

A buckypaper like adsorbent based on amphiphilic graphite nanofilaments for removal of enzyme biomolecules from water

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Abstract

Development of carbon nanomaterials for adsorption thus removal of organic pollutants from water is a progressive research subject. In this regard, carbon nanomaterials with bifunctionality towards polar and non-polar or even amphiphilic undesired materials is indeed attractive for further study and implementation. Here, we created carbon buckypaper adsorbents comprising amphiphilic (oxygenated amorphous carbon ($a\text{-CO}_x$)/graphite (G)) nanofilaments that can dynamically adsorb organic biomolecules (i.e. urease enzyme) and thus purify the wastewaters of relevant industries. Given the dynamic conditions of the test, the adsorbent was highly efficient in adsorption of the enzyme (88%) while permeable to water ($2382 \text{ L}\cdot\text{h}^{-1}\cdot\text{m}^{-2}$), thus holds a great promise for further development and upscaling. A subsequent citric acid functionalization declined selectivity of the membrane to urease, implying the biomolecules adsorb mostly via graphitic domains rather than oxidized, polar amorphous carbon ones. The devised platform i.e. the urease functionalized buckypaper is optimally conductive ($13 \text{ S}\cdot\text{cm}^{-1}$) and can be further employed as a biosensor. Accordingly, water treatment can be linked to biosensing via a nanostructured membrane.

Keywords: Carbon, nanofiber, membrane, urease, biomolecules, water treatment

1. Introduction

As a global challenge, water scarcity is expanding to a major part of the world and threatening the human being's life. This crisis can have different origins but undoubtedly water pollution by industry and from urban communities is a main one. Amongst the variety of water pollutants, the organic ones such as proteins and biomolecules play a determining role. These substances even at a negligible concentration, e.g., less than 1% of the entire pollution in a

river, can use up the oxygen present in water and vanish live creatures from that ecosystem [1]. Water recycling via purification can somewhat remediate this problem but necessitates development of advanced water treatment systems. Micro-, ultra- and nanofiltration membranes are typically utilized for remediation of wastewaters. Their separation action is mainly based on sieving of the pollutants, thus they require a porous structure whose pore size is smaller than the solute size. Other than the membranes, functionalized adsorbents have shown applicability in removal of even molecules and tiny pollutants based on physical/chemical interactions or biological functions [2-6]. Accordingly, there is no need to construction of porous materials with very small pore sizes that could impose high feed pressures. Moreover, a functionalized adsorbent whose surface is equipped to particular functional groups can discriminate or entrap molecules in a selective manner [7].

Electrospun nanofibrous adsorbents have shown promising capabilities for selective water remediation. They possess a structure with high interconnected porosity and huge surface area that in case of functionalization can efficiently separate functional pollutants e.g. ions, dye molecules, organics, etc.. While the high porosity realizes an extraordinary permeability thus energy efficiency, the expansive surface area enables the notable functionalization necessary for highly selective adsorbents. In this regard, biofunctionalized polyurethane, polysulfone, polyacrylonitrile and cellulose nanofibrous membranes have been tested for separation of protein and enzyme (e.g., IgG, BSA, lipase, bromelain, etc.) [7-10]. In our studies [2, 4, 11], we also developed a biofunctionalized nanofibrous adsorbent composed of Bovine Serum Albumin and poly(acrylonitrile-co-glycidyl methacrylate) (PANGMA), as the functional agent and polymer nanofiber, respectively, that could offer a significant metal nanoparticle and biomolecule removal efficiency while being highly water permeable. This adsorbent was synthesized in a simple manner versus its already established counter parts [7, 12]. The separation tests were performed under the most tricky conditions i.e. dynamically and with a trace protein concentration (in the scale of mg/L instead of mg/mL used by the other researchers [7, 9, 10, 13]) and with a size scale of pollutants, potentially passing readily through a macroporous nanofibrous structure. Despite such circumstances, the adsorbent was successful in removal of nanoparticles (97%) as well as proteins (88% BSA and 81% Cal-B). In another study, we devised a nanofibrous adsorbent comprising polyethersulfone (PES) nanofibers that were functionalized by inclusion of vanadium oxide (V_2O_5) nanoparticles [6]. This adsorbent system was successful in removal of methylene blue (MB) dye from water with an efficiency of 85% under alkaline condition and high temperature.

