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(Bio)fouling in Cross-flow Membrane System: Investigating the Role of Transparent Exopolymer Particles (TEP)

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Abstract

There is no doubt that global water consumption in near future will increase exponentially. To meet this upcoming water demand, membrane technologies seem to be one of the most promising ones. Membranes have numerous advantages over other technologies; however, membrane fouling, i.e. the clogging of the pores by organic and inorganic materials and/or the growth of microorganisms, makes these membranes difficult to operate and to be energy inefficient. Fouling slows down the production, requires more operational pressure, cleaning reagents and equipment, and in many cases damages membranes irreversibly. From all fouling types, biological fouling is probably the most difficult to overcome because it involves self developing process - the microbial growth.

Recent discovery revealed the abundance of transparent microscopic sticky substances abundant in natural waters, formed from components excreted by microorganisms (mostly algae). This material has been named transparent exopolymer particles (TEP). It has been observed that these invisible organic substances are usually generated during algal bloom seasons and algae are the main excretor of the TEP. In membrane filtration processes, TEP is suspected to be able to form a sticky layer and facilitate initial attachment of particles and microorganisms on otherwise clean membrane surfaces. The transparent exopolymer particles are therefore recently getting more attention in fouling prevention studies.

This research illustrates how the TEP enhances particular and biological fouling in cross-flow membrane systems (RO and UF). For RO, the studies have been accomplished employing a recently developed tool – membrane fouling simulator (MFS). For UF - a small-scale hollow fiber filtration setup was used. To test the TEP effect on particulate fouling, 1µm polyesterene microspheres have been applied. To test the effect on biological fouling, a pure culture of Escherichia coli (ATCC 25922) as well as a mixed natural microbial culture from the water of North Sea were utilised. In order to attain objective results, the experimental plan has been set up to exclude as many external factors that may interfere with the results as possible, leaving the only variance – the presence and the absences of the TEP. The cultured TEP used in the experiments was extracted from laboratory-grown common strain of seawater diatom Chaetoceros affinis.

To illustrate the effect of TEP on biological fouling, the decline in membrane performance (membrane permeability) over time was monitored and membrane autopsies (TOC, ATP) have been done. The short term tests with pure bacterial culture and the long term tests with natural microbial community have illustrated that the TEP enhances bacterial deposition and growth.
To illustrate the effect of TEP on particulate fouling, the particle concentration that goes in and comes out of the membrane was measured, and deposition of particles on the membranes was evaluated. Surprisingly, it has been shown that the presence of the TEP reduces the deposition of solid non-sticky particles. The backtransport velocity theory was employed to explain this phenomenon.

It has been verified that during the algal bloom periods, TEP should be removed from the feed water of reverse osmosis membranes. Ultrafiltration is an appropriate pre-treatment approach for substantial removal of TEP and minimisation of biofouling in downstream RO system. Moreover, TEP demonstrated flocculant properties on particulate/colloidal materials in the water. Further studies should be carried out to investigate its potential application as a natural coagulant.
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1. Introduction

1.1. Background

The world's water consumption rate is doubling every 20 years [1]. Due to factors like increase in living standards and consumption, industrial growth, shifting of the population to draught and coastal regions, it is anticipated that by the year 2025 water demand will be exceeding supply by 56% [2]. The water demand rate outpaces the population growth by two times. Moreover, human activity endangers fresh surface water and groundwater resources, enhances desertification, and it is believed that the effect of climate change creates unnatural fluctuation of rainfall. The global resources of clean and fresh water are decreasing while water demand for agriculture, industry and people is on the rise. Since the demand is of vital importance and has no substitution, it is accelerating the worldwide need to better manage water resources by applying new policies and new technologies.

Even though the situation is alarming, it is not at all hopeless. Incredibly abundant and continuously available water resources dwell in the oceans and in the seas. The salty and brackish water makes up even 97% of the earth's water of all its forms [3]. Desalination systems installed in the coastal areas for this purpose have the potential to purify plenty of water. It is this source of purified water that is considered to be an important alternative that should cover the future fresh water needs for domestic and industrial uses in many countries [4].

A big part of desalination technologies are the membrane technologies. The membranes are very well suited for water purification. Depending on the membrane type it can make water free from pollutants, bacteria, viruses and dissolved salts. The use of membrane filtration technology in drinking water treatment is increasing because membranes remove pathogens more effectively than conventional filtration processes and without any chemical pre-treatment [5]. Therefore, membranes are also able to make drinking water from the redundant resources like wastewater and polluted water bodies as well as from the salty sea water. According to publication *Sustainability science and engineering* [6], nowadays 63% of the desalination capacity of 44.1 million cubic meters per day is produced from seawater, 19% from brackish water, and 5% from waste water sources. 35% of this water is produced by the reverse osmosis (SWRO) processes.
1.2. Problem statement

Even though the sea water as well as polluted water is normally free, the price of the water from the outlets of the membrane plants is high. The SWRO plants produce water for 0.7 – 1.3 €/m³ [7]. This price is made up of equipment, operational and energy costs. Equipment is becoming ever cheaper, but global energy prices do not tend to decrease. Membrane treatment is an energy costly process and most of its energy is being wasted due to operational problems. Therefore, water desalination is currently developing in wealthy coastal regions such as the Arabian Gulf, the Red Sea, and the Mediterranean Sea. Even 99% of the desalinated Mediterranean Sea water is produced by seawater reverse osmosis technology [6]. In order to implement these technologies in more countries and for more types of demand, minimization of those problems is needed.

The major operational problems in membrane-based water purification systems are scaling and fouling. The membranes are being backwashed to physically remove particles (e.g. MF/UF) and/or cleaned with chemicals (e.g. RO/NF) on a regular basis. Nevertheless, some residues remain on the membrane surfaces and accumulate during long-term operation. Deposition of inorganic matter (scaling), adsorption of organic substances (organic fouling), attachment of microorganisms and growth of the biofilms (biofouling) clog the membranes, reduce their performance and energy efficiency, and even causes irreversible damages of the membranes. The above-mentioned naturally occurring problems make the process operation complicated, reduce machinery lifetime and require additional monitoring, extra staff and equipment. Unwelcome increase in energy demand, which is needed to operate higher trans-membrane pressures when these are clogged, makes membrane technologies hardly feasible and environmentally unfriendly.

