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Functionalization of Synthetic Polymers for Membrane Bioreactors

HAMIDREZA BARGHI

CHALMERS

Department of chemical and Biological Engineering

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Hamidreza Barghi

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School of Engineering
University of Borås
501 90 Borås
Sweden
Telephone: +46 (033)-4354000

Cover: Surface topography of the electroconductive membrane based poly (hydroxymethyl -3, 4-ethylenedioxythiophene-*co*-tetramethylene-*N*-hydroxyethyl adipamide)

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“Anyone who has never made a mistake has never tried anything new”

Albert Einstein

ABSTRACT

Membrane bioreactors (MBRs) show great promise for productivity improvement and energy conservation in conventional bioprocesses for wastewater reclamation. In order to attain high productivity in a bioprocess, it is crucial to retain the microorganisms in the bioreactors by preventing wash out. This enables recycling of the microorganisms, and is consequently saving energy. The main feature of MBRs is their permeable membranes, acting as a limitative interface between the medium and the microorganisms. Permeation of nutrients and metabolites through the membranes is thus dependent on the membrane characteristics, i.e. porosity, hydrophilicity, and polarity. The present thesis introduces membranes for MBRs to be used in a continuous feeding process, designed in the form of robust, durable, and semi-hydrophilic films that constitute an effective barrier for the microorganisms, while permitting passage of nutrients and metabolites. Polyamide 46 (polytetramethylene adipamide), a robust synthetic polymer, holds the desired capabilities, with the exception of porosity and hydrophilicity. In order to achieve adequate porosity and hydrophilicity, bulk functionalization of polyamide 46 with different reagents was performed. These procedures changed the configuration from dense planar to spherical, resulting in increased porosity. Hydroxyethylation of the changed membranes increased the surface tension from 11.2 to 44.6 mJ/m^2. The enhanced hydrophilicity of PA 46 resulted in high productivity of biogas formation in a compact MBR, due to diminished biofouling. Copolymerization of hydrophilized polyamide 46 with hydroxymethyl 3,4-ethylenedioxythiophene revealed electroconductivity and hydrophilic properties, adequate for use in MBRs. To find either the maximal pH stability or the surface charge of the membranes having undergone carboxymethylation, polarity and the isoelectric point (*pI*) of the treated membranes were studied by means of a Zeta analyzer. The hydroxylated PA 46 was finally employed in a multilayer membrane bioreactor and compared with hydrophobic polyamide and PVDF membranes. The resulting biogas production showed that the hydroxylated PA 46 membrane was, after 18 days without regeneration, fully comparable with PVDF membranes.

Keywords: Bioreactor, Functionalization, Hydrophilic, Membrane, Polyamide 46, Synthetic polymer

List of publications:

1- H. Barghi; M. Skrivards; M.J. Taherzadeh, Catalytic Synthesis of Novel Bulk Hydrophilic Acetaldehyde-Modified Polyamide 46, ***Current Organic Synthesis***, 2013, 10, 1-8

2- H. Barghi; M.J. Taherzadeh, Synthesis of an Electroconductive Membrane by Poly (hydroxymethyl 3,4-Ethylenedioxythiophene-*co*-tetramethylene-N-hydroxyethyl adipamide), ***Journal of Materials Chemistry C,*** 1**(39)** 2013, 6347-6354.

3- S. Youngsukkasem; H. Barghi; D.K. Rakshit; M.J. Taherzadeh, Biogas Production Using Compact Multi-layers Membrane Reactor for Rapid Biogas Production, ***Energies***, 2013, **6**, 6211-6224.

4- H. Barghi; M. J. Taherzadeh, Development of Water Permeability of Polyamide 46 via Carboxylation and PEGylation in Continues Phase, submitted.

List of other publications:

1- Majdejabbari, S.; Barghi, H.; Taherzadeh, M.J., Synthesis and Characterization of Biosuperabsorbent Based on Ovalbumin Protein, ***Journal of Macromolecular Science, Part A***, **2010**, 47, 708-715.

2- Majdejabbari, S.; Barghi, H.; Taherzadeh, M.J., Synthesis and properties of a novel biosuperabsorbent from alkali soluble *Rhizomucor pusillus* proteins, ***Applied Microbiology and Biotechnology***, **2011**, 92, 1171-1177.

3- Barghi, H.; Taherzadeh,, M.J., Bulk Hydrophilic Functionalization of Polyamide 46, 2013, ***WO patent*** 2013058702.

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Nomenclatures

AFM	Atomic force microscopy
CNMR	Catalytic non-permselective membrane reactor
CMR	Catalytic permselective active membrane reactor
^{13}C-NMR	Carbon nuclear magnetic resonance
DMSO	Dimethyl sulfoxide
DSC	Diffraction scanning calorimetry
ECM	Electroconductive membrane
ECP	Electroconductive polymers
ESEM	Environmental scanning electron microscopy
FT-IR	Fourier transform infrared spectroscopy
^{1}H-NMR	Proton nuclear magnetic resonance
HMEDOT	Hydroxymethyl 3, 4-ethylenedioxythiophene
ΔH_c	Enthalpy of crystallization
ΔH_m	Enthalpy of melting
LDV	Laser doppler velocimetry
MBRs	Membrane bioreactors
MF	Microfiltration
NF	Nanofiltration
NMR	Non-permselective membrane reactor
PBMR	Packed bed membrane reactor
PHMEDOT	Polyhydroxy 3, 4-ethylenedioxythiophene
PVDF	Polyvinylidene difluoride
PES	Polyethersulfone
PA 46	Polyamide 46
PEG	Polyethylene glycol
pI	Isoelectric point
UF	Ultrafiltration

T_m	Melting temperature
T_c	Crystallization temperature
T_g	Glass transition
TGA	Thermogravimetric analysis

1

Introduction

Rapid high- yield biological production is the main ambition in industrial bioprocesses today. A surge of interest in membrane bioreactors is hence presently opening for new prospects in the bioprocess research agenda. The term membrane bioreactor (MBR) refers to versatile bioreactors used in bioprocesses to attain rapid production and reduced energy consumption. A membrane bioreactor is a biological appliance for carrying out biochemical reactions in the interface between the membranes and the living cells. Membranes consequently play a major role, constituting the porous solid barrier between the microorganisms and the nutrients that helps to extend the biochemical interaction at the interface, but with minimal impact on the living cells. The pores allow nutrient solutions to diffuse and disperse through the membrane, while hindering entrapped cells from freely dispersing into the medium. As a result, the surface of the membranes develops catalytic activity, either by high affinity toward the nutrients or by forced assimilation of the microorganisms [1]. An increased separation aptitude of the membrane improves the catalytic reaction. This is achieved through a selective separation by functioning as an *extractor*, or by a selective sorption, functioning as a *distributor*. This means that in an MBR, these two functions can be attained simultaneously, by controlling pore size as well as polarity. Thus, selective separation is a screening phase based on particle size, whereas selective sorption is a thermodynamic feature of the membrane that is dependent on the polarity of permeates and membrane. The ability of extraction or distribution is related to the affinity of the solutes to the membrane. This means that membranes displaying high interaction with the solutes are able to integrate with water-soluble gradients, while in the case of low interaction, the extraction feature

will dominate. In this context, the hydrophilic property of the membrane in a submerged MBR aids a uniform continuous feeding of entrapped microorganisms. Moreover, any negative charges, like those occurring in cytoplasm membranes (e.g, yeast), are repelled by the negative charges of anionic membranes [2, 3]. This is the most advantageous feature of the negative charged membranes, since it prevents attachment of microorganism onto the surface of membrane. Consequently, the research in this thesis aimed at optimizing the synthesis of functionalized membranes in order to acquire a cross-flow operation system, leading to an increased flux rate along with reduced biofouling.

The purpose was thus to design and synthesize a robust and durable membrane to be exploited in MBRs. Impact on living cells and their environment is a critical quality aspect of membranes in MBRs, and the aim was thus to synthesize polymers that did not have such impact. Selectivity of the membranes was another priority, and was tackled by controlling pore size and surface charge. Stability at high concentrations of electrolytes, high pH levels, and at elevated temperatures during membrane sterilization are other advantages of the synthetic membrane proffered in this theses. Furthermore, the greatly reduced biofouling of the membranes, attained by hydrophilization and negative charges, resulted in a high flux of nutrients and metabolites. Since the synthetic membranes were to be applied in a submerged MBR, capability of welding and fixation in a multilayer bioreactor was other parameters taken into consideration. The hypothesis proposed that retaining of *Saccharomyces cerevisiae* and biogas microorganisms would be particularly effectively retained in a submerged membrane bioreactor. As biogas and CO_2 are being produced during the fermentation or digestion process, an accumulation of gases inevitably occurs inside the membranes. In order to prevent the membranes from being fluidized, fixation by metallic frames kept them below the medium surface. Chemical resistance, durability, reduction of biofouling and bioadhesion, low impact on living cells and on the extent of nutrients flowing through the membrane were the main criteria to be met in producing a synthesized membrane.

The present thesis comprises five chapters, where chapter 2 describes the background and the various conventional MBRs used for batch- and continuous bioprocessing. It furthermore

describes the fouling agents occurring on membranes during biological processes, the background of filtration, mass transport through the membranes, polarity of membranes, biomembranes, synthetic membranes, applications, classification, and methods of preparation (papers I-IV). Chapter 3 put forward the possibility of upgrading polyamide 46, by means of functionalization, bulk and surface, hydrophilically or hydroelectrically. This chapter also covers analytical techniques for assessing the quality of final products, such as surface charges, hydrophilicity, presence of functional groups, heat stability, crystallinity, pore morphology, porosity, and water flux (papers I-IV). Chapter 4 describes the effects of feeding flow and performance of the synthesized membranes on the process in a compact multi layer membrane bioreactor (paper III). Finally, chapter 5 presents conclusions and future work.

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Membrane bioreactors

Application of membranes in bioreactors has been prompted into two directions: first, the capability of producing high flux and defect-free membranes on a large scale, and second, the aptitude of such membranes to function in a compact, high surface area, as well as being constructed as economically viable modules. These demands manifest an ambition to select apt membrane types to be applied in MBRs, paying specific attention to productivity, separation selectivity, shelf life, cost effects, and chemical/mechanical reliability in optimized operating systems. Developing new membrane materials in accordance with these parameters is a key factor for increasing the degree of application of membranes in a multitude of areas. However, for applications in MBRs, acquiring the maximal mass transport through the membrane is the main concern. Generally, two main features are expected from MBRs: functioning as selective separator (extractor) and as selective absorbent (distributor) [4]. Selective separation for screening can be achieved by modifying the pore size of the membranes, and sorption capacity is completely linked to polarity and surface tension of the membranes [1]. Although some types of permselective membranes integrate well with a variety of MBRs, this is not true for all of them. The general goal when constructing membranes for MBRs is less fouling and a high flow rate of effluents. Regardless of the design of the MBR, all membranes used in this type of reactor are expected to accomplish a phase separation between the microorganisms and the medium. Furthermore, one of the advantages microorganisms being entrapped in an exclusive MBR is protection from inhibitors in the feed flow (paper III). The absence of membranes in a reactor increases the risk of bioprocess failure when using industrial wastewaters, due to its content of

inhibitors and toxic materials.[5] The main industrial applications of MBRs comprise aerobic wastewater treatment, and few industries utilize anaerobic MBRs for wastewater treatment.[5] The application of membranes in MBRs therefore mainly concerns pore size distribution and surface charge density. Due to its membranes, the separation process in MBRs is improved in terms of energy and time consumption in comparison with traditional separation methods (centrifugation or sedimentation), making MBRs suitable for water reclamation and desalination in a continuous process at large-scale, as the purpose of the process in this case is to eliminate very small particles [6]. The driving force behind mass transport through membranes is dependent on the concentration of solutes on each side of the membrane, and also on the pressure applied by the fluids on each side of the membrane. The pressure causes metabolites to leak from the entrapped microorganisms into the surrounding medium, which comprises the yield of the MBR. In contrast to conventional high flow rate filtrations using filter-press or centrifuge for separation any particles larger than 10 μm in batch-wise separation, MBRs due to micro pores of membranes are more competent for continuous feeding operation [7].

Figure 2.1 illustrates the configuration of membranes to be used in reactors, showing functionality and position of the membrane [8]. Figure 2.1A shows a catalytic non-permselective membrane reactor (CNMR). In this scheme, an interface contactor provides the separation, selective and steric constraints being placed at the intermediate phases between the membrane (solid) and the fluid (liquid) [9]. Figure 2.1B represents a catalytic membrane reactor (CMR). In this type of MBR, a permselective layer accomplishes the catalytical reactions, either in the form of an inherent catalytic coat, or as an active phase attached on an intermediate polymer layer, or as an active phase embedded in the membrane matrix[10]. Figure 2.1C indicates a non-permselective membrane reactor (NMR) that is passive without any permselective coating. Inside the reactor, membranes, reactants, or microorganisms for chemical/biochemical reactions are distributed, and the selectivity of the permeates is screened only by means of pore size and distribution [11]. Figure 2.1D is a packed bed membrane reactor (PBMR), which is an inert permselective membrane reactor, in which reactants or catalysts are distributed inside the membrane tubes. After systematic reactions, retenates and permeates diffuse through the

membrane in different directions [8, 12]. This type of membrane is only used in a sidestream MBR [13].

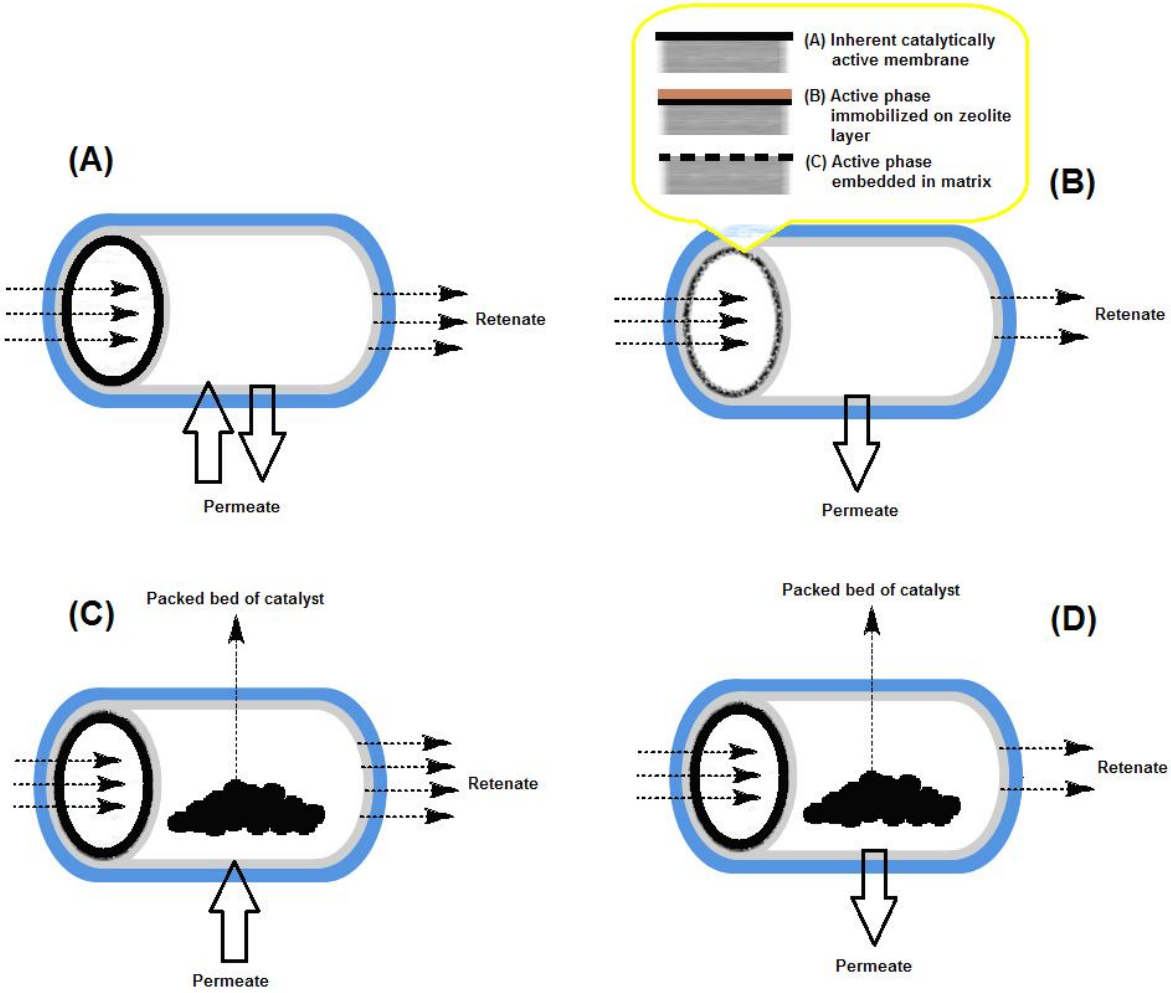

Figure 2.1 Configuration of membranes to be used in reactors: (A) Catalytic non-permselective membrane reactor (CNMR), (B) Catalytic membrane reactor (CMR), (C) Non-permselective membrane reactor (NMR), (D) Packed bed membrane reactor (PBMR).

