UNICORN 5.1 software (GE Healthcare). Peak retention times and peak areas were directly calculated and delivered by the program.

2.6. Amphiphilic and film-forming properties of the polysaccharide-based biomaterial

The amphiphilic property of a material refers to a molecule having both polar, water-soluble groups and nonpolar, water-insoluble groups. The amphiphilic property of the polysaccharide-based biomaterial was measured by using Material Adhesion to Hydrocarbons (MATH) test. This method is based on determination of material hydrophobicity by differential partitioning at an aqueous–hydrocarbon interface and the result yield the hydrocarbon interaction affinity of the material. n-Hexadecane (2 mL) and aqueous solution of the biomaterial (2 mL, 1% (w/v)) were mixed by vortex for 2 min, and stand still for 1 min.