Substantial progress has been made in the field of managing the chemical scaling, which can be relatively easily reduced with chemicals (e.g. anti-scalants, acids). The closely related organic fouling and biofouling are more difficult to control due to their complex nature and hardly predictable development. In membrane research a lot of attention is being paid to gaining more understanding about these two problems. It already led to operational guidelines for different situations, modification of membrane materials, alteration of hydrodynamic conditions, feed water pre-treatment strategies, integrated membrane systems and optimization of membrane cleaning methods. In many cases, the fouling is slightly or considerably mitigated but the reasons for success or failure are however too often the subject of a right guesswork. A general understanding of biofouling as a process itself and the methods how to deal with it are still lacking [8].
1.3. Goals and objectives

Since membrane fouling is a very complex problem, this research will narrow itself to investigate one feed water constituent that is suspected to enhance fouling in initial stages more than any other factors. The suspect of mentioned component comes from recent observations in sea water purification plants. It has been observed that membranes foul more severely in spring seasons (March-May) than in any other time of the year [9]. This problematic period matches with the algal bloom period, when algae concentrations in natural waters reach their peaks. Normally the algae do not enter the membrane systems at all or are removed in the pre-treatment steps. Nevertheless some of the products they produce pass the pre-treatment. These substances (Transparent Exopolymer Particles – TEP) are suspected to enhance the fouling during the algae bloom in particular and during the rest of the time, while they still remain in the waters.

Since the hypothesis itself is not very sound, this study does not aim to provide direct solutions. The objective is to investigate the mentioned material in laboratory membrane units and support or deny some of the assumptions about its contribution to fouling. Given that the nature of various types of the fouling is different, this thesis aims to study the effect that the material contributes to particulate (colloidal) fouling and biofouling separately.

The goals are the following:

Separating as much of the side factors as possible

- to study the effect of TEP contribution to particulate (colloidal) fouling,
- to study the effect of TEP contribution to initial biofouling,
- to study the effect of TEP contribution to long-term fouling.

The proof that the substance enhances the fouling and the figures supporting that proof can be useful for later studies that will aim for solutions.

1.4. Research hypothesis

There are two major properties of the TEP that can make it initiator or enhancer of fouling. The TEP is highly surface active (i.e. sticky) and it has high organic carbon content. Its stickiness is supposed to help particles and bacteria to attach to the membrane. Its natural origin and the carbon source are supposed to act like a bacterial food source and enhance biofouling. Therefore, the main hypothesis is that the TEP enhances both particulate and biological fouling.
2. Literature overview

2.1. Industrial membranes

2.1.1. Definition and classification

A membrane is a thin semi-permeable fabric that can separate a solvent from the components dispersed or dissolved in it. The concept of a membrane is very common in nature. Membranes wrap around every cell, blocking harmful materials from entering but allowing nutrients to come in and the waste to go out. Nowadays industrial membranes are made of polymers and other materials [Figure 1]. They are broadly applied in medicine – blood treatment; food technology – concentration of juice and milk; biotechnology – fractionation of proteins; and have numerous more applications. In water technology membranes accomplish the function of a filter. They retain unwanted components from impure water, letting the clean water pass through. Industrial membranes are differently categorized but the main classification is the pore size, due to which most of the parameters (application range, operational pressure, etc.) depend.

According to the pore size categorization [Figure 2], there are four types of industrial membranes: microfiltration - 0.1-10 µm (MF); ultrafiltration - 0.01-0.05 µm (UF); and nanofiltration - less than 1 nm (NF). The fourth membrane type – reverse osmosis (RO) – has indeed no pores as such. The pressurized water diffuses into the material of the RO membranes from one side and diffuses back into the other side – leaving virtually all the impurities behind. The reason of using a membrane with lower particle removal efficiency (larger pores) is that it requires lower operational pressure to pass the water through and therefore less energy and
smaller operational costs. Different particle removal efficiency with comparison to the size of common objects can be best illustrated by Figure 3.

Figure 3. Filtration scale [10]

MF and UF are low pressure or vacuum driven processes (up to few bars). These membranes remove particulate substances and a part of colloidal materials but not the dissolved ones. MF and UF membranes can be manufactured either from polymeric or ceramic materials. NF and RO are the high pressure driven processes (5 – 80 bars). They are used to remove dissolved solids and can only be polymeric ones [7], [11].

2.1.2. Design and operation

All of the membrane types (MF, UF, NF, RO) can be implemented in different hydrodynamic designs (modules) which also can have different dimensions. Hydrodynamic design is the factor which determines the way membrane operates. The most common designs are the spiral wound, hollow fibre and tubular, each of which have its own operational advantages and disadvantages.
When membranes operate, filtered solution is being forced to move towards the surface of a membrane. The pushing allows water to pass through and retains the dissolved components which are larger than the pores behind. This results in an accumulation of a higher solvent concentration near the surface of the membrane. This type of dissimulation of concentration is called the concentration polarization. Particles are also present in the waters. In membrane filtration, when particles are too large to pass the membrane pores, a kind of sieving process occurs. The retained particles accumulate on the membrane surface in a growing “cake layer”.

If the solution is filtered in perpendicular direction to the membrane surface (dead-end filtration), it highly enhances faster building up of the impurity layer (cake formation) and the effect of concentration polarization. Consecutively, it inhibits the filtration process. In cross flow filtration modules, the fluid motion tangential to the membrane surface may detach the growing cake. The high shear created by the water flowing tangentially to the membrane surface sweeps the particles and accumulated solvents towards the end of the membrane element and longer operation of membrane is possible [12].

Tubular, capillary and hollow-fiber membranes are of the shape of a tube. The only difference in the design is in the size of the internal diameter. The diameter of the tubular is 5 – 20 mm; of capillary – 0.5 – 5 mm and of the hollow fiber – less than 0.5 mm. All of these modules can be operated in a cross-flow and in dead-end modes. These membranes have a simple design and are easy to operate. These types of modules are suitable to be exploited in smaller scale applications and in situations where the filtration efficiency is not high.