2.1. MBR for wastewater treatment

Commercial membrane bioreactores are developed for municipal wastewater treatment[14], and the role of the membranes in these bioreactores is to filter and clarify water. Because of a high flow rate of the effluent, retaining activated sludge might help saving energy as well as stabilizing the biological process. The design of the platform of MBRs is based on the type of effluent. Dorr-Oliver introduced the first commercial MBRs for wastewater treatment in the late 1960s [15]. Further work was published by Bemberis *et al.* in 1971, who used an ultra-filtration (UF) membrane for filtering ship-board sewage [15]. Figure 2.2 shows conventional MBRs for waste water treatment in the form of sidestream and submerged MBRs [14]. In the sidestream platform (Figure 2.2A), the membrane module is located outside the reactor as a discrete unit, and the feed flow is being recycled between two units; the concentration of permeate is then gradually increasing. In contrast, in a submerged MBR, modules are immersed in the medium, leaving the surface module in contact with effluent. Apparently, the microorganisms are in both designs freely fluidized inside the bioreactors, whereupon the membranes are bringing about the separation. It should be noticed that permeates contain either metabolites or feed components. These two methods are suitable for wastewater treatment, where the final metabolites are gases (CH_4 or CO_2) rather than liquids. This motivates research aiming at developing an MBR design for fermentation processes of liquids, such as ethanol fermentation.

One advantage of the sidestream MBR is that it is easy to clean prior to membrane regeneration [16]. This type of MBR is appropriate for large dimension bioreactors with a high flow rate effluent, but has a drawback in that it produces low yield. Challenging the sidestream MBR, the submerged design produces higher yield due to the membrane having a much larger surface in contact with the sewage. This design also lowers the energy consumption (energy being conveyed to the medium) due to a positive pressure of the medium on the surface of the membrane (figure 2.2B). Complicated cleaning and regenerating processes along with operational costs are major disadvantages of this type of MBR [17].

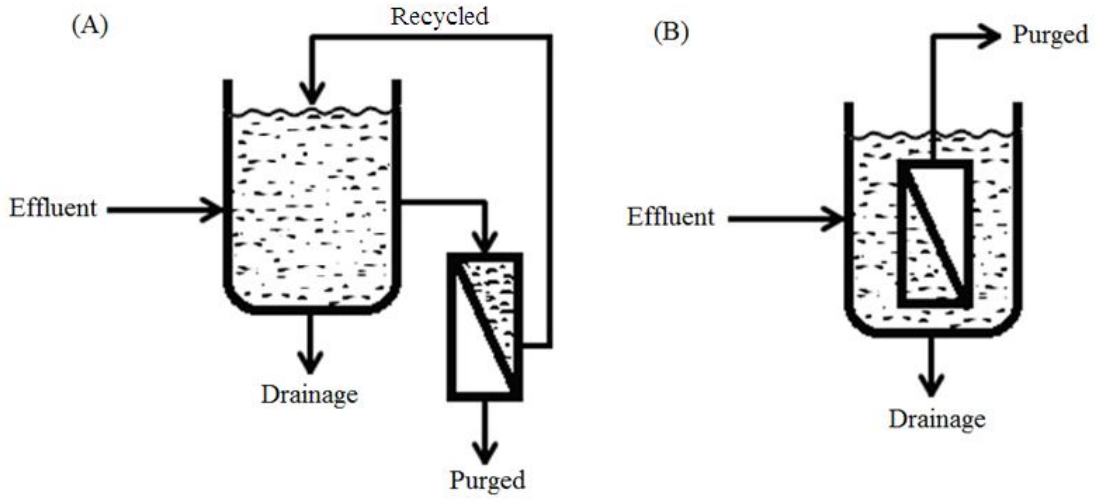

Figure 2.2 Schemes of an anaerobic bioprocess in two types of MBRs: a sidestream membrane bioreactor (A), a submerged membrane bioreactor (B).

The choice of membrane in the MBR designs is founded on flow rate, size of microorganisms, chemical constitution of feed flow, temperature of reactor, and environment. In specific, the membranes introduced have comprised organic, inorganic, and hybrids of organic/inorganic structures [1]. Membranes to be used in MBRs and other types of bioreactors are selected in accordance with the corresponding kinetics of their production, the shelf life of the membrane constitution, their separation selectivity (which is based on the size of particles), and their chemical/mechanical reliability at operational terms, along with capital costs. Applying new membrane materials is however the key for developing MBRs having catalytic properties [1]. The main advantages of MBRs are: high performance, high flow rate of effluents, selectivity in the final products, high flow shocking of the microorganisms is prevented by sufficient adaptation time, as the bioprocess is being retained, which also leads to stability, which is needed in continuous operations [12]. According to the literature, the main motivation for changing the traditional design of MBRs is to enhance the separation performance or to diminish membrane fouling [18]. Fouling of the membranes is one of the problems affecting the commercialization of the MBR technology [12]. Two other factors concerning the performance of the membranes are: (i) the mechanical reliability during cleaning and (ii) stability against chemicals during operation.

The purpose of applying MBRs in bioprocesses is the degradation of residues, or their separation from the downstream formed by the chemical or biological process, in a commercial and feasible process. Since the downstream separation process is expensive because of the high dilution, or is not practicable in some cases, the only alternative in order to defeat the problems involved in industrial implementation, is high yield biocatalytical degradation [19]. Continuous bioprocesses in MBRs hold some advantages in comparison with batch bioprocesses. They are capable of performing at low concentrations of feed, and display tolerance toward inhibitors if they are mixed with the culture medium, due to their low concentrations. They also show a low capital cost.[20] Retaining viable microorganisms is also a main purpose for using MBRs; for prevention of physicochemical shocks, microorganisms need time to adapt [21, 22].

2.2. MBR for ethanol and biogas production

Bioethanol and biogas (methane) are the two main biofuels, which can substitute fossil fuels today. Industrial production of these two fuels can reduce demands for fossil fuels in an ideal situation. Since biofuels can be obtained from biomass such as municipal feedstocks or forest industries, development of a large-scale production line is a promising project in terms of fulfilling future needs of fuel, rendering it great importance. The main criteria for biological production of ethanol and methane are consequently low costs and high yield of final product. In anaerobic digestion, the amount of methane and carbon dioxide produced is reliant on the degree of interaction between digestion cells and organic matters. The digestion process comprises four steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis [5]. The last step, methanogenesis, is a very sensitive step in the digestion process; methanobacters grow very slowly, and are very sensitive to inhibitors, pH- and temperature changes. MBRs have shown to be adequate reactors for the digestion process since wash out is stopped, and the long retention times prevent stockpile of inhibitors [23]. Ethanol is mostly produced by fermentation of starch and various sugars. Lignocellulose hydrolysate is another option to be used as feedstock for fermentation by microorganisms [24], but requires a complete hydrolization into fermentable sugars prior to fermentation. During this process, toxic components with inhibitory properties are

released into the medium. This can be overcome by applying various strategies, e.g. increasing the density of cells [25], utilizing MBRs [26], and detoxification [27].

2.3. Membrane fouling in bioreactors

Membrane fouling is defined as unwanted deposition and accretion of microorganisms, colloids, solutes, and cell debris inside the membrane pores [28]. Because of physical interaction between the suspended living microorganisms and the membrane components, their linkage becomes reversible. This type of complex, formed after adsorption onto the membrane surface, reduces the water fluxes [29]. Fouling increases the energy consumption resulting in high operational costs [18]. Three factors have an impact on the accumulation of living cells on the membrane surface: (i) membrane and module features, (ii) feed constitution, and (iii) operation conditions. When an operating flux turns into a subcritical flux, particles accumulate inside the pores, which with time dramatically reduce the operating feed flows in cross-flow filtration. Furthermore, the biological debris sediments and compiles over the membrane surface, diminishing the permeate flow. The latter problem not only leads to a reduced flow rate of permeates, but also increases the consumption of energy needed for the permeate transfer across the membrane.

Fouling rate has been proven to be inversely proportional to particle velocity [30]. Settling velocity (ω_s) is inversely proportional to shear rate, and is regarded as a function of the *particle Raynolds number*, using the rules of *Stocke* and *Drag* [31]. Consequently, high velocity of gradients causes viscosity reduction due to higher shear rate, which results in higher dispersion of biofilm over the membrane surface. Moreover, because of physicochemical properties of the solute and the membrane constitutions, the membrane flux will diminish over time, even when critical flux conditions are exceeded [2]. This phenomenon is substantially dependent on the charges on the membrane surface, and on the interaction between colloidal particles and the membrane surface in an aqueous medium [32-34].

Physical cleaning, such as air/permeate backwashing and sonicating, are the methods used to remove biofouling. Chemical methods are introduced for any membranes that are resistance

toward chemical reagents. This method inhibits to degrade biofouling using oxidation/reduction for further operation. Finally, an estimation of the deposition rate of biofilm spreading over the membrane surface must take several parameters into account, such as thermodynamic properties, the concentration of culture medium, a physicochemical characterization of the cell debris, and a characterization of the membrane surface.

2.4. Diffusivity and permeability of membranes

Membranes are permeable sheets, and show great diversity in chemical structure and pore size. Membranes are used to separate, purify, and concentrate liquids and gases, consuming minimal amounts of energy. The first study on membranes and osmotic pressure was reported by Nollet in 1748, and concerned extraction of ethanol from other impurities, using porcine urinary bladder. He observed a relationship between osmotic pressure and flux rate in a natural semipermeable membrane [6]. Later, Graham (1833) conducted systematic research on mass transport through membranes [6].

Membranes, being physical barriers, are able to sift by three mechanisms; dead-end filtration, cross-flow filtration, and hybrid flow filtration. Dead-end filtration is the most common filtration mechanism in batch operations (Figure 3.1A). Since feed flow and permeate are unilateral currents, accumulation of filtered matters on the surface of the membrane quickly leads to clogging of the pores. To improve/enhance the flux, it is crucial to apply stages of membrane cleaning in each batch.

Cross-flow filtration is an improved method, as it decreases fouling as well as increases flux rate (Figure 3.1B). This is a suitable alternative method to dead-end filtration when continuous filtration with lower fouling is required. A nearly uniform spreading of the filtered matters over the surface of the membrane prolongs the filtration capacity. To acquire an increased flux rate, the surface area of the membrane as well as the feed flow need to be increased. A higher flow

rate of feed creates a turbulent flow in a tubular system, and will prevent accumulation of filtered matters on the surface of the membrane.

The hybrid flow filtration is a combination of the dead-end and the cross-flow filtrations (Figure 2.3C). A countercurrent feed flow in a closed system is the driving force behind transversal diffusion of permeates, although the consequence of the collision of two feed streams will be an aligned flow of permeates. This method qualifies for the separation of suspended particles in a low concentration feed, particularly in a water treatment process.

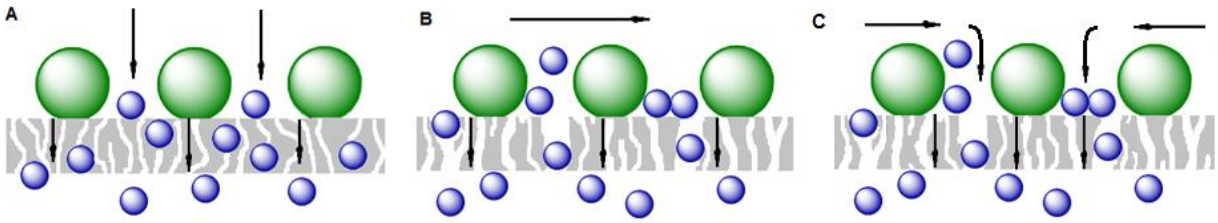

Figure 2.3 Diffusion of permeates through a membrane: (A) dead-end filtration, (B) cross-flow filtration, and (C) hybrid-flow filtration.

Polar membranes are able to promote the flux rate of polar permeates [6]. Non-polar membranes cannot actively bind to the solutes as they have no affinity to them, and consequently, a transverse diffusion is directly dependent on pore size and water flux. Diffusion through polar hydrophilic membranes is not entirely correlated to pore size; affinity of the solutes to the membrane, followed by polarity and the concentration of permeates on both sides of the membrane (the osmotic pressure) are also decisive factors. Diffusion of permeates continues until equilibrium is reached, i.e. the concentrations are equal on both sides of the membrane [35].

The flux rate in a non-polar membrane depends on the viscosity gradient (η), membrane pore tortuosity (τ), pressure gradient (p), and thickness of the membrane (z), as *Hagen–Poiseuille* formulated for a membrane containing parallel cylindrical pores [6].

$$J = \frac{\varepsilon r^2 \Delta P}{8\eta\tau\Delta Z} \qquad (2.1)$$

where,

J is the flux (L/m^{2}h)

ε is surface porosity of the membrane

r is the pore radius in the membrane (m)

η is the viscosity gradient (N·s/m^2)

ΔP is the difference in pressure gradient across the membrane (N/m^2)

τ is the ratio of pore length to the distance between the ends (thickness of membrane), which may be ≥1

ΔZ is the membrane thickness (m) (distance between the ends)

The flux rate of packed sphere membranes is calculated by using the *Carman-Kozeny* equation[36]:

$$J = \frac{\varepsilon_m^3 r^2 \Delta P}{K\eta\Delta Z_m S_m^2 (1-\varepsilon_m)^2} \qquad (2.2)$$

where,

J is the flux rate (L/m^{2}hr)

S_m is the internal surface area of the pore/unit volume (1/m)

ΔP is the pressure difference (N/m^2)

K is the Carman-Kozeny constant

ΔZ_m is the membrane thickness (m)

η is the viscosity of the liquid (N·s/m^2)

ε_m is the surface porosity

The flux rate of the constituents through a polar membrane is not only dependent on the parameters mentioned above, but the chemical nature and electrochemical potentials of the

permeates are also decisive factors in the mass transport rate.[6] This means that the flux rate may be facilitated by permeate and membrane having opposite electrochemical potentials, while having the same electrical charges will impede the flow [34].

