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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.

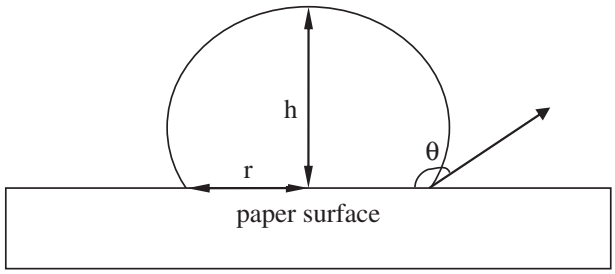

Fig. 2. Contact angle measurement.

Distribution of the biomaterial in both n-hexadecane phase and water phase is checked,

Aqueous solution of the biomaterial (3% (w/v)) was maintained in a plastic petri dish for 72 h at room temperature and room relative humidity. The resulting film was carefully peeled off the inside surface of the petri dish.

2.7. Pigment removal from the polysaccharide-based biomaterial

In order to remove the colored compounds from polysaccharide-based biomaterial, the biomaterial (1 g) was first put into H_2O_2 (30%) for 24 h, and collected after being centrifuged at 4000 rpm and lyophilized.

2.8. Application of the biomaterial as a coating layer on paper

Biomaterial water solution (5% w/v, 1 mL) was sprayed evenly by using a chromatography sprayer (10 mL, Sigma-Aldrich) on pieces of uncoated paper (5 cm × 5 cm, 96 g/m^2, supplied by Kenniscentrum Papier en Karton (KCPK), The Netherlands), and air-dried. 1 mL of Impermax WRP 50C (alkenyl succinic anhydride (ASA) 10% w/v, supplied by KCPK), one of the commercial coating chemical, was sprayed on the same kind of paper and air-dried.

The hydrophobicity of those coated paper sheets was determined by contact angle measurements. A 50 μL drop of Milli-Q water was dropped on the surface of the paper. The change of contact angle with time was recorded by KSV CAM200, and calculated by the formula:

$$\tan(\theta/2) = h/r$$

where h is the height of the drop and r is the radius at the interface of water with the paper surface (Fig. 2).

The change of contact angle with time of a drop of Milli-Q water on the uncoated paper itself was also recorded and measured as a control.

On each piece of paper, the contact angle was measured at 5 different places randomly; the average value and standard deviation were calculated.

3. Results and discussion

3.1. Morphology of polysaccharide-based biomaterial

The yield of polysaccharide-based biomaterial was 160 ± 4 mg/g granular sludge [4]. The recovered biomaterial demonstrates chain-like structure on mica sheet. The width of the chain is around 20 nm (Fig. 3a). The chains extend along the surface and entangle with each other, forming a web-like structure that covers the whole surface of mica. This indicates that the polysaccharide-based biomaterial has a perfect film-forming property and can form a continuous film on the surface. The thickness of the biomaterial film is around 4 nm. In addition to the entangled chains, there are a few globules attaching on the chains and pointing to the air. The height of the globules can reach 15 nm, which is 2 times higher than the thickness of the biomaterial film. Due to the significant difference in height, the globules looked much brighter than the fibers under the atomic force microscope. As the sample was prepared by depositing biomaterial water solution on a surface and air dried, those globules extending out of the surface and pointing to the air must have a hydrophobic property [7–8]. Therefore, the polysaccharide-based biomaterial recovered from aerobic granular sludge is amphiphilic. It has both hydrophilic part and hydrophobic part. When the biomaterial stays at the surface between water and air, the hydrophilic part spreads along the surface, forming a film and the hydrophobic part attach on the film, pointing to the air (Fig. 3b).

3.2. Polysaccharide-based biomaterial composition analysis

The composition of the isolated biomaterial was analyzed by flash pyrolysis–GC–MS. Polysaccharide-derived products such as 5-methylfuraldhyde and levoglucosenone, combinations of amino acids (toluene, styrene, phenol, ethylcyanobenzene and 3-ethylindole) and lipid-derived pyrolysate (fatty acids, wax esters, alcohol moieties and cholestenes) were observed (Fig. 4).