Despite the notable merits of the above mentioned systems in adsorption of various water pollutants, their synthesis and functionalization are multistep and not one pot. As a step forward to meet this need, recently, we developed carbon buckypaper shaped adsorbents based on amphiphilic carbon nanofilaments [14]. The nanofilaments are composed of oxygenated amorphous carbon ($a\text{-CO}_x$) and graphite (G), thus, able to adsorb both polar (e.g. dye) and non-polar (e.g. oil) water pollutants efficiently. Here, we further investigate their applicability in discrimination of biomolecules (i.e. urease enzyme), that as mentioned earlier are of the most important organic pollutants that can adversely affect the water ecosystems, Figure 1. Given the high electrical conductivity of carbon nanomaterials, it is assumed that adsorption of urease can further build up a platform for a related biosensor. This approach starting with water treatment ending up with biosensing is indeed promising and encourages us to more extensive studies after this proof of concept.

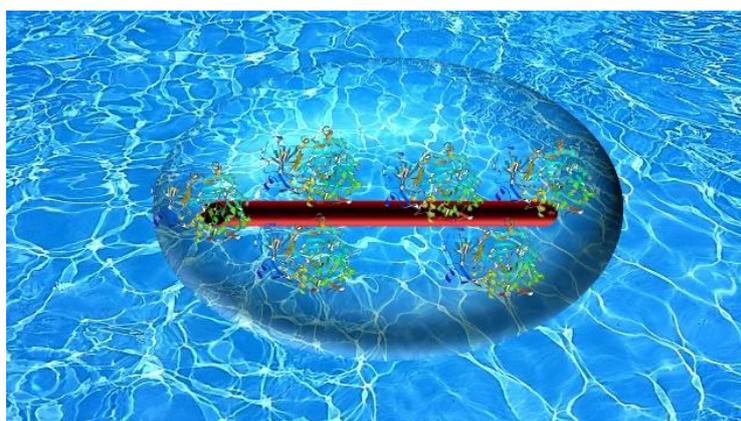


Figure 1. Schematic illustration of adsorption of urease molecules onto amphiphilic carbon nanofilaments

2. Experimental

Materials: polyacrylonitrile (PAN) (molar mass of $200,000 \text{ g}\cdot\text{mol}^{-1}$) and dimethylformamide (DMF) were purchased from Dolan GmbH (Germany) and Merck (Germany), respectively. Urease enzyme was also purchased from Sigma Aldrich (USA). All the materials were used as received.

Synthesis: The precursor PAN nanofibers were synthesized by an electrospinning method. To do so, a PAN solution (8 wt%) was fed with a constant rate ($1 \text{ mL}\cdot\text{h}^{-1}$) into a needle by using a syringe pump (Harvard Apparatus, USA). By applying a voltage of 20 kV (Heinzinger Electronic GmbH, Germany), PAN was electrospun on an aluminum foil. The as-synthesized PAN nanofibers underwent oxidative stabilization and placed in a furnace (Linn Elektro Therm, max $T = 1250 \text{ }^\circ\text{C}$) and heated in air at $250 \text{ }^\circ\text{C}$ for 2 h. Subsequently, the air

oxidized nanofibers were graphitized in argon atmosphere at the temperature of 1250 °C for 30 min and then cooled down to the ambient temperature. The heating and cooling rates were 5 °C min⁻¹.

Due to extreme brittleness of the graphitized nanofibers, challenging their handling as a freestanding membrane, they were immersed in 10 mL distilled water and ultrasonicated for 2 min at a power of 20%. The ultrasonication process chops the a-CO_x/G nanofibers, that were subsequently cast on a circular poly(phenylene sulfide) (PPS) technical nonwoven (diameter = 3.5 cm). The membrane was left to be dried in air overnight. As a control group, a-CO_x/G nanofilaments were also functionalized by citric acid (CA). To do this, CA (300 mg/10 ml) was added to the aqueous suspension and stirred overnight.

Characterization: The a-CO_x/G nanofilaments were characterized in terms of morphology by SEM (LEO 1550VP Gemini from Carl ZEISS) and an atomic force microscope (AFM)(MultiMode™ Atomic Force Microscope from Bruker AXS). Chemical surface analysis of the a-CO_x/G nanofilaments was carried out by FTIR (ALPHA (ATR-Ge, ATR-Di) from BRUKER Optik GmbH). The pore size distribution of the a-CO_x/G buckypaper was measured using an automated capillary flow porometer from Porous Materials Inc.(PMI,USA). The water permeability was determined through a pure water cross-flow filtration. For this sake, the dried a-CO_x/G buckypaper (active filtration area ≈ 7 cm²) was mounted in the membrane module and the water in the reservoir (400 mL) was permeated through by a pump (KNF LIQUIPORT) (feed rate = 62500 L.h⁻¹ .m⁻²). The permeation time was recorded and the flux according to the Equation 1 was calculated:

$$J = \frac{Q}{A \cdot \Delta t} \quad (1)$$

where J is the water flux (L.h⁻¹.m⁻²), Q is the permeated volume (L) of water, A is the effective area of the membranes (m²), and Δt is the sampling time (h). The flux measurement tests were repeated three times.