Membranes can be either biological (natural) or synthetic, and they differ completely in terms of structure, functionality, and diffusivity. Biological membranes are e.g. the outer layer of living cells, while synthetic membranes are hand-made films, easy to manufacture. Using biological membranes as smart devices entail an inherent complexity, due to their specific task of transportation in and between living cells; and selective sorption and desorption, which facilitate transport over biological membranes always consume energy. A vital role of the cell membrane for metabolism in some microorganisms has furthermore been proven [37]. Biological membranes contain a bilayer of anionic phospholipids, where integral proteins are embedded. Water-soluble molecules can only diffuse through the cell membrane via the integral proteins, and fat-soluble molecules penetrate through the membrane via the bilayer of phospholipids. Sensitivity to elevated temperatures and pH-values, weakness pertaining to cleanup, susceptibility to microbial degradation due to their natural origin, are disadvantages limiting the operational use of biomembranes. In contrast, synthetic membranes are not as complex as biomembranes, and their functionality and properties can be manipulated by chemical reactions in order to achieve desired features. Accordingly, the work of the present thesis focused on the application of synthetic membranes in MBRs, where the use of synthetic membranes is technically achievable.

2.5. Synthetic membranes

Solid synthetic membranes refer to selective barriers, and the membrane constitution is prepared by using synthetic polymers for microfiltration (MF), ultrafiltration (UF), or nanofiltration (NF) purposes [38]. An imperative role of membranes as separation barriers is undeniable when striving to manufacture high purity products in the food and pharmaceutical industries, or produce potable water from seawater, eliminating industrial effluents and toxic components, and similarly

to recover valuable ingredients, separate gases from undesired residues, and of course for blood dialysis. The separation occurs by physical means at ambient temperature, without the ingredients undergoing chemical change, which is an imperative feature for biomass extraction, which is sensitive to elevated temperatures. Figure 2.3 demonstrates the classification of the synthesized membranes into homogenous or heterogeneous, symmetric (figure 2.4A) or asymmetric (figure 2.4B), neutral, positive, negative, or bipolar charges, based on constituents, morphology, pore geometry, and the charge distribution [39].

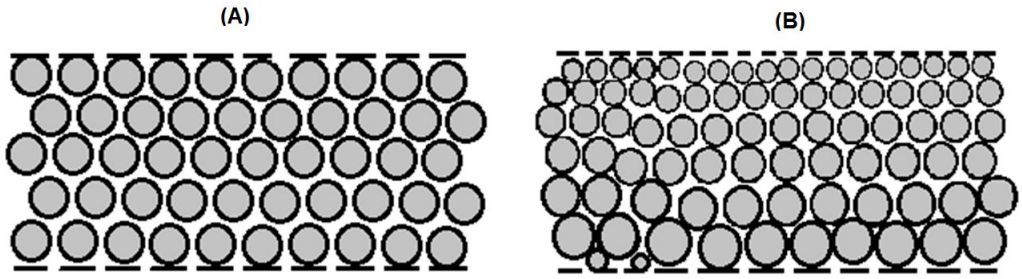

Figure 2.3 Geometry of packed sphere membranes: (A) symmetric (isotropic) membrane, (B) asymmetric (anisotropic) membrane.

Synthetic membranes are furthermore subdivided into organic (polymeric) and inorganic (ceramic, glass, metal) membranes. The operational temperature for organic membranes is 100-300 °C, while for inorganic membranes it is over 250 °C. Inorganic membranes are resistant to chemicals (oxidative and reductive), and display robustness in a wide range of pH-values (acids and bases).

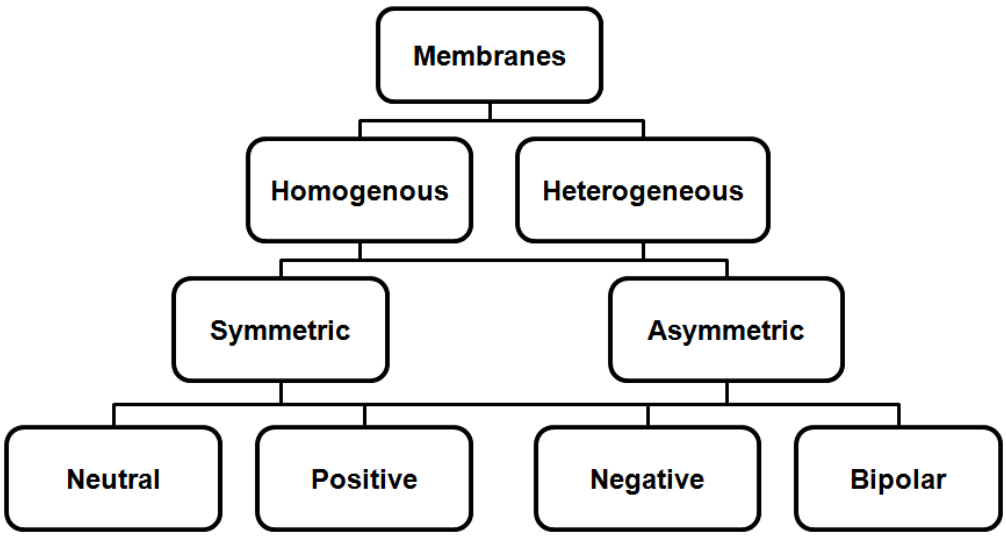

Figure 2.4 Classification of solid synthetic membranes, based on constituents, morphology, pore geometry, and charges.

Homogeneity of a membrane is pertaining to its constitution, which means that polymeric membranes composed of a single component are completely uniform, whereas membranes composed of more than one component lead to heterogeneity. Homogenous (isotropic) membranes may be macroporous, microporous, or nanoporous, being homogeneous in terms of pore size, while heterogeneous (*Anisotropic*) membranes are heterogeneous in terms of varying pore size, layer by layer. Mineral and composite membranes like glass, zeolite, metal, and ceramic, are some examples of anisotropic membranes [7]. Studying pore geometry may aid the prediction of transport and filtration rates through the different membranes. High flux rates are for cost-effective reasons desired in anisotropic membranes. In order to maintain reasonable mechanical properties for conventional purposes, and to avoid defects, the optimal thickness of anisotropic membranes is limited to about 20 μm [7].

Membranes with symmetric structure have maximal homogeneity in terms of porosity and composition. Unlike the casting solution method, the melt casting method always yield a symmetric configuration of the membrane, due to the cooling process being simultaneous and consistent, whereas the solution casting technique most often results in an asymmetric membrane. This probably relates to different rates of solvent evaporation at the air-solution and

cast-solution interfaces. Since the rate of solvent evaporation close to the air interface is much faster, the pore size at the air interface is larger than at the cast interface. Therefore, pore size may be varied in the different layers of nonporous, microporous, and macroporous membranes[38]. Figure 2.5 illustrates reverse osmosis (nanofiltration), ultrafiltration, microfiltration, and macrofiltration using different average pore diameters in standard membranes [7].

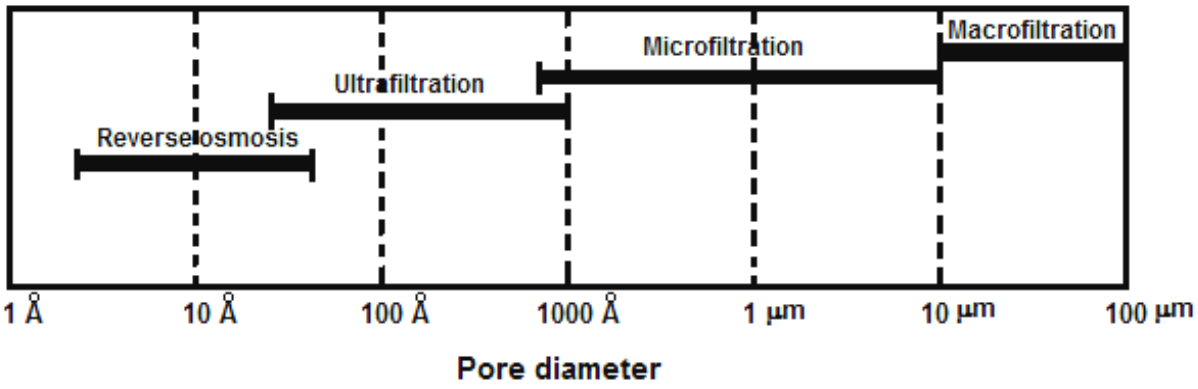

Figure 2.5 Mean values of pore diameters used for standard filtration in reverse osmosis (nanofiltration), ultrafiltration, microfiltration, and macrofiltration.

The electrical charge of the membranes is decided by the type of charge of the free functional groups on their own molecules. Anionic functional groups, such as carboxylate, sulfate or sulfonate groups that are positioned at the side or terminally, exhibit negative charges toward the cations in the surrounding liquid, and are labeled cation-exchange membranes, whereas free cationic functional groups, such as quaternary ammonium, leads to the membrane being positively charged, and these are called anion-exchange membranes. This membrane type is also used in electrodialysis, in order to separate negative and positive electrodes. Separation by means of charge is obtained by exclusion of the same charge, as the ions bind to the membrane, and this is more effective than separation based on pore size. Membranes lacking functional groups will obviously be neutral. In bipolar cell membranes, negative charges are situated at the two surfaces (inside/outside) of the membrane, while positive charges are oriented toward the middle layer of the membrane[38].

Nonporous membranes consist of a compact polymeric film, through which solutes are diffused under osmotic pressure or through the electrically charged gradients, caused by different permeate concentrations at the two surfaces of the membrane.

High cross-flow rate MF and UF membranes are not always made completely hydrophilic for commercial applications, since increased hydrophilicity reduces mechanical properties[14]. Absolutely hydrophobic polymers, such as polyethylene (PE), polypropylene (PP), polytetrafluoroethylene (PTFE), polyvinylidene difluoride (PVDF), polyethersulfone (PES), polyacrylonitrile (PAN), and the polyamide (PA) family, are also not desirable [14], because of low water cross-flow and considerable fouling properties. Hydrophilic functionalization (surface or bulk), post-treatment, or blending with a hydrophilic polymer would enhance the hydrophilic property in such polymers, resulting in semi-hydrophilic membranes, which are well suited for employment in MBRs [14].

Classification of membrane properties is based on membrane nature, geometry, and means of separation. Membranes for commercial applications should have a reasonable price tag, and robustness and flexibility must be adequate, preventing rupture and attenuation. Resistance toward hydrolization of the chemical bonds in the polymeric backbone is another major criterion for use in submerged bioreactors. High polarity of the electrolytes in the medium at moderately elevated temperatures easily causes the polymeric backbone to rupture. Hence, the membranes need to be chemically inert at moderate conditions. However, tolerance toward a wide range of pH-values is advantageous for acid/alkaline cleanup of the bioreactor. PS, PES, and PVDF have shown to be the most robust polymers, chemically as well as mechanically, holding mid-range prices. Limited solubility in common solvents, along with resistance to chemical modification are the main advantages of these polymers when applied in MBRs [14]. Blending with hydrophilic polymers such as polyethylene glycol (PEG) or polyvinylpyrrolidone [39] or wet spinning techniques is recommended [40,41]. However, PVDF has demonstrated obstinacy against hydrophilic modification, and combining the polymers with hydrophilic additives might lead to

weakening of the structure [42]. Thus, the wet spinning technique is recommended for constructing a PVDF membrane [40]. Polymers with a high degree of crystallinity are not fitted for use in MBRs, but hydrophilization or post-treatment can reduce the degree of crystallinity, and might thus be advised for such polymers when used for membrane applications.

The majority of the PVDF membranes have a relatively hydrophobic structure, resulting in their characteristics being very close to unmodified polymers [14]. In addition, minimal biofouling as undesirable deposition achieves when surface of membranes are completely hydrophobic [43, 44]. Biofouling, resulting from a deposit of biomass on the membrane surface, blocks the membrane pores, and may as a consequence dramatically reduce the yield in MBRs. A hydrophilic treatment of the membranes prevents however fouling, resulting in an increased flux rate. Stability against mild-elevated temperatures is another desirable feature of polymeric membranes to be used in MBRs. Pendant groups are easily released from the backbone of polymers at high temperatures (paper I), but cross-linking or cross polymerization can moderate this instability (paper II).

2.6. Methods of membrane fabrication

The characteristics of each membrane depend on the method of preparation. There are two aspects to consider in order to successfully manufacture high performance membranes aimed for specific applications: selection of appropriate materials and the method of fabrication. Selection of materials must be founded on their physicochemical properties. Although techniques for preparing membranes are abundant, the common main methods are recommended, i.e. solution casting, expanded film, track-etching, template leaching, phase separation, interfacial polymerization, solution coating of composite, and plasma polymerization.

2.6.1. Solution Casting

The solution casting method is one of the easiest techniques for lab-scale membrane fabrication. A polymer solution is spread onto a flat glass mold, using a stainless steel casting knife to disperse the highly viscous solution uniformly, simultaneously preventing the solution from running off the edge of the mold. Allow the solvent to evaporate from the polymer solution, leaving behind an even, flat membrane film.[7] Ideal solvents are moderately volatile. Highly volatile solvents result in fragility and a mottled surface, whereas a high boiling point causes absorption of the atmospheric moisture, whereupon the polymer precipitates, forming a hazy and dense surface. After drying the membrane completely, the cast is immersed in water in order to separate it from the cast. If polymers are semi-permeable, adding leachable components is not necessary; if not, adding porosity-inducing agents is inevitable.

The membrane preparations in the work of this thesis were based on the solution-casting method. In this method, 1 g of functionalized polymer was dissolved in 10 mL formic acid and then poured into a glass plate with the area 56.7 cm^2. After the solvent had evaporated at room temperature, the membrane was immersed into tap water in order to separate it from the glass mold. Membranes were dried in an oven at 50 ± 5 °C (papers I-IV).

2.6.2. Expanded film

Some polymers that do not completely dissolve in common solvents at room temperature are better suited for forming expanded film membranes. Polyethylene (PE), polytetrafluoroethylene (PTFE), and polypropylene (PP) are nearly insoluble polymers, and thus, solution casting is not a proper method for making membranes from these polymers. Annealing (heating followed by slow cooling) is performed in order to align the orientation of the crystallite part of the semi-crystalline polymer. After a second annealing, the film is heated and stretched up to 3 times. During the second time, the elongation of the amorphous region results in pores forming between the crystallite parts. Pore size can be controlled by the amount of film being stretched. The polytetrafluoroethylene (Teflon) membrane produced with this method is known as Gore-Tex [45].

2.6.3. Track-etching

The General Electric Company first introduced the track-etch method for manufacturing membranes in the Schenectady Laboratory [7]. In this method a thin polymer film is exposed to high-energy particles, acquired by fission, emitted from a nuclear reactor. They are able to pass through the polymeric film, and are creating a sensitized track due to formation of radicals along their passageway. The tracks are very susceptible to chemicals in comparison with the untracked matrix. The polymer film is subsequently immersed in a solution in order to prompt a reaction with the radicals along the tracks. The chemical composition of this solution is founded on the type of polymer film in question. Pore distribution and pore size depend on the time of exposure to the high-energy particles. The pores will all acquire a uniformly cylindrical shape, with a fixed diameter [7].