The spiral wound design has more complex process layout and requires higher investment costs. Nevertheless, it allows circular flow (tangential to the membrane surface) and reduces concentration polarization and cake formation [Figure 6]. In addition, it has a high surface area to volume ratio and saves greatly the space [Figure 7]. Pressure driven spiral wound membranes are therefore the most popular for the large scale production of drinking water.
2.2. Membrane fouling

2.2.1. Fouling types

The solute or the particles in the feed water attach to the surface of a membrane in a way that it slows down the membrane’s performance. The attachment is virtually unavoidable, but when it increases the net pressure drop (NPD) of the membrane systems by about 15 percent, the unwelcome phenomenon is called fouling. In general the fouling is so troublesome that it is considered to be the most expensive problem for the water industry. Membrane fouling reduces the flux and therefore the amount of the filtered water. When the fouling is not too severe, the flux can be recovered by increasing the pressure. Consequently, the energy consumption rises up and the feasibility of the filtration process declines. If fouling is not treated properly, it would eventually stop the filtration process.

Fouling can be categorized into chemical scaling, particulate (colloidal) fouling, organic fouling and biofouling. When natural water is filtered, it is common for all of the fouling forms to occur simultaneously. Nevertheless, it is useful to categorize fouling in types for better understanding about how to cope with it. The fouling types are described below in an order from probably the least complex to the most problematic one.

Chemical scaling [Figure 9] is a crystallization of solid salts, oxides and hydroxides on a membrane surface that come from the water solutions. The most common examples of chemical scaling are accumulation of calcium carbonate or calcium sulphate. Chemical scaling is a very common problem occurring when filtering hard and salty water. However, it can be already relatively easily controlled by dosing special acids and anti-scalants.
**Particulate (colloidal) fouling** as the name implies is a fouling of particles (>1µm) and colloids (0.001-1µm) that are suspended in the water. These particles can be inorganic (sand, aluminum silicate, iron oxides, magnetite, hematite) as well as organic (large polysaccharide molecules, fulvic compounds, proteins). Particulate fouling is supposed to be easily removed by backwashing of the membranes [see 2.2.2], but with time the resulting surface deposit may harden through processes known as *deposit consolidation* or, informally - *aging*.

**Organic fouling** is a serious problem caused by deposition of natural organic matter dissolved in water. Organic matter is often the main substance why the membranes are needed to be applied for the water treatment. Nevertheless, controlling organic fouling in membrane systems is not an easy task. Organic substances are small; thus, they first block the smallest pores. In the meantime some of the substances attach on the inner surface of larger pores, reduce them and finally clog those as well. If no measures are taken to deal with organic fouling, the layer builds up and stops the filtration.

Biofilm takes place when microbes deposit on a membrane and begin to grow. When biofilm formation causes problems in the membrane system it is called *biofouling* or biological fouling [Figure 8]. Not all biofilms can cause biofouling. Biofilm is a very common phenomenon which takes place virtually on any surface that is exposed to water. The term *biofouling* is operational, referring to membrane performance problems caused by it.

As organic fouling, biological fouling develops in phases. Microorganisms attach to the surface (the induction phase), the growth rate of attached microorganisms becomes much faster than the attachment of new ones (the logarithmic growth phase) and finally biofilm growth and physical detachment rates reach the balance (the plateau phase). This type of form of fouling can be called *alive*. It can increase even after the feed water becomes free of microorganisms. Biofilm consists of microbes and of the extracellular substances they produce. These microorganisms can be of all types - bacteria, protozoa, fungi and algae. Most often microbial community consists of many types of microorganisms.
2.2.2. Fouling treatment

Occurrence and development of the fouling depends on a lot of aspects. These can be the feed water qualities (foulant type and concentration, microbial community, temperature, pH, ionic strength, specific ions), membrane material (roughness, charge, hydrophobicity, surface functional groups) and hydrodynamic conditions (flux, cross-flow velocity, membrane module and spacer design). Numerous studies have been conducted worldwide addressing all the above-mentioned factors and fouling treatment and elimination technology has been improved.

The most primitive and the most common method to eliminate fouling is membrane backwashing. The backwashing is reversing filtration direction and discharging this water away. This approach is normally applied with a higher than operational pressure (or flux) for a short period of time. In most of the cases it removes only a part of a fouling (back-washable) but does not handle the non-back-washable one. The non-back-washable part of the fouling can be cleaned by introducing the chemicals to membrane systems. The chemical reagents remove a part of the fouling that is called reversible fouling, but cannot achieve the same results with an irreversible one.

For the fouling that is not easy to control with backwash or with chemical cleaning, different pretreatment approaches can be considered. Feed water pretreatment reduces certain components in feed water and therefore it results in less membrane fouling. These methods can be cartridge and sand filtration; membrane cascades that progress filtration towards smaller pore sizes; reduction of organic matter concentration by adsorption in processes such as coagulation and flocculation. For solely biological fouling - UV disinfection, advanced oxidation and dosing of the chlorine can be applied as pre-treatment.

Ever increasing water demand and prices of energy co-create the high demand in fouling treatment technology. Nevertheless, fouling is a natural process and cannot be totally eliminated. Materials that do not pass through the membranes must be retained and therefore deposit on membrane surface. However, the room for improvement in fouling prevention and treatment technologies is still big. Better technological designs are essential in order to reduce negative fouling effect and improve water treatment technology. The main fouling causation are the various components in feed water. The knowledge about these components and fluctuations in their concentrations in time are valuable for applying specific pre-treatment actions.
2.3. Transparent exopolymer particles (TEP)

2.3.1. Description and origin

In 1993, an American oceanographer, Alice Alldredge, stained seawater samples with Alcian Blue - a dye used to stain acidic polysaccharides. She then discovered that the samples were full of, until then undetected, microscopic transparent particles that stain with this dye. The discovery of these invisible microscopic materials in oceanic waters has been published in the paper *The abundance and significance of a class of large, transparent organic particles in the ocean* [13]. The chemical composition of these particles suggested that they were formed from phytoplankton excretion products. The discovered substance has been therefore given the name of **Transparent Exopolymer Particles (TEP)**. Transparency of the TEP resulted in escaping detection by microscopy before, but once the method for visualization of the TEP was developed, its high abundances in the oceans, lakes, rivers and reservoirs were shortly revealed [13], [14].