The urease retention efficiency of the buckypapers was assessed using corresponding aqueous solutions in a dead-end mode and by employing a custom-built set-up (shown in ref. [15]). The reservoir of the set-up was filled with 200 mL urease solution (1 g.L⁻¹), permeated through the buckypapers under a feed pressure of 0.5 bar. Based on a constructed standard urease calibration curve, the urease concentration in the original feed and permeates was determined by UV-vis spectroscopy (HITACHI U3000, HITACHI). The urease retention efficiency (RE) was calculated according to the Equation 2:

$$RE = \left(1 - \frac{C_p}{C_f}\right) \times 100\% \quad (2)$$

where C_p and C_f are the urease concentration in the permeate and feed, respectively. The electrical conductivity of the buckypapers as non-functionalized and CA-functionalized before and after urease adsorption was measured by a four-point probe test. At least five measurements were done and the error bars were calculated. The thickness of the samples to be considered in the conductivity measurement was already measured by using a digital micrometer (Deltascop® MP2C from Fischer).

3. Results and Discussion

The developed buckypaper consists of the *a-CO_x/G* nanofilaments, randomly arranged but with no sign of clustering. SEM image, Figure 2a, clearly verifies this fact and preservation of a porous structure that guarantees optimum water permeability. Moreover, as seen in Figure 2b, the nanofilaments' tips are exposed to the surrounding medium and thus raise interactivity of the material with the biomolecule pollutants. In fact, the nanofilaments are able to capture urease through adsorption not only on their body, but also on their cross-sections. Such an interesting feature enables a remarkable potential for adsorption of various functional pollutants. AFM images, Figure 2c, provide insight into dimensions and morphology of nanofilaments individually.