2.6.4. Template leaching

Another process for the production of isotropic membranes is template leaching. This method has been introduced for low solubility polymers, such as PE, PP, and PTFE. A mixture of these polymers and leachable components is melted and extruded in the form of a shapeless paste. The paste is then shaped by means of a cast, forming flat membranes, or by using a nozzle to form tubular, hollow fiber membranes. The films or the hollow fibers is subsequently immersed into suitable solvents in order to release leachable components, thereby creating pores [46, 47]. The pore size can be regulated by means of the molecular size of the leachable components.

2.6.5. Phase separation

In short, this method is very similar to the solution casting, but is utilizing less volatile solvents. The solvents leave the polymer solution after immersing in a non-solvent bath, such as water, results in a phase separation, and the precipitated polymer in form of a film [7]. Since the interface

of the membrane solution is a non-solvent liquid, the rate of solvent extraction differs from layer to layer, leading to the final product having different pore sizes from layer to layer. This results in the membrane morphology being anisotropic, similarly to when using the solution casting method. This method first time is introduced by Loeb-Sourirajan for making high flux, defect-free reverse osmosis membrane from polysulphone [7].

2.6.6. Interfacial polymerization

John Cadotte[48] was the first to introduce interfacial polymerization as a method for manufacturing anisotropic membranes. The method is utilized in order to obtain membranes for reverse osmosis (R.O.), and improves salt rejection and water fluxes in comparison with membranes prepared with the Loeb-Sourirajan technique. Nearly all membranes for reverse osmosis are now made by this method. The membranes introduced by Cadotte were based on polyethyleneimine, cross-linked with toluene 2, 4-diisocynate [48]. A water soluble diamine is soaked in a microporous matrix, such as polysulfone, followed by impregnation with a divalent cross-linker (diacid chloride) in a water-immiscible solvent, e.g. hexane, in order to instigate cross-polymerization between the amine loaded inside the pores and the pore walls. The polycondensation between the amine and the diacid chloride starts at the interface of the two solutions (water-hexane), and a polyamide is obtained, filling the pores. The pore size of the polysulfone matrix is thereby reduced and thus adequate for nanofiltration (NF) as well as for reverse osmosis (R.O.).

2.6.7. Solution-coated composite

Anisotropic membrane composites are made through solution-coating of a thin selective layer of an appropriate microporous matrix. This type of membrane was first introduced by Ward *et al.*[49], and is used for forming narrow pieces of water-casted composite membranes. The solution, comprising a mixture of polymer and additives, is poured onto the water between two Teflon rods in a water cast. The rods then move over the water surface, thereby dispersing the solution

over the water surface. The solvent vanishes from the polymer solution into the water, and a fragile, ultrathin microporous composite membrane forms over the water surface [49].

2.6.8. Plasma polymerization membrane

Plasma polymerization refers to the synthesis of polymers from monomers, and is carried out under non-thermal conditions, using ionized gases, with polymerization occurring in an electric field [50] (Figure 2.6). Plasma polymerization onto the surface of films is usually performed for processes of hydrophilization [51], electroconductivation (paper II), or selectivation [52] of membranes. In order to generate plasma polymerization, radicals of monomers are created on the surface of membranes by using radio frequencies (RF) at 2-50 MHz [53]. Argon or Helium gas at a pressure of 50-100 mTorr is normally used for inert plasma polymerization, whereas oxidative plasma polymerization usually is carried out in the presence of air or oxygen (paper II). In order to accomplish a fully completed reaction, conditions are sustained for 1-10 min in the presence of monomers. Surface grafted polymerization is performed by ionic or radical polymerizations. The monomer susceptibility to plasma polymerization is unpredictable. For instance, acrylic and vinyl monomers polymerize slowly, while uncommon monomers (in this context), such as hexane and benzene, polymerize quickly. The molecular weight of a polymer is dependent on the concentration of monomers, the power or voltage utilized for discharging the atmosphere from the plasma chamber, and the temperature of the substrate. The resulting membranes are frequently obtained as tremendously thin and uniform shells.

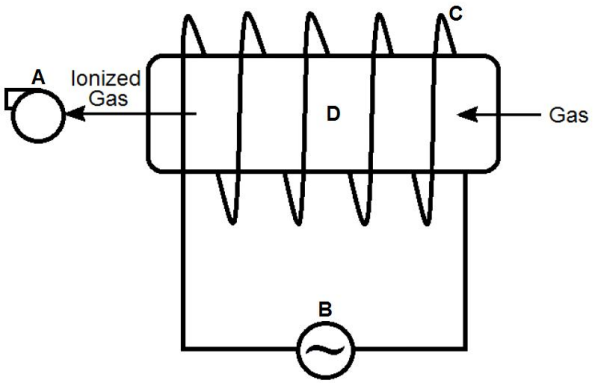

Figure 2.6 Schematic diagram of a plasma reactor containing a vacuum pump (A), power supply (B), RF coil (C), and a glass vessel (D).

3

Functionalization of polymers for MBRs

Polymer functionalization, or post-polymerization modification, represents a valuable means to switch to constitutions, configurations, and properties of the polymers that are not easily accessible by means of direct polymerization of monomers [54]. The strategy involves donation of functional groups to the polymer, when functionalization is not attainable via direct polymerization [55]. The obtained polymers are analogues of the initial polymers, but with new features. The history of functionalization of natural polymers dates back to the early 1840, when Hancok and Ludersdorf independently of each other vulcanized natural rubber with sulfur [55]. Shönbein synthesized nitro and acetate esters of cellulose in 1847, followed by Schützenberger in 1867. Modification of polymers was in the late 19th and the early 20th centuries limited to natural polymers. Polymer functionalization methods switched to synthetic polymers in the middle of the 20th century, using chlorine gas and polystyrene-divinylbenzene [55]. Besides functional groups acquired through substitution, the final products display a changed surface tension. For application in MBRs, functionalization methods embrace two approaches: bulk and surface functionalization, both through either physical deposition (adsorption) or chemical modification. Reversible polymer coating with biomimetic components, without changing the structure of the polymer matrix, is an example of physical modification, whereas chemical modification entails covalent binding of the reagent with active sites on the polymeric backbone, and hence, chemical functionalization of polymers only takes place when the thermodynamic parameters allow it. Functionalized polymers to be used in MBRs should thus be hydrophilized through chemical treatment. Commercial UF and MF membranes for application in MBRs include polytetrafluoroethylene (PTFE)[56], polysulfone (PS)/polyethersulfone (PES)[57],

polyacrylonitrile (PAN), and polyvinylidene difluoride (PVDF) [58]. Since all these polymers constitute a hydrophobic surface on the membrane, they should be hydrophilized prior to using them in MBRs.

3.1. Bulk functionalization

Bulk functionalization of polymers means modification of polymers in a continuous phase, comprising polymer, reagent, and solvent. All components are dissolved in a common solvent, and will appear as a homogenous and continuous phase [59]. After the reaction is completed, the phase inversion method is used to separate the final product (papers I-IV). By this method, inert non-solvents are able to extract impurities and side products, without any chemical effects on the final product. Added functional groups always provide new features to the polymer, and the characteristics of the final modified polymer might end up being a combination of those of the original and the modified polymer, and is dependent on the substitution percentage. Even after processing or reusing, these features persist in the modified polymer, due to covalent bonds between the functional groups and the polymer backbone. Stability of functional groups depends on inherent properties of functional groups as well as on the features of the surrounding phase, and enhances by hindering of functional groups from solvolysis.

Chemical bulk modification of polymers can be accomplished either by copolymerization (extension of molecular length of the polymer via block or graft polymerization) or by functionalization (substitution, using small molecules). Short chains functional groups showed lower stability than those on long chains (papers II, IV), but the employment of a proper linker would be an appropriate strategy for protecting the functional groups on short chains against solvolysis or ageing (paper IV). Unpredictable physicochemical features (like pore size and hydrophilicity) of the obtained copolymers after block copolymerization are undesirable, and the present work therefore focused on functionalization methods for the entrapment of microorganisms in bioreactors.

3.1. 1. Synthesis of poly(tetramethylene-N-hydroxyethyl adipamide)

Hydrophilized synthetic polymers are limited to surface hydrophilized, copolymerized or blended with some hydrophilic polymers such as PVP or PEG, but none of those products meet all requirements such as durability, sustainability, semi-hydrophilicity, robustness, solubility in volatile solvents for solution casting process to manufacture membranes with various thickness (≥ 1 μm). Furthermore, homogeneity in pore size distribution, which is dependent on the sequence of OH-groups on the polymer chain, is required in order to fit all types of MBRs. Moreover, the hydrophilic synthetic polymers on the market, such as polyvinyl alcohol (PVA_{OH}), polyvinyl pyrolidone (PVP), and polyethylene glycol (PEG), posses no mechanical capacity due to a high ratio of OH-groups to CH_2s in the polymeric backbone. Lacking mechanical properties these types of polymers (hydrogels) do not qualify for use in MBRs. A higher ratio of amide to methylene groups made Polyamide 46 the best choice for substitution of OH-groups since the resulting ratio of OH-groups to methylene groups followed suit (as OH-groups bind to amide groups). However, gaps of 4-6 carbons between the OH-groups allow for creation of a flexible, robust, hydrophilic membrane. Studies on other hydrophilizing agents revealed that in some cases, membranes displayed inadequate mechanical traits, forming for instance a sticky paste (when using formaldehyde), while in other cases the membranes showed low water permeability because of larger aldehydes being used, e.g. propyl aldehyde and butyl aldehyde [59]. Among the other hydrophilizers, acetaldehyde showed the best results in terms of producing membranes to be used in MBRs, with satisfactory mechanical and hydrophilic aptitude.

Step-growth polymerization of diamine with diacids results in a polyamide [60]. Polyamide 46 (Stanly TW300, M_n ~ 24 000 g/mol) is registered as having the highest degree of crystallinity (~70%) among the aliphatic polyamides [61]. Durability, flexibility, no impact on living cells and the environment, high mechanical property and resistance to harsh environment created motivation to focus on polyamide 46 as the best choice in the present project. However, high crystallinity leads to limited solubility in common solvents and lower chemical reactivity [55]. PA 46 is no exception from this rule, with solubility in solvents limited to one or two solvents. As

previously mentioned, the main criterion for bulk modification of a polymer is complete solubility in a proper solvent as a continuous phase. All reactions in this study were consequently accomplished in a mixture of formic acid and dimethyl sulfoxide (DMSO), i.e. a binary solvent system. DMSO was in this study used as a co-solvent in order to accomplish a complete dissolution of PA 46 in formic acid, as a continuous phase. When completely dissolved, PA 46 was ready for a nucleophilic attack by acetaldehyde (paper I), or a nucleophilic substitution, using monochloroacetic acid, followed by cross-linking with tartaric acid, and subsequent PEGylation (paper III).

Among the various polymers, polyamides show less reactivity due to the amide being a less reactive functional group. Oxidizability is the weaknesses of polyamides, more or less limiting the modifying reactions to surface modification [62] or copolymerization [63]. Polyamide 46 (Figure 3.1), or poly(tetramethylene adipamide), one of the polymers with highest crystallinity, was chosen for hydrophilization. The ratio of methylene to amide fractions in PA 46 is the lowest (8:2) among the polyamides [61]. This causes a high degree of intermolecular hydrogen bonds, which results in higher crystallinity in the form of a monoclinic crystal lattice. This indicates minimal solubility of polyamide 46, and a resistance to dissolve in common solvents, for bulk modification. Polyamides in an amorphous phase are, due to higher reactivability, capable of undergoing modification [63], and finding a proper solvent for dissolving either amorphous or crystalline phases was thus crucial (paper I). Formic acid was tested as solvent for polyamide 46, but solubility was only limited to the amorphous phase, the reason being that the liquefied PA 46 is restricted to the opaque dispersion in formic acid. In order to complete the polyamide dissolution as a continuous phase for bulk modification, dimethylsulfoxide (DMSO) was added, since it is able to either split inter-hydrogen bonds between the polymer chain or quench the amidic hydrogens [64]. After this procedure, the reaction is able to continue in the presence of acetaldehyde (paper I). Therefore, a mixture of formic acid and DMSO is an appropriate solvent for bulk modification of PA46.

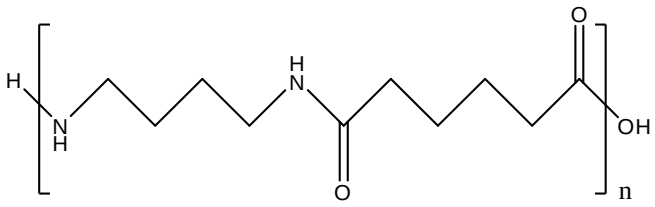

Figure 3.1 Chemical structure of polytetramethylene adipamide (polyamide 46).

In order to substitute the amidic hydrogen with a hydrophilic group, acetaldehyde was protonated in formic acid, forming a carbocation that attacked the amidic nitrogen. This resulted in a 95.65% yield of alcohol functional groups (paper I). In contrast to other aldehydes [59], addition of acetaldehyde to polyamide 46 resulted in semi-hydrophilic capability. Larger aldehydes (e.g. propanal) led to lower hydrophilicity, and the smaller aldehyde (i.e. formaldehyde) formed a pasty hydrophilic polymer [59]. Figure 3.2, illustrating a simulation of the obtained functional polymer, demonstrate that the pendant hydrophilic groups force the polymer chain to bend, forming a nodular aggregation (paper I, II,IV). Studies on monodispersed hydrophilic polymers have disclosed that presence of pendant carboxyl and hydroxyl groups causes deformations on the spherical aggregations[65], which have been observed commonly in hydrophilic biopolymers[66].

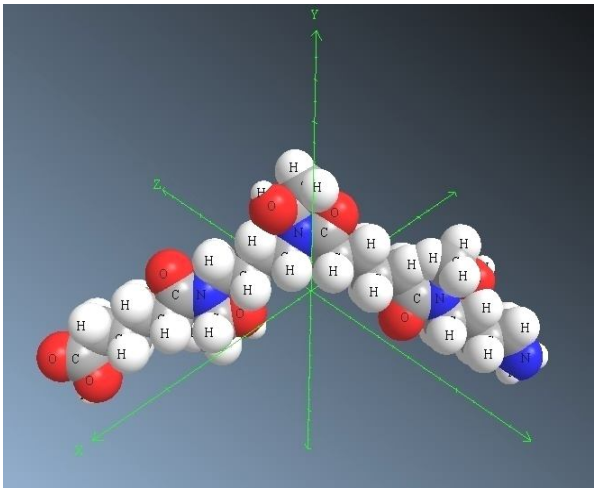

Figure 3.2 A 3-D simulation of a dimer of poly(tetramethylene-N-hydroxyethyl adipamide). Pendant hydroxyethyl groups force the polymer backbone to bend.

ESEM and AFM micrographs have also confirmed this phenomenon (Figures 3.3, 3.4); a nonporous, high crystalline, dense polymer was transformed into porous, lower crystalline, non-dense membranes.