According to our previous research data, the major part of the biomaterial is polysaccharides; while protein content is under the detection limit of Bradford assay (100 mg/L) [4]. The amino acids in the pyrolysate could be due to the amount of proteins present in the biomaterial which is hardly detected by Bradford assay. The lipid content in the biomaterial was measured as 8.2 ± 0.9 mg/g biomaterial. They are enriched with C_{15}–C_{16} fatty acid fractions. These results are in agreement with those from García Becarra et al. [9] that, lipid content in the

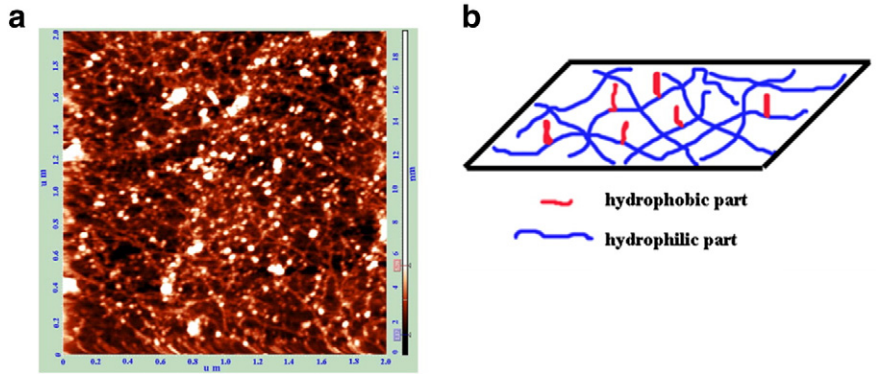

Fig. 3. Morphology of polysaccharide-based biopolymer isolated from aerobic granular sludge by atomic force microscopy (a) and diagram of the biomaterial at the surface between water and air (b). a: The entangled chain-like structure covers the surface and forms film; the globules attach on the film and point to the air. b: The hydrophilic part covers the surface and hydrophobic part points to the air.

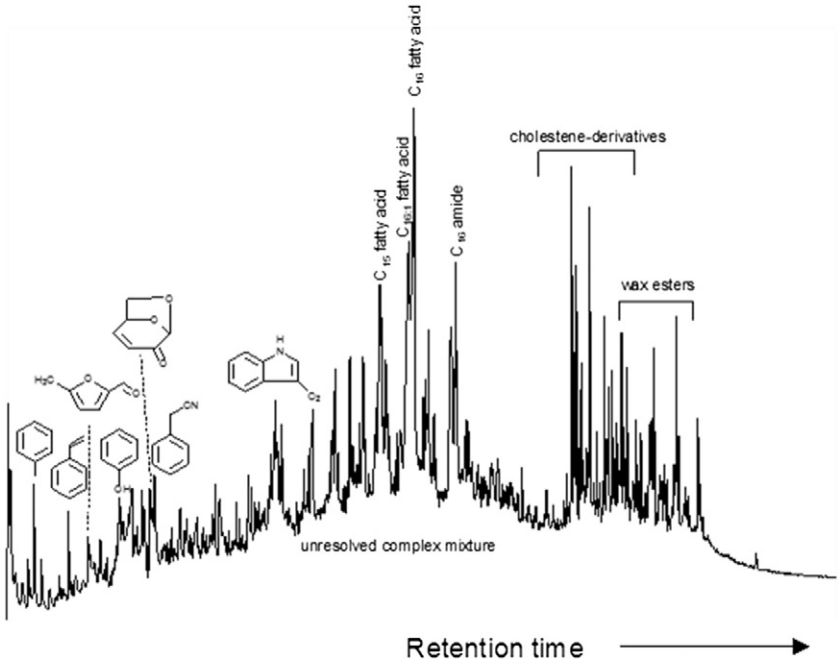

Fig. 4. Pyrolysis–gas chromatograms of polysaccharide-based biomaterial recovered from aerobic granular sludge. Cn and Cn:1 indicate chain length of saturated and unsaturated compounds.

biopolymers extracted from activated sludge by alkaline extraction method is less than 1%, and enriched with C_{15}–C_{16} fatty acid fractions. Therefore, the biomaterial consists of polysaccharides as a major part and lipids as one of the minor parts.

Since polysaccharides are hydrophilic and lipids are hydrophobic in general, considering the morphology in Fig. 3a, it can be assumed that the chain-like structure which forms film on the surface are mostly polysaccharides and those globules pointing towards the air are mostly lipids. In fact, this is in agreement with the idea presented in other studies that the extracted biopolymers from sludge resemble the structure of giant micelles, with lipids adsorbed on the surface of these assemblies [9–12].