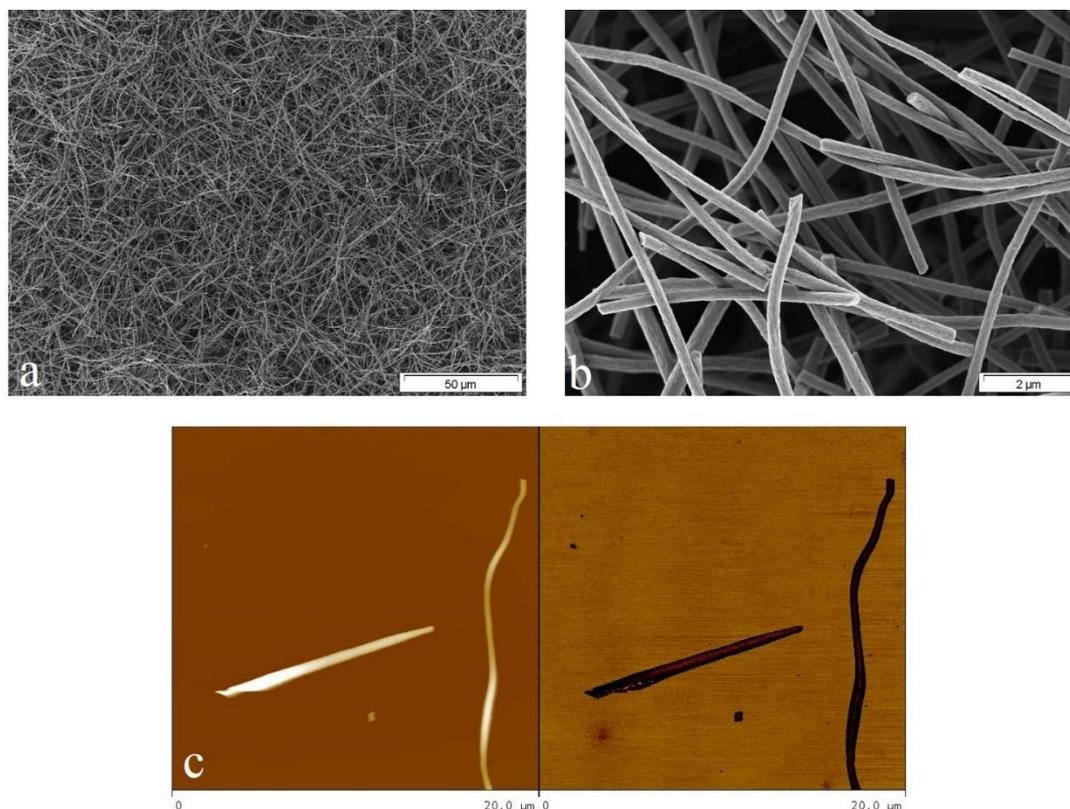


Figure 2. a&b) SEM images show morphology of the nanofilaments at two magnifications. c) AFM micrographs imply the nanofilaments' dimensions and morphology.

Pore size measurement via a bubble point test, Figure 3, implies that the pore size lies in the submicron range as small as 700 nm. This pore size distribution qualifies the structure as a microfiltration membrane [16, 17], that could hardly stop passage of tiny organic pollutants through, particularly under a hydrodynamic pressure.

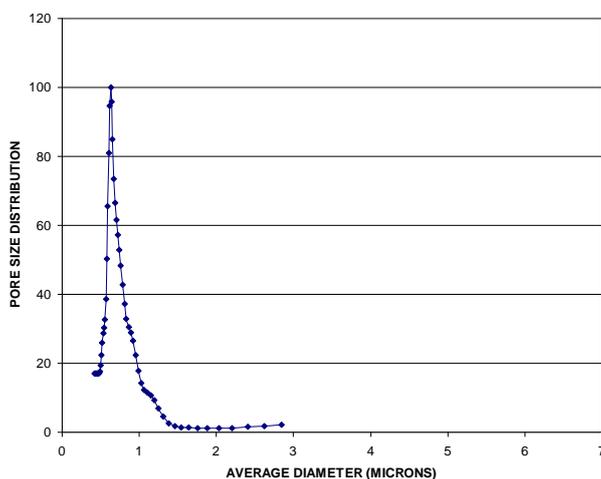


Figure 3. Pore size distribution of the *a-CO_x/G* buckypaper measured by a bubble point test.

In spite of this expectation, the filtration challenges on the buckypaper surprisingly showed a promising separation efficiency. Figure 4a shows that the buckypaper was successful in removal of urease from water. A removal efficiency of 88.5% was recorded after permeation of 150 ml urease aqueous solution. An ascending trend from 50 ml (75%) to 150 ml (88%) in urease removal efficiency is observed. As we previously proved [14], the nanofilaments possess oxygen containing functional groups such as carbonyl and hydroxyl [18] that enable interaction i.e. hydrogen bonding with amino acid units of urease [19-21], thus their adsorption. In addition to hydrogen bonding, the positively charged amine groups of urease and the negatively charged oxygen containing functional groups of *a-CO_x* segments can electrostatically interact [21, 22]. On the other hand, major graphitic regions allow for π - π interaction with non-polar domains of urease. For a similar DNA-CNT system, van der Waals forces have been introduced as an adsorption driving factor with a larger impact than hydrophobic forces [23]. For the urease molecules, several intramolecular bonding between different functional groups could be also envisaged. Accordingly, some molecules interact through their less polar and non-polar zones with the nanofilaments [21]. Thus, collectively, different parts of the nanofilaments are able to adsorb urease molecules via interaction with their corresponding regions. This feature can stabilize the enzyme on its substrate and prevent

its conformational change that can lead to loss of enzyme activity, beneficial for a further application as e.g. a biocatalyst [21, 24, 25]. Moreover, huge surface area of the buckypaper minimizes the diffusion pathway for the reaction products, thus enhancing the efficiency of the immobilized enzyme [26]. ATR-FTIR spectra, Figure 5, clearly witnesses the adsorption of urease onto the nanofilaments. Before the adsorption, the strong peak located at 1589 cm^{-1} represents the unoxidized sp^2 C=C groups of the graphitic segments of the nanofilaments, resulted from the aromatization process during the thermostabilization of PAN nanofibers [27, 28]. The second evident groups at $1000\text{--}1300$ (two bands) and 3800 cm^{-1} imply C-OH bond [14]. After the adsorption, main chemical bonds related to urease emerge on the nanofilaments. The main characteristic peaks of urease are tabulated in Table 1.