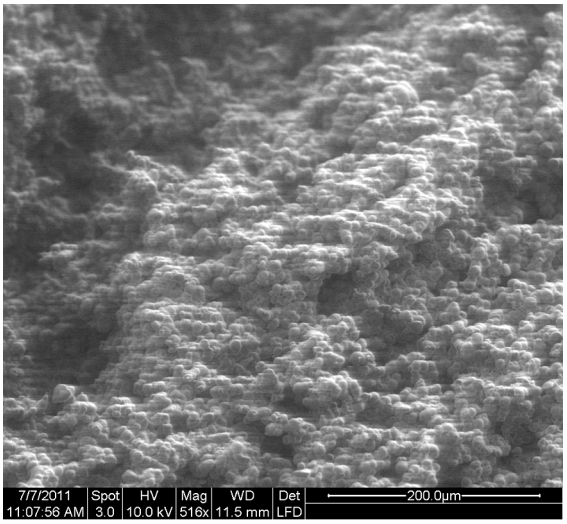

Figure 3.3 ESEM micrograph of the nodular structure of a poly (tetramethylene-N-hydroxyethyl adipamide) membrane.

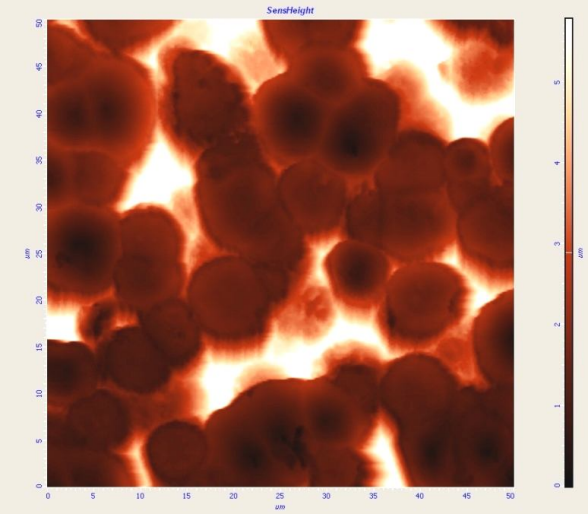

Figure 3.4 AFM micrograph of the nodular structure of a poly (tetramethylene-N-hydroxyethyl adipamide) membrane.

3.1.2. Synthesis of carboxymethyl polyamide 46

Carboxymethylation is one of the carboxylation methods used to introduce $–CH_2COOH$ [67]. Carboxymethylation has been performed, using monochloroacetic or monobromoacetic acid as carboxymethylating reagents [68]. Depending on the desired chemical functionality of the polymers, processing methods aimed at developing functional characteristics might be needed prior to the carboxymethylation process. For instance, substitution of any functional groups along the polymer chain requires the presence of free hydroxyl or amine groups [69]. Studies must be undertaken to establish the hydrophilic aptness and the distribution of negative charges. Nucleophilic substitution reactions can be carried out by treating polymers with haloacetic acids, and upon the completion of the reaction, halo acids (HCl, HBr) are eliminated. Non-toxicity, low chemical cost, weak anionic property of the pendant COOH groups, have resulted in this method being widely developed for modification of most biopolymers [68, 70] employed in biological research [70-72].

Synthetic carboxylated polymers include carboxylated styrene[73], slightly carboxylated polyester via extension of terminal groups[74], carboxylated polysulfone[75], carboxylated polyolefine[76], polyacetylene[77], carboxymethyl polyvinyl alcohol hydrogel[78], and carboxymethyl PA 46 (paper IV) [59]. All these polymers have a negatively charged surface; the intensity of the charge depends however on the groups linked to COOH. Electron withdrawing groups increase the polarity of COO^-, while electron donating groups reduce the concentration of charges around COO^- [79]. Therefore carboxylation using monochloroacetic acid leads to substitute carboxylate anion with mild negative charges for prevention highly hydration and ion exchanging. For this reason, polyamide 46 should rather undergo a nucleophilic substitution with monochloroacetic acid in the presence of a mixture of solvents (Formic acid and DMSO). Dimethyl sulfoxide (DMSO), shown to be a proper proton quencher [64], facilitates the elimination of hydrochloric acid [80], the rest product formed from the monochloroacetic acid. However, to prevent the reaction from reversion, HCl (byproduct) and formic acid (solvent) were at the final stage gently neutralized with sodium hydroxide in methanol to pH=6-7. A yield of 89.2% indicated that carboxymethylation of PA 46 is less complete than after hydroxyethylation. This could be related to the final product being hydrolyzed by HCl which is formed during the reaction. In order to

improve the mechanical property of the carboxylated derivatives of PA 46 for membrane application, tartaric acid, being a dihydroxy diacid, intertwined two polymer chains (Figure 3.5). Du to esterification of the carboxylated polyamide 46 with linker, all hydrophilic groups in the polymer chains were blocked, resulting in a robust, nonporous film. The reason for choosing tartaric acid as a linker was its four functional groups. The two hydroxyl groups at the tartaric acid linked two carboxylated PA 46 chains, and the two carboxylic acid residues of the linker (tartaric acid) remained intact, available to react with two PEG chains in the next step (paper IV).

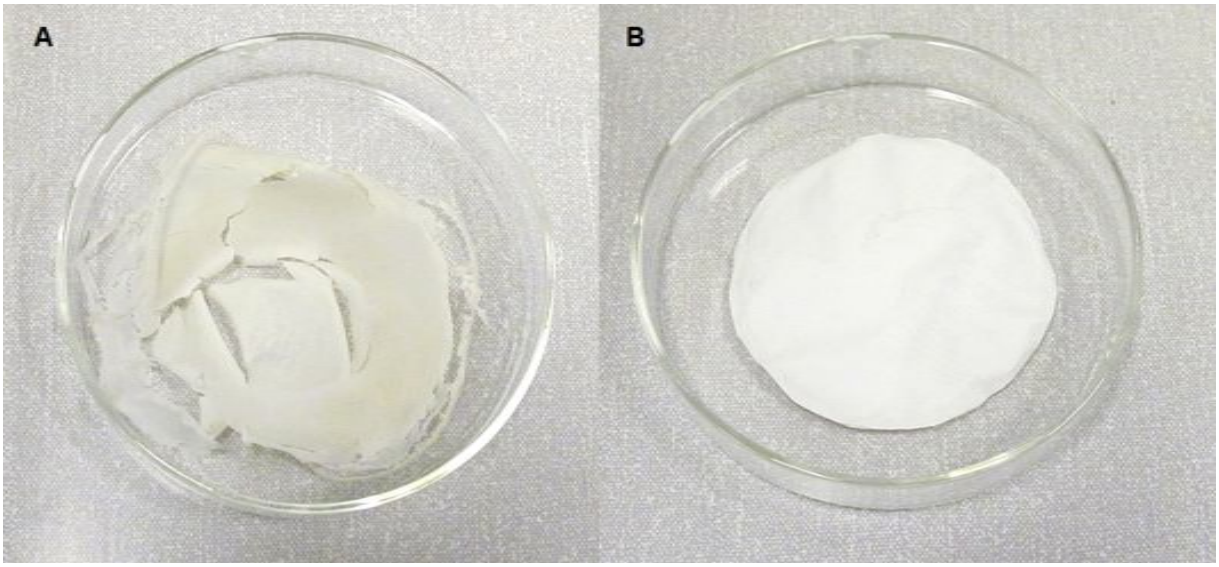

Figure 3.5 Study on the effect of linkers, used to improve the mechanical traits of carboxylated PA 46 membranes, applying the solution casting technique: (A) Non-cross-linked carboxylated PA 46 membrane, (B) Cross-linked carboxylated PA 46 membrane, using tartaric acid as cross-linker.

3.1.3. **PEGylation of carboxymethyl polyamide 46**

PEGylation is a route to covalent attachment of polyethylene glycol (PEG) to other molecules. The substitution reaction includes the elimination of potential, leaving groups such as HCl or H_2O [81]. The attached PEG improves the hydrophilicity of hydrophobic substrates, masking the surface of host molecules [82]. PEGylation has been applied as post-modification, in order to improve the biomedical efficiency of therapeutic proteins [83], biocompatibility, low enzymatic degradation (extended half-life), and minor toxicity have resulted in manufacture of PEGylated pharmaceuticals for many years [83]. There are some reports on PEGylation of proteins[82],

cellulose[81], and other biological molecules [84], but none so far concerning research on PEGylation of synthetic polymers. This is most probably related to limited application for such type of polymers, or to the challenge it entails to create active sites for PEG conjugation, which would increase the functionality of the synthetic polymers. In addition, hydrophilic functionalization of hydrophobic synthetic polymers requires dissolution of the synthetic polymers in an aprotic solvent, allowing transfer and elimination of polar leaving groups (HCl or H_2O). In accordance with our accumulated knowledge, PA 46, or its derivatives, acquired by dissolution in the aprotic solvent formic acid, is our preference among the synthetic polymers. Results showed that PEGylation is an appropriate method for improving hydrophilicity of macromolecules[81], and solubility of single molecules[85], due to the mass ratio between PEG and host molecules.

There are some reported methods for activating either PEGs or the reagents for PEGylation. One of these methods involves the activation of PEGs for amino group conjugation. In polypeptides, the reactive functional groups engaging in conjugation are nucleophiles, such as α- or ε- amino groups, thiols, carboxylates, and hydroxylate residues [82]. Hydroxylate and carboxylate groups in proteins are coupled inter- or intra-molecularly at neutral pH, and consequently, the challenge lies in the transformation of the terminal hydroxyl groups of PEG, that will allow reaction solely with free amino groups [86, 87]. Hence, the activation of PEGs is limited to a terminal hydroxyl transformation[88]. The derivatives obtained are PEG-aldehyde (PEG propionaldehyde)[89], tresylated PEG [90], PEG epoxide [82], and acylated PEG [91]. Another method for PEGylation is to activate the reagents in order to make them capable of esterification of the terminal OH-groups of PEG [92, 93]. PEGylation of propionic acid in the presence of formic acid as a catalyst has been reported by Kozlowski *et.al* [94], and PEGylated fatty acid (PEG-150 distearate) is e.g. used for diagnostic purposes [95].

In the present study, PEGylation of polyamide 46 was preceded by carboxylated groups being introduced to the polyamide, followed by cross-linking with tartaric acid, thus improving the mechanical aptness of the polymer, which in turn was followed by esterification between the cross-linked carboxymethyl PA 46 and PEG in the presence of formic acid. Formic acid, being a strong hygroscopic organic acid, catalyzes the esterification process at elevated temperature.

Repeated units of O-CH_2 in PEG improve the hydrophilicity of the final molecule (Figure 3.6). In order to attach the carboxylate PA 46 to PEG, tartaric acid (as a quaternary linker [96, 97]), was used by the esterification, thus bettering the arrangement of the molecule and yielding robustness to the membrane (Figure 3.7).

Hydrophilic cross-linkers not only adhere to polymer chains, but are also able to compensate for low hydrophilicity of polymers [98]. Tartaric acid [99], citric acid [100], and glycolic acid [101] are some of the hydrophilic cross-linkers.

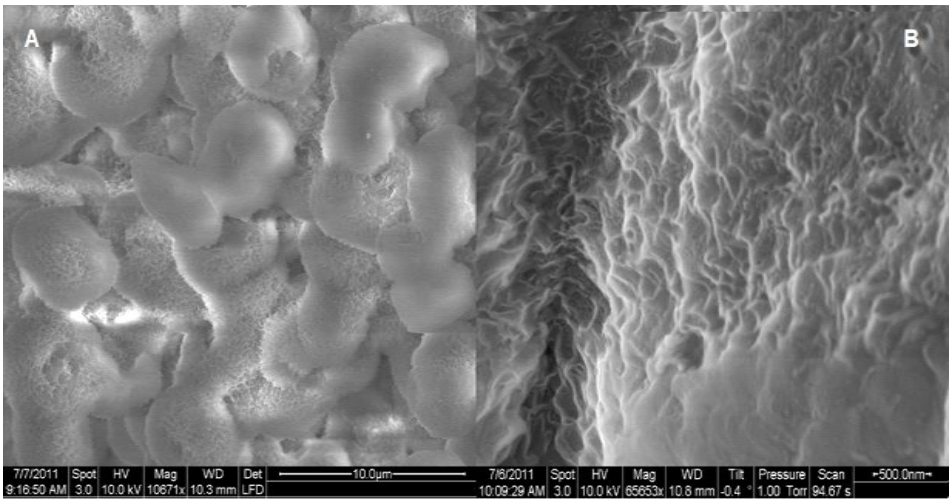

Figure 3.6 Surface morphology study of the rough globular surface of PEGylated carboxymethyl PA 46: (A) globular aggregation of PEGylated carboxymethyl PA 46 (Magnification 10671X), (B) Surface of globular PEGylated carboxymethyl PA 46 (Magnification 65653X).

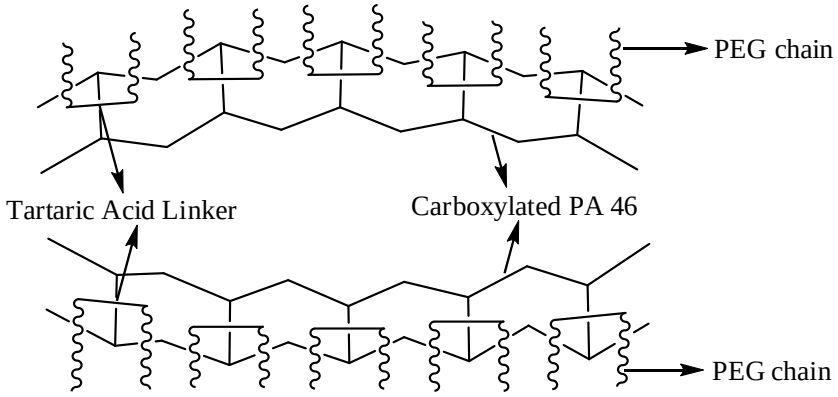

Figure 3.7 Schematic structure of globular PEGylated polyamide 46.

3.2. Surface functionalization

Surface functionalization of polymers is a method for changing the outer layer of the polymer, and is performed by substitution with a thin layer of functional groups. However, the low thickness resulted in non-durability and weakness of the modified surface, and the method was thus deemed unsuitable. Appropriate engineering, by combining bulk features with functional surface features, can expand the aptitudes of materials.

The principle of surface functionalization is localization of polar functional groups on the membrane surface. This opens for development of wettability, paintability, and biocompatibility as well as permission or prevention to adhere materials or biomass onto the surface. Several methods are available to achieve this aim, e.g. corona discharge, plasma treatment (paper II), flame treatment, and irradiation with UV-light or γ-rays in the presence of ionizable gases[102]. The techniques for surface functionalization are classified as either physical deposition (adsorption) or chemical modification. Physical deposition leads to reversible changes of the surface morphology and rheology, without chemical reactions, while the chemical binding to the surface form irreversible covalent bonds.

Graft polymerization is a versatile technique for both bulk and surface modifications. Chemical initiators (peroxides, diazos) or ionizing agents (plasma, γ–radiation, UV-light) are in this method applied when the surface of the polymer is passive and physical treatments have no effect on the surface. If the surface of the substrate has active functional groups, such as OH, NH_2, COOH, etc., the monomers are anchoring to the surface, after which the chemical initiator or the ionizing agent starts propagating them (paper II), resulting in the formed polymer being localized on the surface in a right angle transverse orientation (Figure 3.8). The molecular weight of grafted polymers is dependent on the shelf life of the radicals and the concentration of the monomer.