3.3. Molecular weight of the polysaccharide-based biomaterial

The size distribution profile of the polysaccharide-based biomaterial by size exclusion chromatography is shown in Fig. 5. There are 5 fractions with different elution volumes. The fraction with the elution volume of 7.83 mL separates well with other fractions. The molecular weight of this fraction is more than 70 kDa. Three fractions with elution volumes between 13 mL and 17 mL co-eluted and a minor fraction with very low molecular weight was eluted after 20 mL. The molecular weight of these 5 fractions and their percentages are listed in Table 1. It can be clearly seen that the molecular weight of 1/3 of the biomaterial is higher than 70 kDa, and the molecular weight of almost other 1/2 of the biomaterial is around 6 kDa. In comparison, Seviour et al. detected three peaks with the molecular weight ranging from 1 to 1000 kDa [12], in the study of extracellular polysaccharides isolated from aerobic granular sludge with alkaline extraction. The difference in the range of molecular weight distribution between this research and the research of Seviour et al. is likely due to the different extraction methods, eluent and standards used. Molecular weight distribution of a polymer is one of the key factors influencing film formation property [13]. In the polysaccharide-based biomaterial, the fraction which has molecular weight higher than 70 kDa may contribute to the formation of the web-like structure in Fig. 3 and consequently a continuous film.

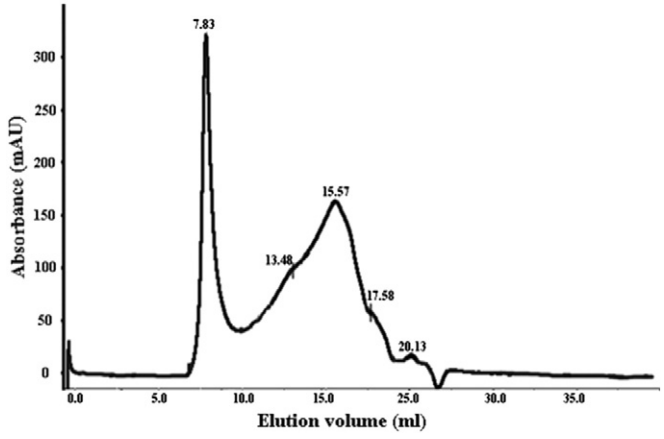

Fig. 5. Size distribution profiles of polysaccharide-based biomaterial isolated from aerobic granular sludge by size exclusion chromatography.

3.4. Amphiphilic and film-forming properties of polysaccharide-based biomaterial

When the aqueous solution of the recovered biomaterial was mixed with n-hexadecane, it dispersed in both n-hexadecane phase and water phase, staying at the interface between n-hexadecane and water (Fig. 6a). Apparently, although polysaccharides are the major

Table 1
Molecular weight of different fractions in polysaccharide-based biomaterial.

Elution volume of the peak (mL)	Molecular weight (kDa)	Percentage of the fraction (% peak area)
7.83	>70	29.74
13.48	14.4	18.82
15.57	5.79	45.15
17.58	2.15	4.42
20.13	0.656	1.87

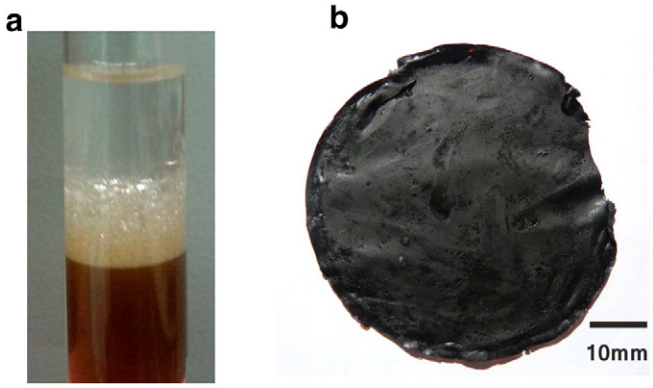

Fig. 6. Amphiphilic property and film-forming property of the polysaccharide-based biomaterial recovered from aerobic granular sludge (a: the polysaccharide-based biomaterial has amphiphilic property, staying at the interface between n-hexadecane and water; b: the polysaccharide-based biomaterial forms flexible film).

part of the biomaterial, due to the fact that it consists of lipids as one of the miner parts, this biomaterial is amphiphilic.

In granular sludge, components of the extracellular biomaterials are polysaccharides, proteins, lipids and even some intracellular polymers. All these biomaterials are cross-linked forming a compact polymeric matrix for bacteria to be embedded in [14]. Although it is possible to isolate specific biomaterial with one component as the dominant part, e.g. polysaccharide-based biomaterial, attachment of the other components as minor parts is almost unavoidable. Sometimes, the presence of those minor parts provides additional properties to the biomaterial, which leads to additional applications. In this study, the amphiphilic property of the polysaccharide-based biomaterial is directly linked to the presence of lipids. Removal of the lipids fraction by further purification may destroy the amphiphilic property of the whole biomaterial. Therefore, it is significantly important to understand the physical and chemical properties of the raw biomaterial recovered from granular sludge. At this respect, purification of the biomaterial is not always necessary. It totally depends on which property of the biomaterial is intended to be kept.