The precise composition of TEP is unknown. The TEP are chemically diverse and heterogeneous. The TEP is unlike the common particles, because most of the particles in theory are settable. The better definition of TEP is hydro gels. They compose around 99 percent of water, are not solid and are often not settable. The size of a separate TEP is hard to determine because it can easily deform.

TEP are operationally defined as particles retained on polycarbonate filters, which stain with the cationic dye Alcian blue [15]. These particles are deformable, gel-like, suspended in the water mass and appear in many forms: amorphous blobs, clouds, sheets, filaments or clumps [16]. As described in the oceanographic and limnological literature, TEP range in size from about 0.4 μm to about 100–200 μm.

Like the extracellular polymeric substances that form the matrix of aquatic biofilms, TEP are mostly composed of polysaccharides (that take up the Alcian Blue dye). Because TEP are surface active, many other substances, like acidic polysaccharides, proteins, nucleic acids or trace elements may be associated with these gelly particles [16]. Many TEP are intensely colonised by bacteria that find them both a convenient and a nutritional platform on which to grow [17].

The biggest part of the TEP originates from polysaccharides, released by microalgae and bacteria. Most of the TEP are initially released into the water as dissolved polysaccharides, which subsequently coagulates to form the TEP in later formation stages. The chemical composition of phytoplankton and bacterioplankton excretion products is known to vary.
between species and it results in different TEP properties [13]. Some TEP can also be produced from the gelatinous, mucous envelopes surrounding bacterial cells, diatoms, cyanobacteria and various other algae [16]. Polysaccharides are also exuded or lysed out from macro algae (seaweed) and some higher marine organisms and can also form TEP [18].

In summary, TEP may be characterized as microscopic (>0.4 μm) transparent particles, ubiquitous in marine and freshwaters, that constitute a subgroup of planktonic EPS.

### 2.3.2. TEP formation methods

TEP can be formed in aquatic systems by either abiotic or biotic mechanisms. In biotic pathway TEP are released by some organisms directly. TEP are mainly formed by the abiotic pathway which depends on types and abundance of TEP precursors as well as on environmental factors (turbulence, ion density, concentration and of inorganic colloids) [9].

The abiotic mechanism starts when aquatic organisms excrete dissolved acidic polysaccharides to aquatic system as a reaction to a specific environmental stress. Before releasing these polymers, microorganisms assemble them into packages of colloidal dimensions taking the form of extremely thin ribbons or fibrils called TEP precursors [19]. Moreover, these fibrils can originate from both capsules of active bacteria [20] and intracellular substances from damaged cells [21].

The precursors form cationic bridges and hydrogen bonds promoted by cations in the water (e.g. Ca$^{2+}$ which is in sea water ~400 mg/l). Finally, these submicron gels coagulate further to form TEPs. This process is mainly enhanced by two mechanisms: firstly, application laminar or turbulent shear as it brings fibrils into alignment and accelerates formation of larger particles, and, secondly, by adsorption onto scavenging particles. The formation of particulate matter (TEP) by scavenging depends on size, dimension, concentration and settling velocity of the scavenging particle as well as TEP precursor concentrations.

The formation of TEP as a result of microbial activity varies with species composition, growth conditions and activity; and the amount of TEP generated by macroalgae (seaweed) depends on light, temperature and age of the algae [13].
2.3.3. TEP properties

TEP exhibit the characteristics of hydrogels, and consist predominantly of acidic polysaccharides. The role of TEP in aquatic systems differs from other forms of EPS, because as individual particles not only they can aggregate but they can also be collected by filtration; whereas dissolved substances can only mix with the surrounding water [13].

There is no general chemical structure of the TEP. The chemical TEP composition is specific to the species. In natural waters TEP composition is highly dependent on the microorganism that releases them. Although TEP seem likely to consist predominantly of water, all measurements of the carbon content of the TEP indicate that it can be very high. Estimates of TEP-C calculated from microscopic or colorimetric determinations suggest that the carbon content of TEP lies in the same range as that of phytoplankton (on the order of 230 ± 150 µg C/l) [22].
The physical properties of TEP, such as volume and stability also depend on environmental conditions. In contrast to solid particles, TEP exhibit the properties of gels, such as high flexibility and their volume to mass ratios depend on environmental factors [13]. Significant physical characteristic is that TEP are highly surface active - sticky. It was found that the acidity of these particles is mainly due to sulfate ester groups (R-OSO3-) [23].

The interactions between TEP and other particles are primarily determined by the high stickiness (probability that two particles remain attached after collision) of TEP. It is extremely difficult to measure stickiness of TEP directly, because it is difficult to isolate them. Because the overall stickiness of natural particles of different stickiness is determined by the few sticky particles, the stickiness of TEP may be estimated from measurements of a combined stickiness coefficient ($\alpha$) by evaluating the relative contribution of TEP to overall stickiness [9].

The combined stickiness coefficient of a sample containing different particles, such as phytoplankton, bacteria, detritus and TEP, is determined by measuring the aggregation rate and the size frequency distribution of all particles in an environment of constant and known laminar shear, e.g. in a Couette flocculator. Such determinations of combined $\alpha$ indicate that during diatom blooms the overall stickiness of particles is largely determined by the high stickiness of TEP, which exhibit an alpha larger than 0.1. The stickiness coefficient of old, bacteria-covered mucus particles, which were generated by a batch culture of Coscinodiscus sp. are also very high ($\alpha = 0.7$). Stickiness of most solid particles, including detritus, sediment and phytoplankton, has generally been estimated to be low with attachment probabilities 1% ($\alpha = 10^{-2} - 10^{-4}$) [9].

The stickiness, which by definition is a high probability to attach upon collision, indicates that the TEP are likely to play an important role in coating natural surfaces. Once TEP adhere to the surface, they can provide a nutritious substrate for microbial growth and the establishment of biofilm.

### 2.3.4. Methods of TEP concentration estimation

TEP may be quantified either microscopically or colorimetrically. Although data based on measurements using one or the other of these methods are not directly comparable, the results are consistent. Both of these methods are based on staining TEP with Alcian blue. Firstly stained particles may be enumerated and sized microscopically. Alternatively, the amount of stain bound to particles is acid-extracted from the filters and measured colorimetrically.