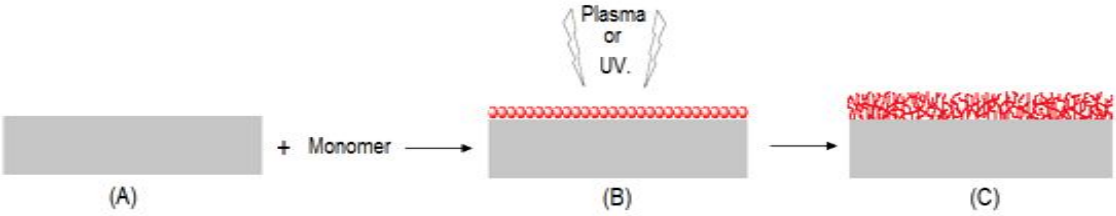

Figure 3.8 Surface graft polymerization steps: (A) Polymeric substrate, (B) Graft polymerization of anchored monomer is initiated after exposure to plasma or UV-light, (C) Polymeric substrate after surface graft polymerization.

3.2.1. Surface graft polymerization of polyamide 46 copolymer

Graft polymerization of PA 46 was performed in order to produce an electroconductive membrane (ECM) (paper II). Prior to bulk graft polymerization, PA 46 endured hydroxyethylation (paper I) in order to create active sites for binding with an inherent electroconductive monomer (hydroxymethyl 3,4-ethylenedioxythiophene). The obtained bulk graft copolymer subsequently took part in a surface graft copolymerization, in order to acquire an electroconductive membrane (paper II). The liquid phase polymerization (LPP) method was applied for the surface graft polymerization. The membrane was immersed in a HMEDOT monomer solution, after which propagation was initiated by exposure to plasma radiation.

3.2.2. Electroconductive Membrane (ECM)

Polyaniline, as the first electroconductive polymer, was synthesized in 1834 by Runge[103]. Henry Letheby described the electrical properties of PANI in two forms: oxidized quinoid and reduced benzoid, in 1862 [104]. This characteristic opened up for a new initiative: to substitute metals with organic complexes [105]. At the time, most scientists concentrated on other conjugated polymers, like polyacetylene, polypyrroles, polythiophenes, and polyphenylenevinylenes [106, 107]. Conjugated polymers contain conjugated π-bonds, but with no resonance. Doping these polymers

with electron withdrawing groups, initiates electron resonance, and electrical conductivity ensues. Polyacetylene was the simplest structure among the conjugated polymers, and was 1977 widely studied by Shirakawa *et al.*[108] Investigations on electroconductive membranes date back to 1985, when Wagner wanted to find another application for polyacetylene [109]. From then on, all the methods have been based on coating a polymeric matrix with different types of conjugated monomers. Supplementary development of electroconductive membranes (ECM) was also carried out by integration of electroconductive polymers (ECPs) with porous electrically insulated polymers [110]. Dipping a cellulosic substrate in aniline monomers followed by in situ polymerization yields ECM, but with pore sizes varying tremendously, and being out of control [111]. Furthermore, coating PVC membranes by using polyaniline was studied by Shishkanova[112]. Fabrication of ECM by coating paper with carbon nanotubes is yet another coating technique [113], as is coating nylon-6 with carbon nanotubes[114]. In all these methods, ECPs are promoting conductivity of porous substrates. However, none of them is suitable for use in membranes for MBRs, due to low mechanical properties, and inappropriate binding between ECPs and non-ECPs, resulting in low durability. This is why none of them currently is being used in the industry. In spite of showing the most superior electrical properties in comparison with other conjugated polymers[115], polyacetylene is not possible to copolymerize with other polymers, simultaneously retaining its own electrical capacity. For this reason, the work in this thesis focused on 3, 4-ethylenedioxythiophene (PEDOT) and its derivatives, based on its electrical property, ability to copolymerize in a wide range of solvents, and its biocompatibility [116]. Moreover, PEDOT and PA 46 have displayed biocompatibility [116, 117], meeting our requirements for use in MBRs. PA 46 is one of the polymers with highest crystallinity, and possess superior physicochemical properties [61]. However, lack of active sites prevents reaction with HMEDOT. Hydroxyl groups make suitable anchor sites starting copolymerization between EDOT monomers and cellulose [118, 119]. Thus, we concentrated on an introductory hydrophilization of PA 46, followed by copolymerization with hydroxymethyl 3, 4-ethylenedioxythiophene (HMEDOT) (paper II).

The obtained semi-electroconductive copolymer in reality showed to be inherently hydrophobic, as a result of the hydroxyl groups of the hydrophilized PA 46 being occupied with HMEDOT in

an etherification reaction (paper II). The hydrophilic capability of the obtained copolymer was therefore limited to forming low hydrophilic ether bonds between HEPA 46 and HMEDOT. In order to create pores in the bulk of the semi-electroconductive copolymer, urea was applied. The urea was completely dissolved in the copolymer solution as a continuous transparent phase, and easily leached out after drying the polymer solution. The selection of urea as a pore maker was based on the *Biginelli* reaction [120]: during the reaction with a thiophene derivative, urea binds to the aromatic rings forming an intermediate. The reaction will never proceed to ring formation being created, due to absence of another reagent (ethyl acetoacetate) [121]. The urea is hence easily washed out and hydrolyzed by water wash. By this strategy, nanopores were obtained, and the copolymer was ready for surface polymerization with HMEDOT. The final product is a surface hydrophilized and electroconductivated membrane suitable for MBR purposes.

3.3. Characterization of modified polyamide 46

Functionalized polyamide 46 and poly(hydroxymethyl 3,4-ethylenedioxythiophene) were characterized through chemical and physical analyses. Surface morphology and pore size distribution were measured by means of AFM, and a Zetasizer instrument was used to analyze surface charge domains of carboxylated and cross-linked, carboxylated membranes. Surface tension and contact angle were determined by using a Dynamic Absorption tester. Chemical structures and reaction yields were investigated by ^{1}H-NMR and ^{13}C-NMR. Thermal properties of the functionalized polymers were tested with a differential scanning calorimeter (DSC) and a thermogravimetric analysis (TGA) was carried out, measuring crystallization temperature, melting point, and energy content of each transition, as well as uncovering volatile functional groups and assessing thermal consistency. Porosity and pore size were studied, using the bubble point method. Finally, water flux was measured by a dead-end filtration cell.

3.3.1. Surface morphology of the electroconductive membranes

Surface morphology of the electroconductive membranes was inspected by means of atomic force microscope (AFM/SPM NETGERA prima, NT-MDT Inc., Russia), measuring pore size, nodule size, porosity, pore size distribution, and roughness. The mean value of the roughness (R_a) of both membranes was used for determining the roughness of coated as well as uncoated membranes, and was, due to surface dimensions being dependent on the center plane, calculated as:

$$R_a = \frac{1}{L_x L_y} \int_0^{L_x} \int_0^{L_y} f(x, y)\, dxdy \qquad (3.1)$$

Where *f(x,y)* is roughness height on the surface, while L_x and L_y are the surface dimensions (dependent on the center plane [122]. The AFM micrographs revealed that surface polymerization resulted in roughness increasing by 11.81%, and a simultaneous increase in membrane thickness by 12.61%. It is reasonable to believe that these increases were an effect of the implemented method. It stands to reason that the surface of membranes should be impregnated with monomers prior to plasma treatment, in order to safeguard anchoring of the monomers to the surface, and consequently, the starting point of polymerization is limited to the amount of monomer available. If vaporized monomers are injected continuously to the plasma chamber, the layer of monomers on the surface increases, resulting in improved electroconductivity. The plasma vapor-phase polymerization (PVPP) method could however not be employed in the present work due to instrumental limitations. The dark area in figure 3.9 implies absence of homopolymer on the surface of the electroconductive membrane (paper II).

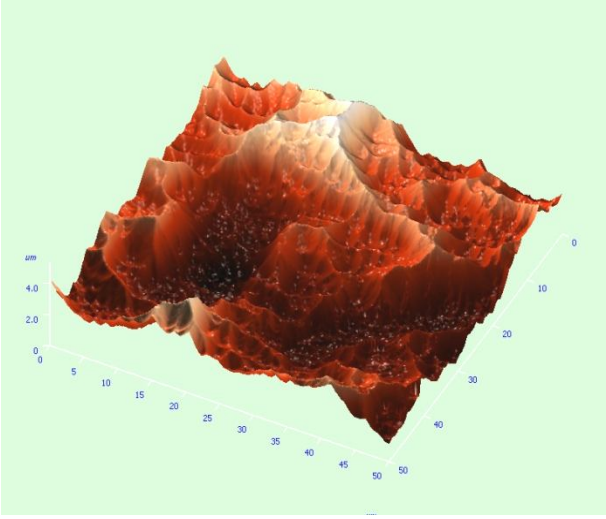

Figure 3.9 Surface topography of the electroconductive membrane after plasma polymerization based on poly(hydroxymethyl-3,4-ethylenedioxythiophene-co-tetramethylene-N-hydroxyethyl adipamide)

3.3.2. Charge domains on the membrane surface

Determination of surface charge of polymers can well be performed by means of for instance *surface potential imaging* (SPM) and *electric force imaging* (EFM) modes in atomic forced microscopy (AFM). Notwithstanding, one of the most effective techniques for measuring charge domains on the surface of polymer particles suspended in water involves the measurement of the speed with which the particles move in an electric field. For the study of charge domain on the membrane surface, examining the substitution of COOH in bulk and membrane surface films, Zeta potential (ζ) measurements were carried out. Measurements of Zeta potentials were also, along with measurements of isoelectric points (*pI*), carried out in order to study the electrokinetic behavior at the solid/liquid interface. A Zetasizer instrument of the model Nano-ZS (Malvern instruments, UK), applying the Laser Doppler Velocimetry (LDV) technique, was used for the measurements. This instrument calculates the zeta potential by measuring the electrophoretic mobility, using the Henry equation (3.2):

$$U = \frac{2\varepsilon z f(k_a)}{3\eta} \qquad (3.2)$$

where,

U is the electrophoretic mobility,

ε is the dielectric constant of water,

η is the viscosity (cp) of water at a certain temperature,

$f(k_a)$ is Henry's function, which is equal to 1.5 following the Smoluchowski approximation [123]. Conversion of Henry's law into the Smoluchowski equation yields the Zeta potential (ζ):

$$\zeta = \frac{4\pi\eta}{\varepsilon} \times U \times 300 \times 300 \times 1000 \qquad (3.3)$$

where,

ζ = Zeta potential (mV)

$U = v$ / (V/L)

v = Speed of particles in the electrical field (cm/sec)

V= Voltage (V)

L= Distance between electrodes (cm)

The polarity of carboxylated PA 46 was measured using a Zeta analyzer. The analysis conducted by this equipment is based on light scattering of colloidal polymeric particles in a solution, at different pH-values. The velocity of the particles is correlated to the amount of surface charge; the higher the surface charge, the faster the speed of the particles in the electrical field. This technique was thus utilized for measuring the Zeta potential and the isoelectric points. This system includes a modulator for laser beams, and an oscillating mirror detects the position of the particles. The change in position of particles is monitored by a change in the angle of the reflected beams (Figure 3.10).

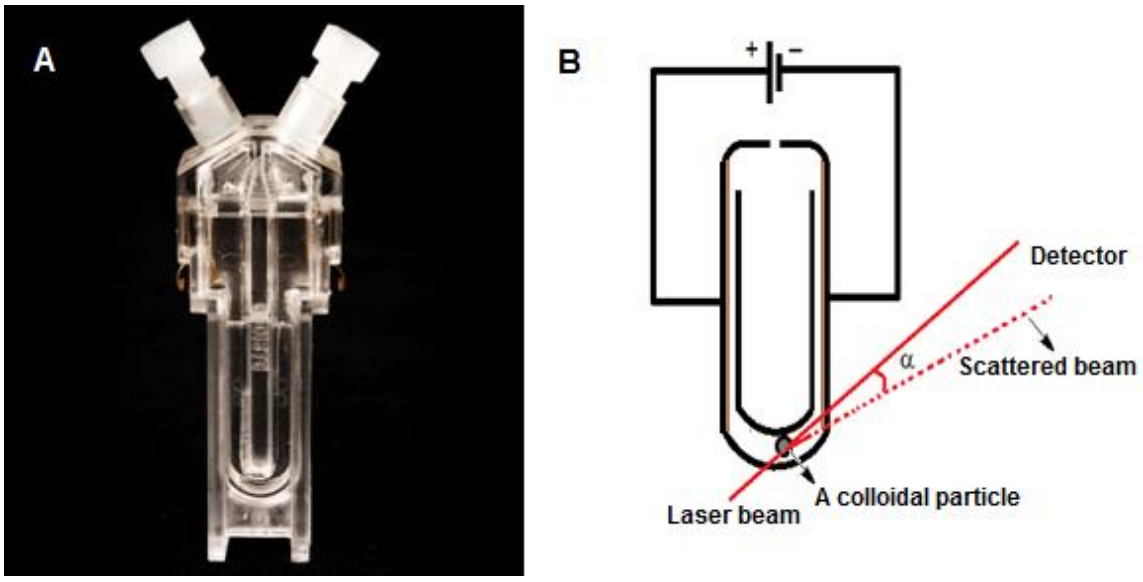

Figure 3.10 (A) Malvern capillary cell. (B) The conceptual mechanism of a Zetasizer Nano includes a Laser Doppler Velocimeter.

Since polyamide 46 derivatives are completely soluble in formic acid, the pH was at the starting point set to zero for all samples. The analysis revealed that the negatively charged COO^{-} of cross-linked carboxylated PA 46 moved fast toward the positive charge. This might be due to the concentration of COO^{-} in the cross-linked polymer being lower than in the carboxylated PA 46, which in turn might be due to most of the carboxylic groups being consumed during cross-linking. In PEGylated PA 46, the terminal OH-groups showed a tendency to convert into the negatively charged O^{-}, the amount of zeta potential is being higher than the carboxylated as well as the cross-linked carboxylated derivatives (-4.9 mV). Polyamide 46 showed a positive zeta potential (12.8 mV) due to presence of amidic nitrogen groups. The absolute amounts of zeta potential of polymers did not change in polyamide 46 and cross-linked carboxylated PA 46 after their respective isoelectric point. PEGylated PA 46 and carboxylated PA 46, on the other hand, showed positive potential at higher pH-values, indicating amphoteric properties (paper IV). We can conclude only polyamide 46 and crosslinked polyamide 46 are resistance to pHs changes. Since polyamide is not hydrophilic polymer, crosslinked carboxylated PA 46 is the best choice for MBRs due to hydrophilicity and stability at wide range pHs.

3.3.3. Surface tension and contact angle

To assess the wetting behavior of the surfaces when in contact with fresh deionized water, contact angle and surface tension were investigated, using captive bubbles. The surface tension was investigated with the Dynamic Absorption Tester (DAT) model Fibro's DAT 1100 (Thwing-Albert Instrument Co., USA), applying the pendant drop method. The instrument software was set in accordance with the Owens-Wendt model[124], which is obtained from the Young-Dupré equation, which is defined as the relation between the contact angle and the surface energy:

$$\gamma_L(1 + \cos\theta) = 2\sqrt{\gamma_S^D}\sqrt{\gamma_L^D} + 2\sqrt{\gamma_S^P}\sqrt{\gamma_L^P} \qquad (3.4)$$

where θ is the contact angle between the liquid and the solid, γ_L is the surface tension of the liquid, γ_S^D is the dispersive component of the substrate (Lifshitz-Van der Waals interactions), and γ_S^P is the non-dispersive component (polar interactions, Lewis acid/base) [125]. The instrument was calibrated at 20 °C each time, using water as the dispersive liquid, having a surface tension at 72.8 mN/m, and employing diiodomethane as the non-dispersive liquid, its surface tension being 50.8 mN/m [126].