3.5. Potential application of polysaccharide-based biomaterial as a surface coating on paper packaging

The polysaccharide-based biomaterial isolated from aerobic granular sludge is amphiphilic, with both hydrophobic and hydrophilic groups. This biomaterial is easily drawn into homogeneous and flexible film (Fig. 6b). These two properties indicate that the biomaterial has the potential to be used as a coating material, which deposits as a film on the hydrophilic surface to enhance the water resistance of the surface.

Paper is widely used in packaging applications. It consists of a porous cellulose structure made up of microfibrils. The hydrophilic nature of cellulose, due to the OH sites in the basic unit of cellulose ($C_6H_{10}O_5$) and fiber network porosity, limits the water-barrier property of paper [15]. Therefore, paper is often associated with other materials to have better water-barrier property.

Polyolefins are generally chosen as paper coating materials to overcome porosity and hygroscopicity of paper (e.g. alkenyl succinic anhydride (ASA), one of the commercial coating material, is olefins from crude oil). Unfortunately, the obtained paper-polyolefin material loses its biodegradation and recyclability characteristics due to the addition of non-biodegradable layers of polyolefins.

Renewable biomaterials such as polysaccharides, proteins, and lipids or combinations of those components can be used as barrier coatings on paper [16]. Those components offer favorable environmental advantages of recyclability and reutilization compared to conventional petroleum-based synthetic polymers. The possibility of using the isolated polysaccharide-based biomaterial as a water-barrier coating on paper was investigated in this research. Water resistant property of paper with and without coating was evaluated by measuring the contact angle of water droplets on the surface (Fig. 7). To a paper surface without coating, once a drop of water was dripped on, it was absorbed immediately by the paper and spread around (Fig. 7a). The contact angle of water droplets on this surface was zero (Fig. 7d). In comparison, to a paper surface which was coated by the recovered biomaterial, the water drop still kept the shape of a drop without spreading (Fig. 7b).The initial contact angle of water drop on the coated surface was higher than 100, which indicates that the polysaccharide-based biomaterial indeed provides a water barrier to the paper. In addition, as implied by the curve of contact angle–time in Fig. 7d, it can be concluded that, 5%

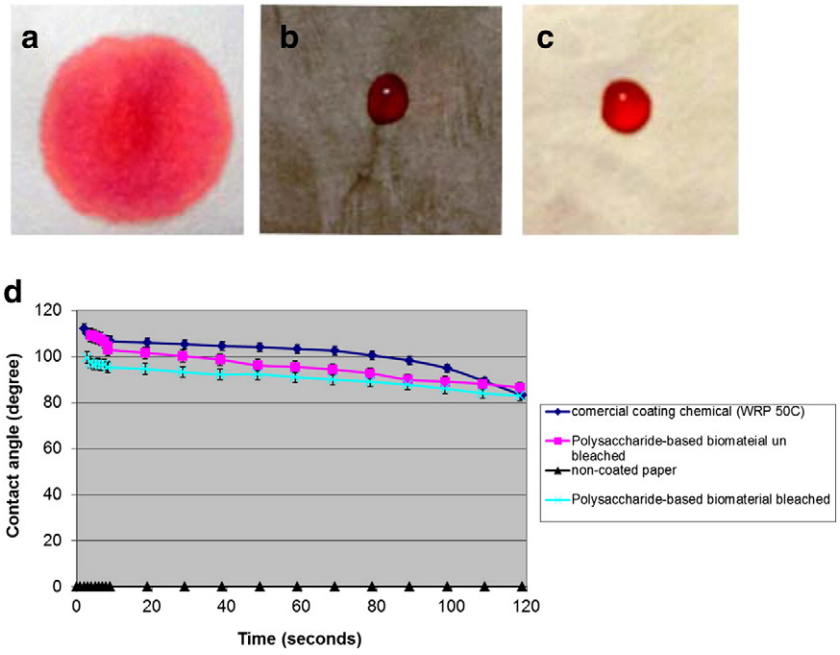

Fig. 7. Water droplets on paper (a): uncoated paper; (b): paper coated with 5% polysaccharide-based biomaterial; (c): paper coated with 8% bleached polysaccharide-based biomaterial; and (d) contact angle–time curves of water drops on the surface of paper sheets (the red color in a, b and c comes from a water soluble pigment).