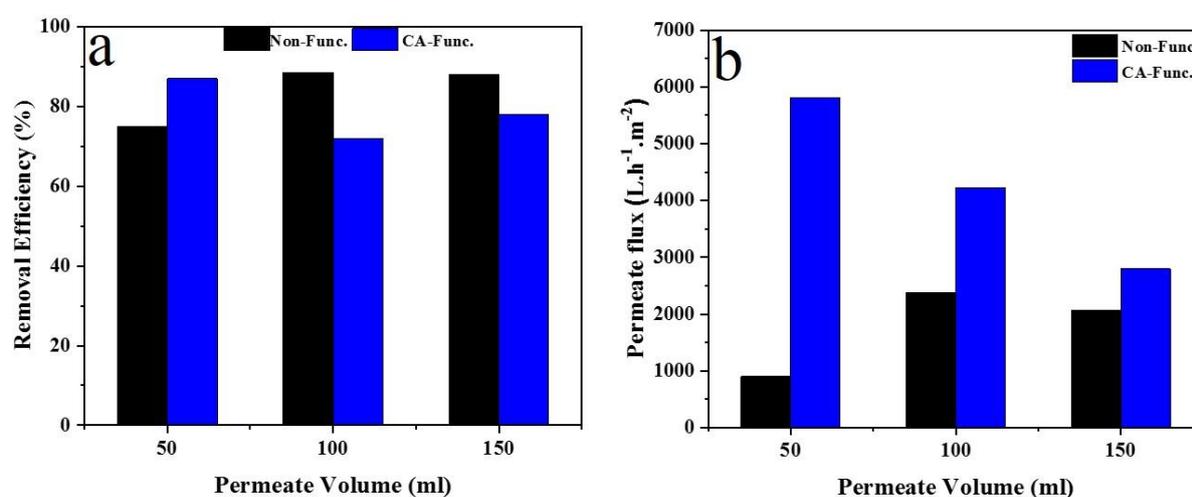


Figure 4. a) Urease removal efficiency, b) water permeability of the buckypapers in two classes of non-functionalized and CA-functionalized.

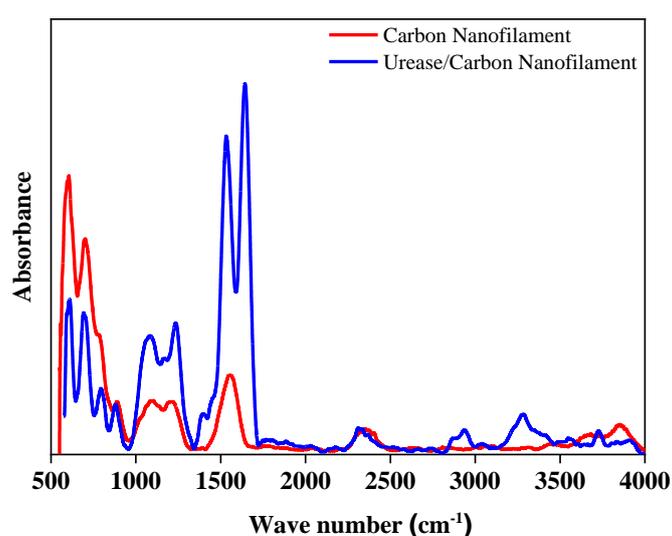


Figure 5. ATR-FTIR spectra compare surface chemistry of carbon nanofilaments before and after urease adsorption.

Table 1. FTIR peaks assignment for urease adsorbed on the carbon nanofilaments [29]

Wavenumber (cm^{-1})	Peak assignments
3343	N-H stretch of primary and secondary amines and amides
2967	-C-H stretch of alkanes
1698	-C = O stretch of carboxylic acids
1562	N-H bending of amine and C = O stretch of ketones and C = C of benzene
1320	-C-N stretch of aromatic amines
1172	C-N stretch of aliphatic amines
1011	C-N stretch of aliphatic primary and secondary amines, amide (peptide)
938	O-H bending of carboxylic acids