Hydrophilicity is one of the crucial membrane features. Water flux and biofouling are directly correlated to the degree of hydrophilicity of the membranes. Measuring the contact angle at which the water interacts with the surface of the solid is a useful method for investigating hydrophilicity in terms of wettability of the substrate surface, and the Owens-Wendt equation determines the level of interaction energy between the solid surface and the water droplets. The sessile drop method (Figure 3.11) was used to verify the surface hydrophilicity of the control and the modified film. The angles with which droplets of water (with a surface tension of 72.8 mN/m) and diiodomethane (with a surface tension of 50.8 mN/m) [droplet size 5 (paper II) or 10 (paper I) μL] interact with the membrane surface within 10 sec at 20 °C were studied by using the Dynamic Absorption Tester (Fibro's DAT 1100, Thwing-Albert instrument Co., USA) and a Tensiometer (KRUSS G10, Germany). Mean values were calculated from three (paper I) or ten

(paper II) repetitions of each measurement. Increasing surface tension and decreasing contact angle indicates improving hydrophilicity after functionalization (papers I, II, IV).

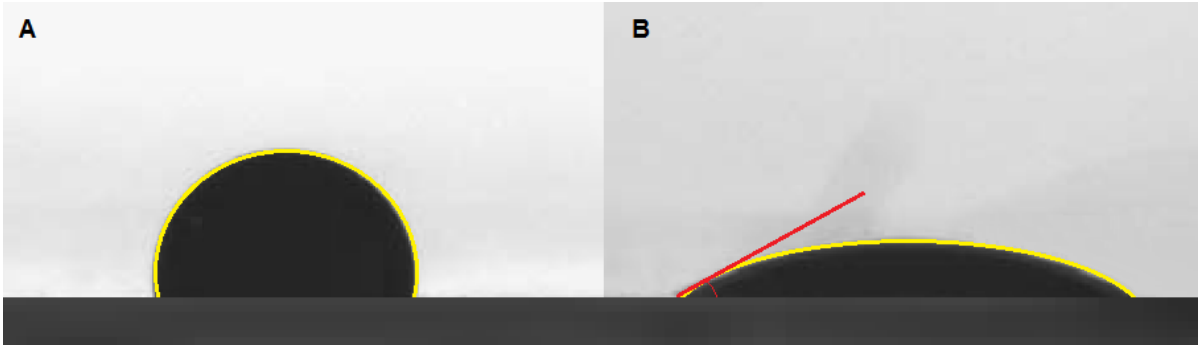

Figure 3.11 Camera views of water droplets on the surface of poly(tetramethylene-N-hydroxyethyl adipamide) in a contact angle analyzer: (A) the water droplet on the membrane film at the time zero, (B) the water droplet on the membrane film after 10 sec.

3.3.4. Nuclear magnetic resonance (NMR)

Nuclear magnetic resonance is a sensitive technique capable of determining the chemical structures of materials in bulk, qualitatively as well as quantitatively. The molecular information is provided by the nuclear resonance frequency being altered when a molecule is placed in an external magnetic field. Since each nucleus has an intramolecular magnetic field around itself (due to the nuclear spin), atoms in the external electromagnetic field are able to absorb electromagnetic radiation at a certain frequency, characteristic of the isotope in question (^{1}H, ^{13}C, ^{15}N, ^{31}P, ^{19}F). The proportion of energy absorbed correlates to the signal intensity. The atoms in a molecule are identified by the interaction of intramolecular magnetic fields of the neighboring atoms. Different nuclei have different neighbors, and thus resonate at different frequencies. The nuclear resonant frequency, when compared with a reference [usually tetramethylsilane, $Si(CH_3)_4$], is termed the *chemical shift* (σ). NMR is an appropriate instrument for detecting functional groups only in a bulk modification of a polymer chain, in spite of the minute weight ratio of the functional groups to the polymer backbone.

3.3.5. Thermal properties

Thermal degradation was studied by using a thermogravimetric analyzer (TGA) model Q500 (TA Instruments, Delaware, USA). This instrument evaluates the stability of the polymer at elevated temperatures, based on weight loss in the absence of oxygen. The thermogravimetric analysis is a suitable method for determining the percentage of functional groups in the modified polymer in comparison with a non-modified polymer. The degree of crystallinity and the enthalpy of fusion (i.e. the melting point, which decides the degree of crystallinity) were studied by employing a differential scanning calorimeter (DSC) (model Q2000, TA Instruments, Delaware, USA). Pure nitrogen was injected as the inert purge gas to clean the furnace chamber from any residues of oxygen and water.

The term differential scanning calorimetry (DSC) is used for the quantitative comparison of heat absorption/desorption between samples and reference under isothermal conditions, expressed as a function of temperature or time, whereas the thermogravimetric analysis (TGA) technique is used for analyzing thermal consistency of the molecules at different temperatures.

The thermal analyses in the present study (utilizing DSC) disclosed a decrease of glass transition (T_g) and crystallization (T_c) in hydroxyethylated, carboxymethylated, and PEGylated PA 46. This resulted in increased amorphism due to scanty formation of spherical units (papers I, IV). The thermal analyses furthermore revealed that after functionalization, polyamide 46 showed reduced T_g and T_c, as well as lowered T_m (i.e. phase transition enthalpy) of melting (ΔH_m) and crystallization (ΔH_c). This could be related to spherical aggregation being curtailed, resulting in reduced order of the dense structure of PA 46. Since bulky pendant groups, such as hydroxyethyl, carboxymethyl, and polyethylene glycol, create gaps between contiguous nodules, the convergence of polymeric chains is hampered, thus resulting in reduced glass transition (T_g) and crystallization (T_c), and lowered melting temperature (T_m).

Thermal gravimetric analysis (TGA) is one of the appropriate techniques for estimating stability of polymers at elevated temperatures, and during the analysis in the present study, mass changes of the polymers were monitored under nitrogen gas. The polymer in the present study lost no weight due to oxidation, and thus, the process might be labeled carbonization. The pendant groups on the functionalized polyamide 46 in this study displayed instability. Thermograms revealed that while carboxylated PA 46 exhibited the lowest stability, cross-linking of the carboxylated PA 46 resulted in higher stability (paper III). Molecular studies on carboxylated compounds have uncovered that when carboxyl groups are attached to a carbon situated adjacent to electron withdrawing groups, e.g. amidic nitrogen, the molecule becomes unstable [127], which supports the results in the present study that increased molecular weight of functionalized polyamide increased molecular stability. This might explain development of internal hydrogen bonds in polymers, and also that PEGylated derivatives become entangled. Entanglement depends on the length of the polymer chain and also its polarity, and affects the stiffness of the chain [128].

3.3.6. Porosity and pore size distribution

There are several techniques for determination of pore size. The main ones are described below:

(a) Bubble point: This method was first presented by Bechhold [129].A membrane is fixed into a membrane holder (Figure 3.12). Compressed air is passed through a closed system that is open to the outside solely via the membrane holder. A pressure gauge is used to monitor the gas pressure. A very thin layer of water is allowed onto the membrane in the membrane holder. After opening the valve, thus increasing the pressure, the first bubbles are observed in the middle of the membrane (see Figure 3.12). The pressure at which this occurs is put into equation 3.5:

$$r = \frac{2k\gamma\, cos\theta}{\Delta p} \qquad (3.5)$$

where,

r is the membrane pore diameter in μm,

Δp is the bubble point pressure in psi,

k is the conversion factor (Table 1),

γ is the liquid surface tension in dyn/cm,

θ is the Liquid-membrane contact angle in degrees.

At complete wetting, θ is equal to 0^{o}, and thus, cos θ is equal to 1.

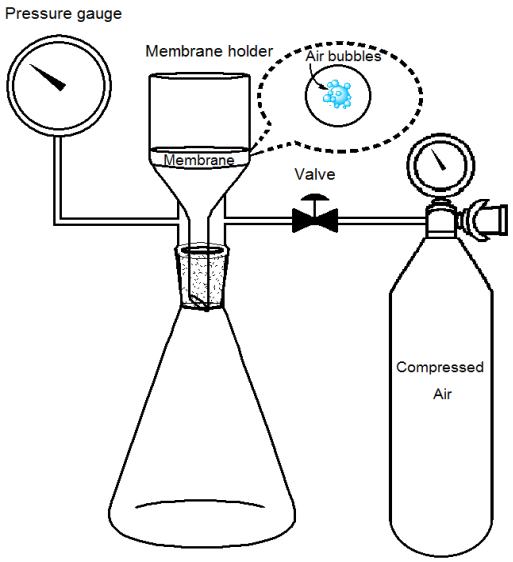

Figure 3.12 Bubble point measurement device: pressure gauge, membrane holder, valve for controlling gas pressure, and cylinder with compressed air

Table 1. Surface tension of some liquids versus conversion factor (k) [7]

Liquid	Surface tension (dyn/cm)	Conversion factor (k)
Water	72	42
Kerosene	30	17
Isopropanol	21.3	12
Silicon fluid[a]	18.7	11
Fluorocarbon fluid[b]	16	9

[a]Dow corning 200 fluid, 2.0 cSt.
[b]3M Company, Fluorochemical FC-43

(b) Direct visualizing technique: Various types of microscopy techniques are employed for visualizing pores in membranes, e.g. SEM (scanning electron microscopy), ESEM (environmental scanning electron microscopy), FESEM (field effect scanning electron microscopy), TEM (transmission electron microscopy), STEM (scanning transmission electron microscopy), and AFM (atomic force microscopy). Supplementary software is required for measuring pore diameters by AFM.

(c) Adsorption-base techniques: This technique is based on adsorption and desorption of an ideal gas (following thermodynamic rules) under isothermal conditions. Gas pressure and temperature are monitored, and results are evaluated, using the Kelvin equation.[130] This method allows measurement of the total volume of pores (porosity).

(d) Thermoporometry method: Changes in crystallization enthalpy (H_c) of liquids inside pores are measured by means of DSC (diffraction scanning calorimetry) [131]. Since change of enthalpy is dependent on the amount of fluid, estimation of the ΔH_c requires a comparison between a control prototype and a sample saturated with liquid, which will indicate the amount of localized fluid inside the pores. This method also reveals the porosity of membranes.

(e) NMR method: This method entails analysis of water contents in saturated membranes, using ^{1}H-NMR, and thus indicates the porosity of the membrane [132]. Excess of water should by centrifugation be removed from the membrane prior to the NMR study, and thus this technique reveals the amount of water being entrapped inside the pores.

(f) Swelling ratio: Porosity was also determined by investigating the water-swelling capacity (percentage) of the membranes, as this gives the total volume of pores. Dried membranes were immersed in Milli-Q water at 20 °C overnight, attaining equilibrium swelling. Excess water was removed from the membranes by using a tissue, after which the membranes were promptly weighed (W = mass symbol in equation). The membranes were subsequently dried for 2h in an oven at 105 ºC, after which they were weighed (= W_0). The water uptake capacity at 20 °C was calculated, using equation 3.6:

$$\text{Water uptake (20 °C)} = \frac{W - W_0}{W_0} \qquad (3.6)$$

where W and W_0 correspond to the weights of wet and dry membranes, respectively [133]. To determine porosity, the volume (mass converted into volume expressed as cm^3; $d_{H2O} = 1$) of the retained water in the membrane is divided by the total volume of the membrane, multiplied by 100, thus yielding porosity (3.7):

$$\text{Prosity \%} = \frac{\text{Retained Water}}{V} \times 100 \qquad (3.7)$$

where *V* is the total volume of the membrane [134].

3.3.7. Water flux

The water flux through membranes at 14.5 psi (1 bar) was studied by using a dead-end cell. Three types of membranes with 64 mm Ø and of various thicknesses (0.11, 0.125, and 0.13 mm) were fixed inside the cell. Membranes were immersed in Milli-Q water for 3h, after which a tissue was used to remove excess water. Water flux (J_w) was then calculated by following equation (3.8):

$$J_w = \frac{V}{A\Delta t} \qquad (3.8)$$

where,

V is the volume of permeated water (L)

A is the area of membrane (m^2)

Δt is the permeation time (h) [135].

4

Functionalized polyamide 46 in membrane bioreactors

The designing of a submerged bioreactor was based on cross-flow diffusion in a multi-layer membrane module for uprising feeding flow. In the bioreactor, minimal gaps (1.5 cm) between the membrane beds forms very narrow tunnels, thus yielding maximal membrane surface area, and thereby providing maximal mass transfer, which in turn results in higher efficiency. Each bed comprises five parts: two membrane films, two stainless steel sieve supports, and a rubber O-ring (Figure 4.1). The rubber O-rings are located between the two membranes, creating a 2 mm gap into which the microorganisms are loaded. Two stainless steel supporters are by means of six long screws compressing the two membranes. After all the membrane beds have been joined together (by the six screws), the membrane module is installed in a double layer polymethylmethacrylate vessel (Figure 4.2). An entry to the reactor allowed injection of the medium, and two outlets provided for circulation of the medium and for sampling of the produced biogas. The bioreactor was also connected to a control system, furnished with a computer that recorded the acquired data, and to an *Automatic methane potential test system* (AMPTS) that analyzed the obtained biogas. The reactor was designed for obtaining maximal surface contact with the medium, and used 17 submerged packed beds for the uprising plug flow. The beds could all be fixed inside the reactor, since the gaps between the membranes were only 1.5 cm, and the reactor was subsequently plugged over the culture medium, which contained fatty acids (propionic, butyric, and acetic acids), glucose, and methanol. The working volume and the head space volume were 800 mL and 1.5 mL, respectively. The total height and diameter of the reactor were 220 mm and 120 mm, respectively. The anaerobic digestion in the reactor

was controlled by circulation of warm water through the reactor jacket, keeping the temperature of the reactor at 55 ± 5 ºC, and allowing circulation of the medium through the vessel.

The produced biogas accumulated at the top of the reactor, after which it passed through the Automatic Methane Potential Test System (AMPTS). Different types of synthetic membranes were tested in this study, by loading 9 g of inoculum onto each bed between two membrane sheets (Figure 4.3).

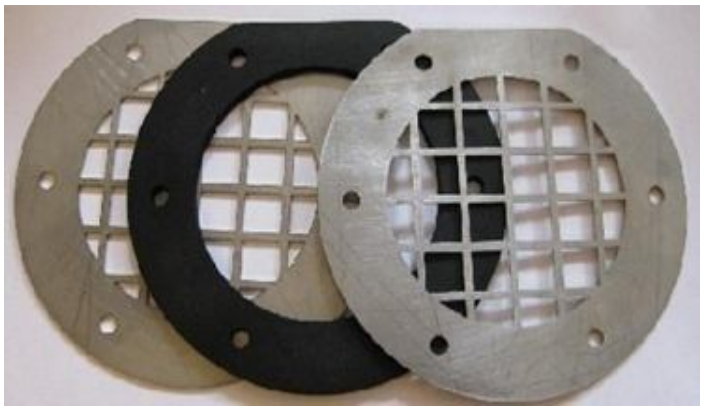

Figure 4.1 Stainless steel supporters for the compression of the membranes.