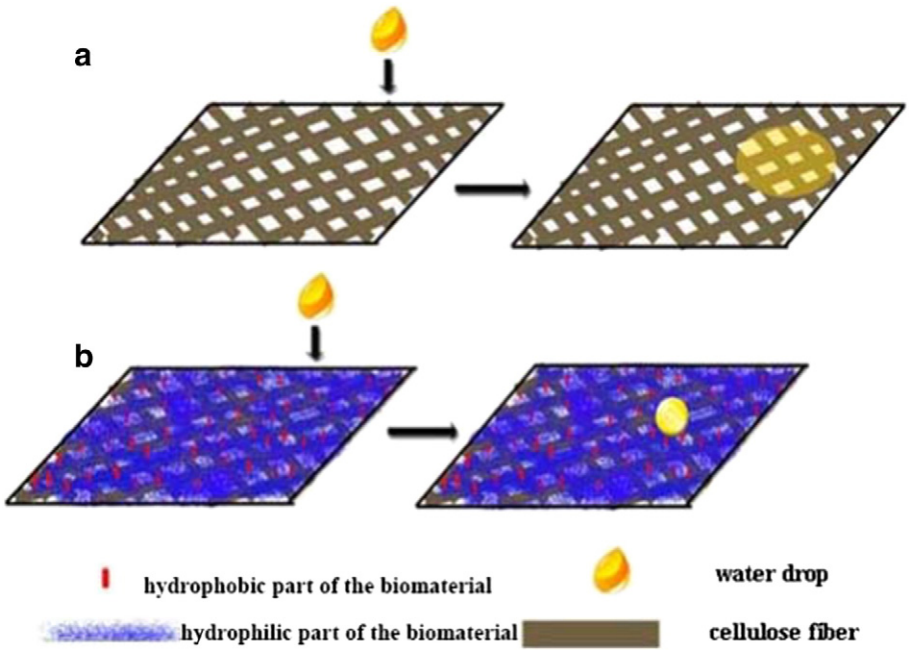

Fig. 8. Diagram of the water barrier effect of polysaccharide-based biomaterial on cellulosic fiber. a: Cellulosic fibers are porous (there are empty voids between the fibers), water is easily wet and penetrate the fiber network. b: The polysaccharide-based biomaterial is chain-like with 20 nm in width of the chain, which is at least 1000 times thinner than cellulosic fiber. The polysaccharide-based biomaterial forms film on cellulosic fiber. The hydrophobic groups attach on the chain and point to the air. Due to the repulsion force from the hydrophobic globules, the water droplet keeps the shape of a drop without running through the fiber.

(w/v) polysaccharide-based biomaterial water solution provides the same water resistant property to paper as the commercial coating product (10% (w/v) alkenyl succinic anhydride (ASA)) does.

The raw polysaccharide-based biomaterial has brown color. Bleach the biomaterial with H_2O_2 removed the brown pigment, while still maintained the amphiphilic property of the biomaterial (Fig. 7c and d).

The width of the cellulose fiber is around 20 μm [17], when a sheet of paper is formed, significant amount of empty voids are present between the fibers. Besides, cellulose is hydrophilic. Thus, water easily spreads on cellulose fibers and runs through the empty voids, which makes paper get wet (Fig. 8a). In comparison, the width of the chains of the polysaccharide-based biomaterial is only 20 nm, which is 1000 times thinner than the cellulose fiber. Those chains entangle with each other and form a web-like film which covers the surface of both the cellulosic fibers and the empty voids. Moreover, the hydrophobic groups attaching on the hydrophilic chains form globules pointing to the air. When a water droplet is dripped on the coated paper surface, it is first in contact with the hydrophobic globules from the biomaterial. Due to the repulsion force from the hydrophobic globules, the water droplet keeps the shape of a drop (Fig. 8b).Therefore, the biomaterial layer acts as a water barrier of the cellulosic fiber.

Therefore, the association of biomaterials recovered from aerobic granular sludge to paper provides interesting functionalities while maintaining environment-friendly characteristic of the material. Sludge granulation is a novel biotechnology, and it is estimated that there will be hundreds of wastewater treatment plants using sludge granulation technology in the coming 5 years globally (e.g. Nereda®; www.royalhaskoningdhv.com/nereda). Consequently, large amount of granular sludge are produced. If the polysaccharide-based biomaterial is recovered from waste granular sludge and applied, a sustainable source of biomaterial will be provided.

4. Conclusions

The polysaccharide-based biomaterial recovered from aerobic granular sludge is amphiphilic due to containing both carbohydrates and lipids. It 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.

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