The first method developed to measure TEP was by microscopic “enumeration”. TEP is filtered and stained with Alcian Blue dye. The stained TEP are transferred into a glass slide, immersed
with oil, and covered with cover slip. Finally, TEP particles are counted, either manually or semi-
automatically. The semi-automatic approach is quantifying by calculating accumulated volume
or surface area. However, microscopic enumeration is time consuming and difficult to apply.

To overcome the disadvantages of microscopic enumeration, Passow and Alldredge [24]
developed semi quantitative technique based on spectrophotometry. In this method water
containing TEP is filtered, the TEP is retained on a membrane and the membrane is stained with
the alcian blue dye. The stained filter is rinsed with distilled water to remove excess dye and
then the filter is transferred into a 25-ml beaker. Afterwards, the rinsed filter is soaked H₂SO₄
and after two hours the absorption is measured in 1-cm cuvette at 787nm wavelength. This
total absorption is adjusted for absorption of filter blank, which may vary with filter type and
stain batch, and sample blank. Finally, the TEP concentration is calculated in terms of gum
xanthan equivalent based on calibration experiments with gum xanthan solutions.

Although the spectrophotometric method is simple to use, it cannot be applied for samples that
contain other suspended materials in the water that are hardly destroyed by sulfuric acid which
may interfere with TEP measurement (sand, suspended materials including algal cells, iron,
etc.). To solve this problem, each water sample can be measured in a series of dilution rates.
Absorption of each dilution rate is corrected for filter blank and for unstained turbidity blank.
Subsequently, corrected absorption values are plotted against the corresponding amount of
sample on the filter. That should give a straight line with a slope represent the uncalibrated
value in the original sample. Lastly, TEP concentration readings are standardized using gum
xanthan as mentioned before [9].

Two new techniques were developed, namely: rapid spectrophotometric method [25] and acid
polysaccharide (APS) method [26]. These techniques share a lot of similarities. In both methods,
alcian blue (AB) is added to water samples to form AB-TEP complexes that precipitate.
Thereafter, these complexes are separated and the retained liquid part is analyzed for excess
alcian blue concentration. These values are proportional to TEP concentration by inverse linear
relationship. The main difference between the two methods is the separation step where AB-
TEP are removed by centrifugation in the rapid spectrophotometric method and a filter is used
in the APS method.

All above techniques have been developed by scientist from Limnology and Oceanography
disciplines "due to the central role of APS in water column biogeo-chemistry" [26].
Nevertheless, other authors (Liberman and Berman, 2006; Kennedy, 2009; Villacorte, 2009)
raised the need for an acceptable analysis to monitor TEP in water treatment systems [27] [28].
Villacorte et al. (2009) expanded the spectrophotometric method described above to measure
TEP in the range (0.05-0.4) µm.
2.3.5. TEP role in aquatic ecosystems

The availability of simple methods to measure TEP provided researchers with a growing understanding of the role of TEP in aquatic systems. In all waters, whether fresh or marine, TEP has been found to be associated with particles > 5 μm varying in concentration between 1 and 8000 ml\(^{-1}\) and particles > 2 μm varying in concentrations between 3000 and 40000 ml\(^{-1}\) [9]. TEP abundances in fresh and marine waters are in the same range as those of phytoplankton, with peak values occurring during phytoplankton blooms. Higher TEP concentrations have been found in coastal areas compared to an open ocean [29]. In the open Atlantic Ocean values were two orders of magnitude lower compared to the ones in coastal areas [9].

It is evident that TEP in seawater are mostly formed by phytoplankton [9]. Bacteria, which do not generate significant amounts of TEP, may however have an impact on the production of TEP by phytoplankton. Bacteria might compete with phytoplankton for the nutrients. Some algae (especially diatoms) release the TEP to capture nutrients and to keep the nutrients close to them. Interactions between bacteria and phytoplankton have rarely been investigated, so the relative importance of each, and the importance of their interactions for the production of TEP cannot yet be evaluated.

TEP also play a role in the structuring of food webs of small animals and microorganisms. For example as free exopolymer material, TEP and their precursors can be ingested directly and utilized as food by small protozoan (filter feeding microorganism) and Appendicularia [32]. Appendicularia (a solitary free swimming filter feeders found throughout the world’s oceans) remove TEP from seawater at rates typical for grazing on colloids. Because new TEP are formed from colloidal matter at levels similar to their removal described above, such grazing activity does not result in a rapid decrease of TEP concentrations.

The presence of TEP has been also observed to provide additional food for euphausiids (small shrimp-like animals found in all the oceans of the world). TEP-rich microaggregates, consisting of pico- and nano-plankton are readily grazed, thus permit the uptake of particles that would otherwise be too small to be grazed directly [9]. Therefore, TEP- microaggregates provide a link between the microbial loop and the traditional food web by enabling large zooplankton to ingest bacteria-sized particles. Bacteria utilize TEP as well, but the quantitative importance of microbial degradation is still under debate.
3. Research methodology

For the goals and objectives of this thesis (see section 1.3), materials by which the membranes would be fouled have been selected as follows: for the particulate (colloidal) fouling - tiny spheres; for initial biofouling - a pure culture of known bacteria; for long-term fouling – the water with known concentrations of particularly important compounds. All fouling experiments have been done using the same membranes from the same provider and trying to exclude as many external factors as possible.

3.1. Materials

3.1.1. Artificial sea water

Artificial seawater (ASW) with similar ion concentrations to the average world seawater was being prepared as the standard matrix of the inflow for the most of the experiments [Table 1][11]. To prepare this ASW, appropriate amount of every other salt was step by step dissolved in a bucket of stirred milli-Q water [see Annex 1].