The increasing trend of urease removal efficiency witnesses that the adsorption of urease is robust and after further passage of the solution does not result in its release into water. This enhancement of removal efficiency can be attributed to a strong intermolecular interaction between the adsorbed urease molecules and solutes via peptide-peptide interactions [30]. Interestingly, adsorption of urease molecules enhances water permeability of the buckypaper, due to a hydrophilization effect, Figure 4b. In contrast to the non-functionalized samples, CA-functionalization slightly lowers removal efficiency as far as the filtration is continued. While a high efficiency of 87% is seen at onset of the experiment, it declines to 78% at 150 ml permeate volume. The reason could be sought at less available binding sites for urease molecules or even release of the previously adsorbed ones because of less graphitic regions that most likely have played a more important role in stable adsorption of urease molecules rather than polar groups (hydrogen bonding or electrostatic interaction). However, still efficiency is as promising as 78%. Noteworthy, water permeability for CA-buckypapers are significantly higher than that for the non-functionalized ones due to their hydrophilicity. The descending trend of water flux in this class of adsorbents could be attributed to their declined hydrophilicity compared to the neat or fresh CA-functionalized samples due to adsorption of the urease molecules. Slightly enhanced hydrophobicity along with accumulation of the

adsorbed molecules on the nanofilaments that lowers pore size, cooperatively increase resistance against water permeation.

The adsorption experiment performed here can be regarded as a proof of concept witnessing applicability of the buckypaper adsorbent in removal of urease molecules as a model for biomolecule pollutants from water. In this regard, taking into account the effect of environmental factors such as pH, temperature, ionic strength, adsorption time, and urease concentration, further experiments are in progress. The results will be later used in isotherm, thermodynamic and kinetic calculations.

As an extra bonus, the enzyme immobilization successfully performed here can be promising for further applications of the buckypaper with respect to biosensing, e.g. The buckypaper adsorbent can potentially act as a biosensor, as well. Adsorption of urease can change electrical conductivity of the nanofilaments thus the entire buckypaper. To validate this proof of concept, electrical conductivity of the buckypaper before and after adsorption of urease was recorded. As shown in Figure 6, CA functionalization can lower electrical conductivity of the buckypaper, due to inclusion of carboxyl groups that act as electron withdrawing elements, raising electrical resistivity [31]. In contrast, adsorption of urease enhances electrical conductivity notably, but with a lower rate for CA-functionalized buckypapers. One reason for the enhancement of conductivity could be formation of electron transfer bridges between the nanofilaments by urease molecules. This observation can be interpreted in another way i.e. bridging between enzyme molecules (i.e. the biosensing element) by the carbon nanofilaments. This phenomenon i.e. the direct electrical contacting of redox enzymes and electrodes through carbon nanomaterials have been reported earlier. Patolski et al.[32], showed this behavior by alignment of glucose oxidase enzymes on the SWCNTs' tips that were structured as an array on a conductive substrate. Exposure of the enzyme immobilized buckypaper to urea, often monitored in blood to track kidney diseases, can alter the electrical conductivity and be considered as the sensing mechanism for such an analyte. It is worthy to note that immobilization of enzymes is indeed the simplest technique that can tackle the bottleneck of their high solubility [33]. Enzyme immobilization allows tailoring of the bioreactions' conditions, thus enables a continuous process with minimum pollution by the reaction products, an extremely desirable characteristic in the food industry. Moreover, it guarantees an improved stability, lifespan and ease of separation of the enzyme from the reaction mixture at the end of the process [34], enabling cost efficiency and reuse of the enzyme. As mentioned earlier, immobilization can also lead to stabilization of biocatalysts, prevent their unfolding and immune the polypeptide bonds against rupture [25].

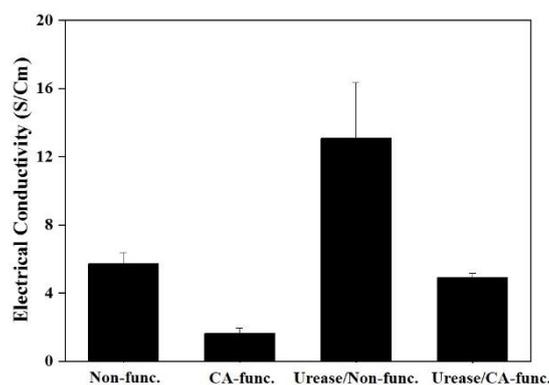


Figure 6. Electrical conductivity of the various classes of buckypapers before and after adsorption of urease measured via a 4-Probe test

Taken together, here, we devised a buckypaper adsorbent based on amphiphilic carbon nanofilaments that could separate urease molecules from water effectively. The separation tests were performed under dynamic conditions that could challenge the adsorbent more strictly. Promising selectivity and permeability of this novel adsorbent/membrane hold great promise for further development of the system for practical applications. Furthermore, firm immobilization of urease on conductive nanofilaments can assure us about efficiency of a potential biosensing system for a second further application.