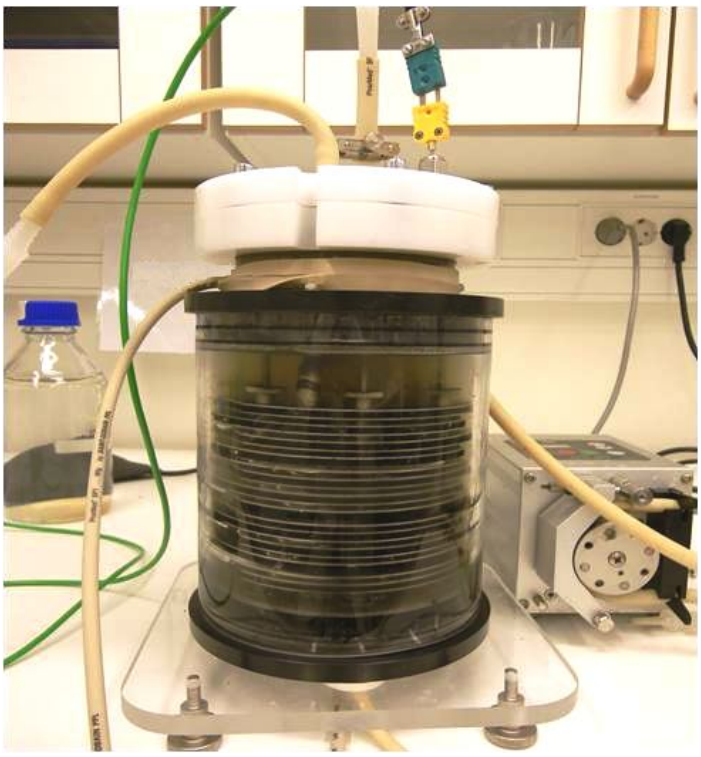

Figure 4.2 A compact multi-layer membrane bioreactor (MMBR).

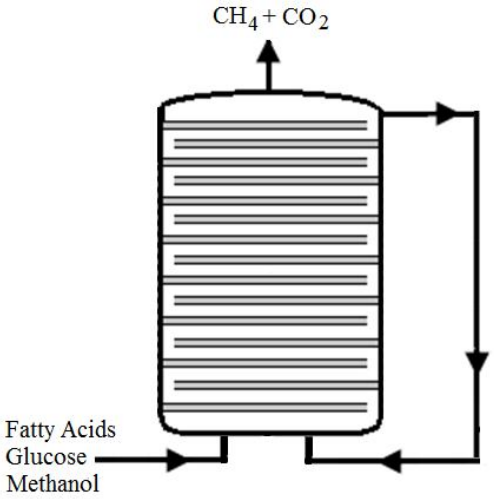

Figure 4.3. Scheme of an anaerobic multi-layer membrane bioreactor (MMBR), used for rapid biogas production (paper III).

4.1. Membranes in biogas production

Cross-flow of water-soluble components is prohibited through unmodified polyamide 46 since it, due to lack of hydrophilic functional groups, is not permeable. Polyamide 46 is highly resistant to common solvents, such as terpenes and tetrahydrofuran (THF), which might be present in wastewater, and as a consequence, the water treatment process might be thwarted due to microorganisms being killed by these solvents. In order to develop permeability of PA 46, two methods were used. Polyamide 46 was dissolved in a solvent together with a hydrophilic polymer (such as PVP) that acted as pore maker. The other method entailed functionalization of polyamide 46 into a homogenous hydroxylated derivative, which improved capillary flow. The difference between these two methods was that the latter method changed the polarity of the polymer. Hydroxyl groups, being moist groups, intend to bind to polar solvents by hydrogen bonding, while unmodified polyamide 46, because of its low polarity even in the presence of electrolytes, reduced the cross-flow rate due to presence of biological foulants. The hydroxylated polyamide 46 in this study displayed, thanks to the presence of hydroxyl groups, a high flow rate for nutrients and biogas production up to 18 days (paper III). After 18 days, however, the OH-groups in the hydroxylated PA 46 probably started to react with the anions of the carboxylic fatty acids, resulting in blockage and neutralization of the hydroxyl groups on the polymer chain, and consequently, bio-foulants started to stick onto the surface of the membrane in the same manner as when unmodified PA 46 is used. Accordingly, the flow of nutrients and biogas across the membrane gradually decreased (Figure 4.4). In accordance with this hypothesis, the reaction should be reversible by washing the membranes with very dilute non-oxidative mineral acids, such as hydrochloric or sulfuric acids, for regeneration of the membranes. However, this phenomenon was not observed when using PVDF membranes, due to their unwillingness to react and their lack of affinity toward anions. The rate of permeate flow through membranes is governed (directly or indirectly) by Fick's Law, involving parameters such as temperature, size of molecules, surface area of membrane, concentration of gradient, and polarity of both membrane and gradient [136]. Figure 4.5 illustrates diffusivities of fatty acids through the membranes used in the present study. Diffusion under isothermal conditions, using equally sized membrane areas was correlated to size of the fatty acids and the polarity of the membrane. Initially, diffusion of acetic acid was rapid in all membranes, due to high portability. However,

once the concentration of gradient was equal on both sides of the membrane, consumption as well as diffusion rate decreased dramatically, and was eventually dependent only on the microorganisms' consumption of acetate anions. In terms of the other fatty acids tested in the present study, the initial permeate flow was lower than for acetic acid, most likely due to their larger molecular size and their lower mobility. The different permeate flows over the three membranes was correlated to polarity of the membranes[136], whereas the driving force behind mass transport trough the membranes was regulated by the chemical potential of permeate, cell consumption and osmotic pressure. Since the microorganisms were of the same type in all tests, they were not involved in the control of permeate flow or mass transport [136]. The reason for lower accumulation of propionic acid than of butyric acid when using hydroxylated PA 46 was most likely related to the propionate anion having a lower ratio of molecular weight to charge number than the butyric acid anion, results in higher diffusivity of propionate anions. Notwithstanding, as the butyrate anion possesses less polarity, and therefore has a lower affinity to the hydroxyl groups in hydroxylated PA 46, the diffusivity of the butyrate anion is inferior to the propionate anion diffusivity. Unlike hydroxylated PA 46, the PVDF membrane does not interact with acetate, propionate, and butyrate anions. Hence, lateral diffusivity of chemicals over this membrane is only correlated to molecular weight or mobility of permeate[137] and osmotic pressure. To sum up, due to lower molecular weight and higher mobility, propionate displays a higher diffusion rate than butyrate, resulting in very minute accumulation in the medium (paper III).

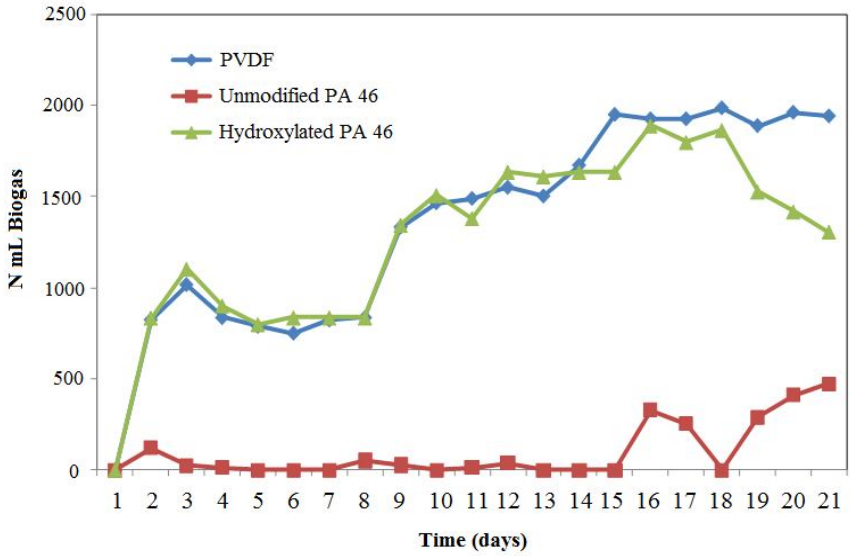

Figure 4.4. Volume of biogas production vs. time, using PVDF, hydroxylated PA 46, and unmodified PA 46 membranes.

As is shown in figure 4.4, hydroxyethylated PA 46 competed with PVDF up to 18 days, after which fouling of the membranes resulted in decreasing biogas production. The advantage of this type of design is that multi-unit membranes create a large surface area that maximizes mass transport of the medium through the membrane, in form of compact submerged membrane reactor (paper III).

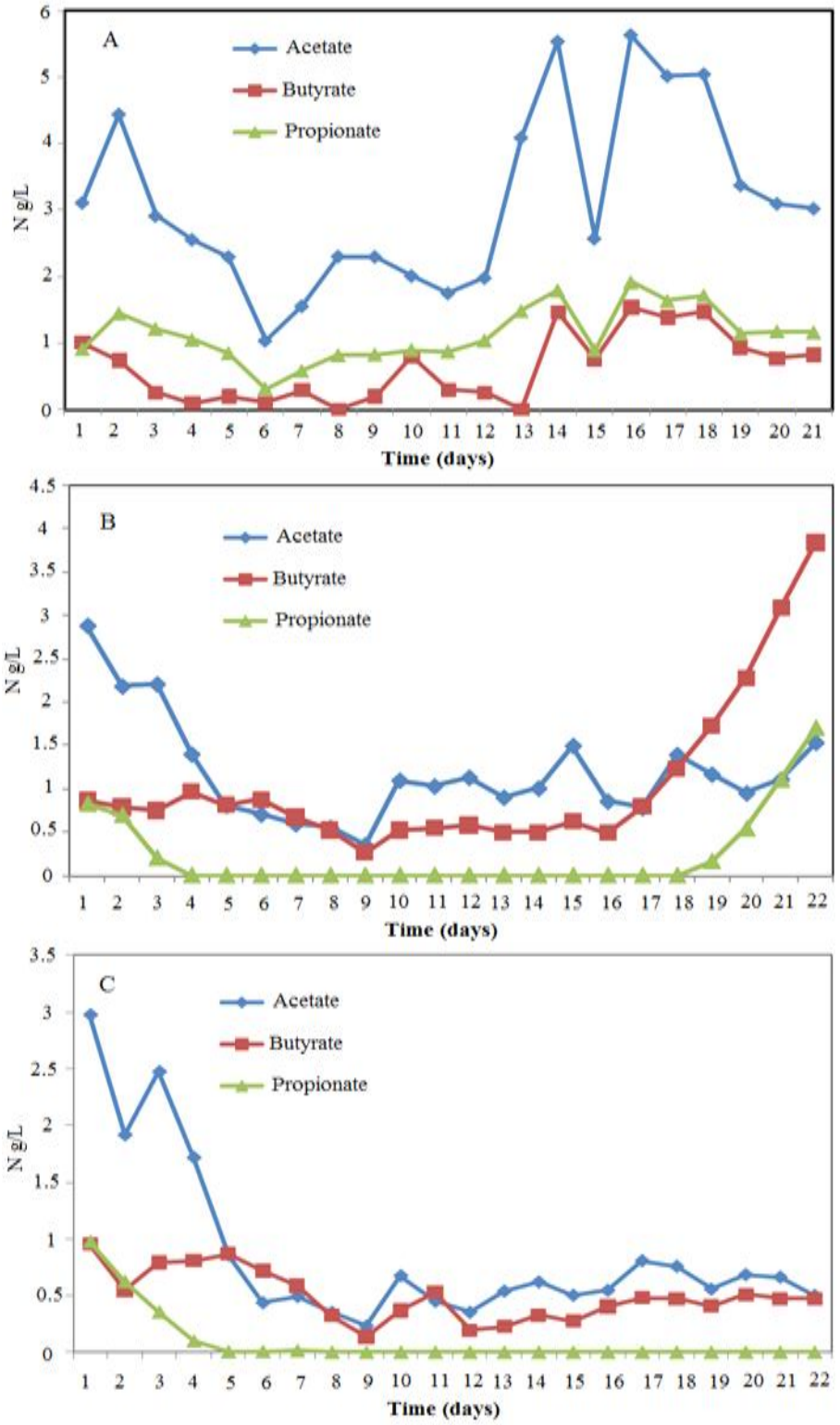

Figure 4.5. Fatty acids consumption (Acetate, butyrate, propionate) in MMBR: (A) Unmodified PA 46 membrane, (B) Hydroxylated PA 46 membrane, (C) PVDF membrane.

5

CONCLUSIONS AND FUTURE WORK

5.1. Conclusions

The present thesis has brought to attention membranes based on polyamide 46 and their use in submerged MBRs (multi-layer membrane bioreactors). Studying the processes of synthesizing this type of membrane aimed at obtaining high performance membranes for fermentation processes in MBRs, through which a continuous and distributive feeding of medium would be optimized. Increasing the hydrophilicity of bulk membranes or the membrane surface would reduce biofouling and as a result increase the flux of nutrients and metabolites through the membranes. Highly porous and hydrophilic membranes revealed high yield in the bioreactor due to the surface being unable to attach microorganisms that otherwise would be blocking the membrane pores. Results also disclosed that increased hydrophilicity of membranes caused decreased crystallinity and lowered the mechanical properties of the membranes. However, significantly fewer such observations were noted for polyamide 46, a highly crystalline polymer. Copolymerization of hydrophilized PA 46 with an electroconductive monomer HMEDOT yielded a robust electroconductive polymer. Thus, hydroxyethylated PA 46 is a suitable choice for manufacturing a mechanically highly apt electroconductive membrane, useful for many applications. The negative charge of this type of membrane is very high, but entrapping living

cells minimized biofouling. In addition, plasma polymerization might be a suitable technique in order to coat any type of membrane of minimal thickness, and still retain high effectiveness.

5.2. Future Work

The method of functionalization of polyamide 46, thereby obtaining modified semi-permeable polyamide has in the present thesis proved its eligibility for further applications in various areas as synthetic cellulose. The hydrophilized polyamide show physical properties similar to cellulose, but is much stronger. Moreover, durability (due to ability to dissolve in common solvents) and recyclability are other advantages after functionalization of this polymer. Future potential applications consist of:

- Anti-bacterial membranes for water treatment in MBRs
- Gas or heavy metal sorption in MBRS.
- Reactive membranes to adsorb toxic materials from effluents.
- Ionomer membranes to facilitate hydrogen exchange in fuel cells or biofuel cells.
- Catalytic membranes for promotion of chemical reactions, including separation processes.
- Inherent electroconductive membranes can be developed in other areas; e.g. smart membranes to be used in: the human body, electrobioreactors, solar cells, and fuel cells, as an alternative to the expensive Nafion membrane.
- Fluffy functional polyamide 46 is suitable for wastewater treatment, heavy metal removal, gas adsorption, water decoloration, and deodorizing.
- Biocompatibility of hydrophilized polyamides provides motivation for implantation of its derivatives in the form of an artificial kidney or liver.
- Embedment of drugs in functionalized polyamides is suitable for dermally administered drugs and for healing patches.
- Ability to synthesize superhydrophobic polymers, using the method described in this thesis might be an alternative to Teflon.

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