<table>
<thead>
<tr>
<th>Chemical ion</th>
<th>Concentration (g/L)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorine, Cl⁻</td>
<td>19.2</td>
<td>55.1</td>
</tr>
<tr>
<td>Sodium, Na⁺</td>
<td>10.7</td>
<td>30.6</td>
</tr>
<tr>
<td>Sulfate, SO₄²⁻</td>
<td>2.7</td>
<td>7.7</td>
</tr>
<tr>
<td>Magnesium, Mg²⁺</td>
<td>1.3</td>
<td>3.7</td>
</tr>
<tr>
<td>Calcium, Ca²⁺</td>
<td>0.42</td>
<td>1.2</td>
</tr>
<tr>
<td>Potassium, K⁺</td>
<td>0.39</td>
<td>1.0</td>
</tr>
<tr>
<td>Hydrogen carbonate HCO₃⁻</td>
<td>0.16</td>
<td>0.44</td>
</tr>
<tr>
<td>Carbonate, CO₃²⁻</td>
<td>0.001</td>
<td>0.0</td>
</tr>
<tr>
<td>Bromine, Br⁻</td>
<td>0.05</td>
<td>0.0</td>
</tr>
<tr>
<td>Total</td>
<td>34.3</td>
<td>100</td>
</tr>
</tbody>
</table>
3.1.2. Natural sea water

The natural sea water for the long term (bio)fouling tests has been collected in Jacobahaven UF-RO see water desalination plant in Zeeland that belongs to Evides water company. The 20 litre buckets of water have been taken in the inflow of the plant (only after the 50 µm strainer) and in the effluent of ultrafiltration unit. The water has been collected on purpose when the highest concentrations of algae in the sea have been recorded (see section 4.3.2.1).

3.1.3. Cultured TEP

Diatoms are especially well known for excreting copious quantities of polysaccharides during all phases of their growth [33]. A strain of common seawater diatom *Chaetoceros affinis* (CCAP 1010/27) was cultured in the laboratory to collect TEP for experiments.

The TEP extraction procedures were performed by Yuli Ekowati (MSc) as follows:

1. A medium was prepared from artificial seawater containing basic nutrients and trace elements necessary for the strain to grow rapidly and simulate an algal bloom [Annex 2].

2. A round bottom flask of 2 L volume used as a reactor containing the medium which were sterilized by autoclaving for 30 minutes and then left to cool down to room temperature.

3. The air outlets of the reactors were covered with cotton to allow air circulation inside the reactor while minimizing contamination from the air.

4. *Chaetoceros affinis* (CCAP 1010/27) was inoculated (5 ml/50 ml of medium) in the above prepared media at room temperature.

5. An artificial light source (by fluorescent lamps) was supplied above the reactor for 12 hours per day. The ideal light intensity range, that was suggested by the supplier of the strain, was used (30 to 50 µmol /m²·s).

6. To keep cells in suspension and effectively utilized available nutrients in the medium, a continuous mixing condition was provided to the reactor using a shaker (90 RPM).
7. After 10-12 days under the above-mentioned conditions and ambient temperature of 20±3°C, the liquid phase was extracted by allowing the diatom cells to settle for 24 hours and then siphoning the supernatant of the reactor to a clean flask. The collected supernatant solution is the TEP stock solution.

8. The cultured TEP stock was filtered with 10 µm filter to further remove diatom particles and then stored in a cooling room at 5°C for later use in membrane filtration experiments.

9. Biopolymer carbon content was measured using liquid chromatography – organic carbon detection (LC-OCD) method for each batch of solutions [Annex 3].

3.1.4. Microsphere suspensions

In total two types of microscopic spherical particles (acquired from Polysciences, Inc.) have been used:

1. Polybead® Black Dyed 1 µm microspheres as a 2.5% pure water aqueous solution;

2. Polybead® Carboxylate 1 µm Red dyed microspheres as a 2.5% aqueous solution.

The both types of particles have been chosen because they apparently did not aggregate and did not seem to settle in the artificial sea water. However, it was difficult to distinguish whether these particles in the artificial sea water behaved like colloids or not. A colloid is a substance microscopically dispersed evenly throughout another substance. According to one of the colloid definitions, the colloids are the particles in the size rage of approximately 0.001-1µm and the particles bigger than 1µm are not colloids. The particles used for the experiments were of an intermediate size.

The selection of 1 µm Polybead® microspheres was based on the following criteria:

- The size of the particles represents colloids/particles that generally pass the pre-treatment steps (e.g. dual media filters) and may cause the fouling in the real filtration plants;
- The particles form a stable solution and within the period of few days apparently do not coagulate nor settle in artificial sea water;
- The particles dispersed in the water provided sufficient turbidity and any small change in particle concentration could be detected with available Dr. Lange® turbidity meter.
Polybead® Carboxylate Red dyed microspheres have been used to test whether the nature of particle surface materials is responsible for the particle deposition factors with and without the TEP. The carboxylic coatings on the microspheres are normally applied in science to imitate the bacterial surfaces.

The carboxyl groups (COOH⁻) – functional groups found in all carboxylic acids and amino acids - are negatively charged. Each hydroxyl (OH⁻) can make hydrogen bonds to three different water molecules. The hydrogen can bond to a pair of valence electron on the oxygen of water and each of the two pairs of valence electrons of the hydroxyl can bond to a hydrogen of water. Therefore carboxyl groups make hydrogen bonds with water and the molecules that dissolve or interact with water are said to be hydrophylic.

### 3.1.5. Microbial culture

For initial biofouling experiments, a pure culture of harmless *E.coli* strain ATCC 25922 has been used (cell size 2-3 µm). The *E.coli* has been chosen as a very common species of microorganisms, which can be found in the sea water as well. Other microbes could have been chosen (like vibrio), but they were absent in the laboratory and the strict safety rules prohibited using them. The advantages of using the *E.coli* strain were that it was easy to get, the procedure of working with it in the laboratory was perfected with time and the results of these procedures (like concentration estimation) were reliable. The procedure of working with the *E.coli* culture minimised the chances of contaminating the samples with other organisms in a way that would disturb the results. Prior to the test it could have been taken for granted that neither the experimental water nor the used equipment contained coliform and the membranes did not have to be disinfected from all the microorganisms that could have been present in it. The *E. coli* was grown on coliform agar [Annex 4] and it was almost sure that no other microbes could grow on the plates and certainly sure that grown collaries of other microorganisms could not have been confused with the experimental *E.coli* strain.

The practical reason of bacterial deposition experiments was not the try to simulate biological activity of the bacteria, but rather the physical characteristic of a bacterial surface to stick.

### 3.1.6. Membranes (Hollow fiber UF & RO)

The hollow fiber membranes were produced by *Pentair X-Flow® Seaguard*. The inner diameter of the fibers was 0.8 mm, the molecular weight cut-off (MWCO) 7kDa. The membranes were commercially designed for filtering both fresh and sea water.
The slices of reverse osmosis membranes and the spacers for the fouling simulator were manually cut from a new spiral wound membrane (DOW, BW30). The membrane and the spacers needed to be fixed between two plastic templates of MFS (8.5 x 0.26 x 5.5 cm). The height of the feed spacer (0.74 mm) was measured using an accurate calliper (Electronic Micrometer HBM Machines).