Author Contributions

S.H. conceived the idea, prepared samples, performed characterizations and drafted the manuscript. M. E. was involved in development of the idea and analysis of the results.

Acknowledgements

M.E. appreciates the financial support provided through Aalto University, Academy of Finland, and Helmholtz Association (Grant No. VH-NG-523). The authors would like to acknowledge Kristian Bühr for the design of the water flux measurement set-up, and Joachim Koll for the bubble point test.

References

- [1] S. Ramakrishna, K. Fujihara, W.-E. Teo, T. Yong, Z. Ma, R. Ramaseshan, Electrospun nanofibers: solving global issues, *Materials Today* 9(3) (2006) 40-50.
- [2] S. Homaeigohar, T. Dai, M. Elbahri, Biofunctionalized nanofibrous membranes as super separators of protein and enzyme from water, *Journal of Colloid and Interface Science* 406(0) (2013) 86-93.

- [3] S. Homaeigohar, Y. Davoudpour, Y. Habibi, M. Elbahri, The Electrospun Ceramic Hollow Nanofibers, *Nanomaterials* 7(11) (2017) 383.
- [4] S. Homaeigohar, D. Disci-Zayed, T. Dai, M. Elbahri, Biofunctionalized nanofibrous membranes mimicking carnivorous plants, *Bioinspired, Biomimetic and Nanobiomaterials* 2(4) (2013) 186-193.
- [5] S. Homaeigohar, M. Elbahri, Nanocomposite Electrospun Nanofiber Membranes for Environmental Remediation, *Materials* 7(2) (2014) 1017-1045.
- [6] S. Homaeigohar, A.U. Zillohu, R. Abdelaziz, M.K. Hedayati, M. Elbahri, A Novel Nanohybrid Nanofibrous Adsorbent for Water Purification from Dye Pollutants, *Materials* 9(10) (2016) 848.
- [7] Z.W. Ma, M. Kotaki, S. Ramakrishna, Electrospun cellulose nanofiber as affinity membrane, *Journal of Membrane Science* 265(1-2) (2005) 115-123.
- [8] C.H. Bamford, K.G. Allamee, M.D. Purbrick, T.J. Wear, Studies of a Novel Membrane for Affinity Separations .1. Functionalization and Protein Coupling, *Journal of Chromatography* 606(1) (1992) 19-31.
- [9] Z. Ma, M. Kotaki, S. Ramakrishna, Surface modified nonwoven polysulphone (PSU) fiber mesh by electrospinning: A novel affinity membrane, *Journal of Membrane Science* 272(1) (2006) 179-187.
- [10] H. Zhang, H. Nie, D. Yu, C. Wu, Y. Zhang, C.J.B. White, L. Zhu, Surface modification of electrospun polyacrylonitrile nanofiber towards developing an affinity membrane for bromelain adsorption, *Desalination* 256(1-3) (2010) 141-147.
- [11] M. Elbahri, Sh. Homaeigohar, T. Dai, R. Abdelaziz, R. Khalil, A.U. Zillohu, Smart Metal-Polymer Bionanocomposites as Omnidirectional Plasmonic Black Absorbers Formed by Nanofluid Filtration *Advanced Functional Materials* 22(22) (2012) 4771-4777.
- [12] Z. Ma, Z. Lan, T. Matsuura, S. Ramakrishna, Electrospun polyethersulfone affinity membrane: Membrane preparation and performance evaluation, *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences* 877(29) (2009) 3686-3694.
- [13] P. Lu, Y.-L. Hsieh, Lipase bound cellulose nanofibrous membrane via Cibacron Blue F3GA affinity ligand, *Journal of Membrane Science* 330(1-2) (2009) 288-296.
- [14] S. Homaeigohar, T. Strunskus, J. Strobel, L. Kienle, M. Elbahri, A Flexible Oxygenated Carbographite Nanofilamentous Buckypaper as an Amphiphilic Membrane, *Advanced Materials Interfaces* 5(8) (2018) 1800001.

- [15] S.Sh. Homaeigohar, H. Mahdavi, M. Elbahri, Extraordinarily water permeable sol gel formed nanocomposite nanofibrous membranes, *Journal of Colloid and Interface Science* 366 (2012) 51-56.
- [16] R. Baker, *Membrane technology and applications*, 2nd edition ed., Wiley 2004.
- [17] S.Sh. Homaeigohar, K. Buhr, K. Ebert, Polyethersulfone electrosponed nanofibrous composite membrane for liquid filtration, *J Mem Sci* 365 (2010) 68-77.
- [18] C.A. Santini, A. Sebastian, C. Marchiori, V.P. Jonnalagadda, L. Dellmann, W.W. Koelmans, M.D. Rossell, C.P. Rossel, E. Eleftheriou, Oxygenated amorphous carbon for resistive memory applications, 6 (2015) 8600.
- [19] C. Mateo, J.M. Palomo, G. Fernandez-Lorente, J.M. Guisan, R. Fernandez-Lafuente, Improvement of enzyme activity, stability and selectivity via immobilization techniques, *Enzyme and Microbial Technology* 40(6) (2007) 1451-1463.
- [20] D.N. Tran, K.J. Balkus, Perspective of Recent Progress in Immobilization of Enzymes, *ACS Catalysis* 1(8) (2011) 956-968.
- [21] R. Pogorilyi, I. Melnyk, Y. Zub, G. Seisenbaeva, V. Kessler, Immobilization of urease on magnetic nanoparticles coated by polysiloxane layers bearing thiol- or thiol- and alkyl- functions, *Journal of Materials Chemistry B* 2(18) (2014) 2694-2702.
- [22] R. Pogorilyi, V. Goncharyk, L. Kozhara, Y. Zub, The influence on the activity of the adsorbed urease of structural and adsorption characteristics of polysiloxane matrices containing surface layer with 3-aminopropyl groups, *Surface* 1(16) (2009) 35-45.
- [23] H. Gao, Y. Kong, D. Cui, C.S. Ozkan, Spontaneous insertion of DNA oligonucleotides into carbon nanotubes, *Nano Letters* 3(4) (2003) 471-473.
- [24] A.M. Azevedo, D.M. Prazeres, J.M. Cabral, L.S.P. Fonseca, Stability of free and immobilised peroxidase in aqueous-organic solvents mixtures, *Journal of Molecular Catalysis B: Enzymatic* 15(4-6) (2001) 147-153.
- [25] P. Villeneuve, J.M. Muderhwa, J. Graille, M.J. Haas, Customizing lipases for biocatalysis: a survey of chemical, physical and molecular biological approaches, *Journal of molecular catalysis B: enzymatic* 9(4-6) (2000) 113-148.
- [26] A. Borole, S. Dai, C.L. Cheng, M. Rodriguez, B.H. Davison, Performance of chloroperoxidase stabilization in mesoporous sol-gel glass using in situ glucose oxidase peroxide generation, *Applied biochemistry and biotechnology* 113(1-3) (2004) 273-285.
- [27] M. Hu, B. Mi, Enabling graphene oxide nanosheets as water separation membranes, *Environmental science & technology* 47(8) (2013) 3715-3723.

- [28] G. Singh, D. Rana, T. Matsuura, S. Ramakrishna, R.M. Narbaitz, S. Tabe, Removal of disinfection byproducts from water by carbonized electrospun nanofibrous membranes, *Separation and Purification Technology* 74(2) (2010) 202-212.
- [29] S.F. D'Souza, J. Kumar, S.K. Jha, B.S. Kubal, Immobilization of the urease on eggshell membrane and its application in biosensor, *Materials Science and Engineering: C* 33(2) (2013) 850-854.
- [30] J.N. Barisci, M. Tahhan, G.G. Wallace, S. Badaire, T. Vaugien, M. Maugey, P. Poulin, Properties of carbon nanotube fibers spun from DNA-stabilized dispersions, *Advanced Functional Materials* 14(2) (2004) 133-138.
- [31] N. Lala, V. Thavasi, S. Ramakrishna, Preparation of Surface Adsorbed and Impregnated Multi-walled Carbon Nanotube/Nylon-6 Nanofiber Composites and Investigation of their Gas Sensing Ability, *Sensors* 9(1) (2009) 86-101.
- [32] F. Patolsky, Y. Weizmann, I. Willner, Long-range electrical contacting of redox enzymes by SWCNT connectors, *Angewandte Chemie* 116(16) (2004) 2165-2169.
- [33] H.E. Schoemaker, D. Mink, M.G. Wubbolts, Dispelling the myths--biocatalysis in industrial synthesis, *Science* 299(5613) (2003) 1694-1697.
- [34] I.R. Tebeka, A.G. Silva, D.F. Petri, Hydrolytic activity of free and immobilized cellulase, *Langmuir* 25(3) (2009) 1582-1587.