### 3.1.7. Experimental set-up

#### 3.1.7.1. Cross-flow UF system

The main element of the mini UF cross-flow filtration setup was a hollow fibre UF membrane. The system for making membranes ready to operate has been made manually: two units of 100 cm length fiber membranes were positioned in a plastic tube. Both ends of the tube were glued with sealing resins and cut in a way that the incoming water could only enter into the inner side of a fiber. In addition, a T-connector was fixed in the middle of the module to collect a permeate outflow [Figure 10].

![Figure 10. Two identical plastic tubes with the two hollow fiber membranes inside each. The sides are sealed in a way that the inflow, coming from the side, can only enter inside the fiber. The concentrate (outflow) can only escape hollow fiber from the other side. The open hole in a T-connector (middle) is the place where filtered (permeate) water collects.](image)

The prepared tubes were integrated into a set-up. The other main parts of the set-up were peristaltic pump, pressure gauge, manometer, recording scale and computer. Later the liquid manometer was changed with another pressure gauge (one for measuring feed channel pressure drop; another – pressure of a concentrate in the end of the tube). The cross flow velocity and permeate flux could be adjusted using control valve on concentrate stream that regulates concentrate flow. The permeate flow was collected into a beaker that was positioned on a scale. The scale, together with the pressure gauges, was connected to a computer through a communication box. Special software translated the signal from the communication box and through Macros delivered data to Microsoft Excel. The algorithm in Excel was prepared converting the data of weight into the data of flux under the given membrane dimensions. The data of pressure gouges could have also been translated into feed channel pressure drop or the trans-membrane pressure given the proper algorithm. [Figure 11].
Note: This is the principal UF cross-flow filtration setup. During experiments the set up could have changed without changing the principal. Another similar setup was build with time, which measured the pressure in the beginning and at the end of the tube, instead of the pressure difference and the pressure in the end.

![Diagram of laboratory UF installation](image)

**Figure 11.** The scheme of laboratory UF installation. (A) and picture of it (B). The feed water is filtered through hollow fiber membrane. The pressure drop over the hollow fiber is measured with a pressure sensor, a digital balance is connected to the computer to register permeate weight. Manometers are used to monitor concentrate and permeate pressure readings; both – the pressure and the permeate weight data are registered through the communication box in the computer.

### 3.1.7.2. Membrane fouling simulator

Membrane fouling simulator (MFS) is a tool developed for the validation of membrane fouling on the spacers of spiral wound membranes. The MFS uses the same membranes and spacers as present in commercial spiral-wound reverse osmosis and nanofiltration membrane elements, has similar hydrodynamics and is equipped with a sight window [34, page 82]. Using the MFS, fouling can be monitored by operational parameters like feed channel pressure drop and analysis of coupons sampled from the membrane. The main reasons for working with MFS is that it requires small amounts of water and other research resources, does not require high pressure and is claimed to represent spiral wound membranes well enough. Previous comparison studies of the MFS and spiral-wound membrane elements showed the same pressure drop development in time and the same fouling accumulation [34].
Figure 12. Scheme of the MFS installation. (A) and picture of it (B). The feed water (in) pressure is reduced using a pressure reducer (c). Chemicals can be dosed (d) to the feed water of the MFS. The pressure drop over the MFS (e) is measured with a sensitive pressure drop over the MFS (e) is measured with a sensitive pressure transmitter (Δp). The flow is regulated using a flow controller (f). [34]

The disadvantage of MFS is that it does not have permeated flow. The fouling mostly develops along the spacer channel because of the lateral flow of the water. Therefore no increase in trans-membrane pressure drop and no decrease in membrane permeability could be measured. Nevertheless, feed channel pressure drop rate can be evaluated that occurs due to fouling in membrane spacer and on the membrane surface. The pressure drop readings on a digital display of the pressure transmitter have been recorded manually.

### 3.2. Experimental methods

#### 3.2.1. Particle deposition experiments

The aim of the particle deposition experiments is to illustrate how the particles foul or deposit on the membranes together with TEP, in comparison to when no TEP are involved. The applied setups were UF hollow fiber cross flow [Figure 11] and reverse osmosis membrane fouling simulator (RO MFS) [Figure 12]. UF cross flow setup represents the membrane processes in hollow-fibre membranes; membrane fouling simulator – in spacers of spiral wound membranes. To prepare particles in ASW, $10^7$ of 1µm diameter microspheres per 1 ml were dispersed in it [Figure 13].
Since membrane fouling simulator has no permeate flux, only the feed channel pressure drop has been registered. When operating without permeate flux, less fouling is expected and therefore the experiments were planned to run for a longer period of time – 56 hours each [Figure 14].
3.2.2. *E.Coli* deposition experiments

The aim of the bacterial deposition experiments is to illustrate how *E.coli* sticks to membranes together with TEP, in comparison to when no TEP are involved. The experiments for bacterial deposition (initial biofouling) have been done according to the following plan:

![Experimental plan of initial biofouling in UF cross-flow filtration setup](image)

3.2.3. Biofouling experiments

The biofouling experiments can be divided in two major types: laboratory water with set concentrations of TEP [3.2.3.1] and natural sea water collected during the algal bloom period [3.2.3.2].

3.2.3.1. Laboratory prepared TEP

The aims of the biofouling experiments with laboratory prepared TEP were as follows:

- To illustrate how the microorganisms foul the membranes together with TEP, in comparison to when no TEP are involved;
- To illustrate whether in these conditions nutrients present in water enhance biofouling;
- To investigate whether the TEP can be consumed by microorganisms when no other carbon source is available.
The experimental plan includes both setups, UF cross flow filtration and membrane fouling simulator.

![Figure 16. Experimental plan of laboratory prepared water biofouling in UF cross-flow filtration setup](image)

![Figure 17. Experimental plan of laboratory prepared water biofouling in RO MFS](image)

### 3.2.3.2. TEP from algal bloom impacted waters

The aims of the biofouling experiments with the algal bloom impacted sea water were as follows:

- To illustrate the extent of biofouling in case raw sea water filtered,
- To compare this extent when the same water is pre-treated with a UF pre treatment step (Jacobahaven UF-RO desalination plant).

The experiments have been conducted in UF cross-flow filtration setup [Figure 18] as well as in reverse osmosis membrane fouling simulator[Figure 19].
3.3. Computational methods

3.3.1. Permeate flow

In all UF cross-flow filtration experiments, the flux has been chosen to be $15 \text{l/(m}^2 \cdot \text{h)}$. When the membranes foul, the flux naturally decreases. When the tiny valve openings block with impurities, the pressure of the concentrate side increases, in turn increasing the flux as the driving pressure for water permeation. During the experiments the permeate flow needed to be as often as possible adjusted to its original value.

The orriginal value of the filtered water (permeate) flow has been calculated with equations 1 and 2:

$$A_m = \pi \cdot d \cdot l \cdot n \quad [m^2] \quad (1)$$

Where:

- $A_m$ – membrane surface area,
- $d$ – the internal diameter of a hollow fiber. $d = 0.0008 \text{ m},$
- $l$ – the length of one fiber. $l = 1 \text{ m},$
- $n$ – the number of fibers. $n = 2.$

$$A_m = \pi \cdot 0.0008 \cdot 1 \cdot 2 = 0.005 \text{ m}^2$$
\[ Q_p = J \cdot A_m \quad [\text{L/s}] \] (2)

Where:

- \( Q_p \) – permeate flow,
- \( J \) – the flux. \( J = 15 \text{ L/(m}^2\cdot\text{h)} \).

\[ Q_p = 15 \times 0.005 = 0.075 \text{ L/h} = 1.26 \text{ ml/min} \]

3.3.2. Cross flow velocities

3.3.2.1. In the Cross-flow UF system

In all UF cross-flow filtration experiments the cross-flow velocity has been chosen to be 0.15 m/s. In order to maintain this cross-flow velocity, the inflow needed to be maintained constant.

The feed flow has been calculated with the following equations:

\[ Q_f = Q_p + Q_c \quad [\text{m}^3/\text{s}] \] (3)

Where:

- \( Q_f \) – feed flow,
- \( Q_p \) – permeate flow. \( Q_p = 1.26 \text{ ml/min} = 2.08 \times 10^{-8} \text{ m}^3/\text{s} \),
- \( Q_c \) – concentrate flow [m\(^3\)/s].

The cross flow velocity in a hollow fiber membrane is not constant. As the amount of the water inside the fiber decreases, so does the speed of the flow. When the membrane fouls, the feed side normally fouls more than the concentrate side and therefore the amount of water filtered throughout the membrane may equalize. The cross flow velocity has been referred to the average water flow in the fiber.
\[ CFV = \frac{Q_{\text{aver}}}{A_{cs}} \quad [m/s] \quad (4) \]

Where:

- \( CFV \) – cross flow velocity,
- \( Q_{\text{aver}} \) – average flow in the hollow fiber \([m^3/s]\),
- \( A_{cs} \) - area of a hollow fiber internal cross section \([m^2]\).

\[ Q_{\text{aver}} = \frac{Q_f + Q_c}{2} \quad (5) \]

\[ A_{cs} = \frac{\pi d^2}{4} \cdot n \quad [m^2] \quad (6) \]

Where:

- \( d \) – internal diameter of a hollow fiber. \( d = 0.0008 \) m,
- \( n \) – the number of the fibers. \( n = 2 \).

\[ A_{cs} = \frac{\pi \cdot 0.0008^2}{4} \cdot 2 = 10^{-6} \quad m^2 \]

(4) and (5) gives:

\[ CFV = \frac{Q_f + Q_c}{2} \cdot \frac{1}{A_{cs}} \quad (7) \]

Inserting (3) into (7) and rearrangement gives:
\[ Q_c = CFV \cdot A_{cs} - \frac{Q_p}{2} \]  \hspace{1cm} (8)

\[ Q_c = 0.15 \cdot 10^{-6} - \frac{2.08 \cdot 10^{-8}}{2} = 1.396 \cdot 10^{-7} \text{ m}^3/\text{s} = 8.38 \text{ ml/min} \]

Incoming flow can be finally calculated by the equation (3):

\[ Q_f = 1.26 + 8.38 = 9.64 \text{ ml/min} \]

### 3.3.2.2. In the MFS

To find the flow needed to set up certain cross flow velocity in a membrane fouling simulator, effective cross sectional area of the feed channel is needed. Not all cross-sectional area of the channel is effective because the membrane spacer reduces it. Effective area can be defined by the feed channel porosity:

\[ A_{eff} = \varepsilon \cdot h \cdot w \text{ [m}^2\text{]} \]  \hspace{1cm} (9)

Where:

\( A_{eff} \) – effective cross-sectional area,

\( \varepsilon \) – spacer porosity,

\( h \) – spacer channel height [m],

\( w \) – spacer channel width [m].

\[ A_{eff} = 0.89 \cdot 0.00074 \cdot 0.01 = 6.59 \cdot 10^{-6} \text{ m}^2 \]

The needed flow can be calculated:

\[ Q_f = CFV \cdot A_{eff} \text{ [m}^3/\text{s]} \]  \hspace{1cm} (10)

\[ Q_f = 0.15 \cdot 6.59 \cdot 10^{-6} = 9.89 \cdot 10^{-7} \text{ m}^3/\text{s} = 59.3 \text{ ml/min} \]

Where:

\( Q_f \) – flow of the feed [m\(^3\)/s],
CFV – cross flow velocity [m/s].

3.3.3. Membrane permeability (Kw) and relative permeability (Kw/Kw0)

Membrane permeability is a very practical unit to compare the change in membrane performance over time. The physical meaning of the permeability is a volume of filtered water per area of a membrane surface per applied pressure per time. It therefore gives an objective comparison for different experiments even if fluctuating fluxes at compared times were observed.

The membrane permeability can be calculated as follows:

\[
K_w = \frac{1}{\text{TMP}} \left[ \frac{m^3}{m^2 \cdot P \cdot h} \right] \tag{11}
\]

Where:
- \(K_w\) – membrane permeability,
- \(J\) – the applied flux \([m^3/(m^2 \cdot h)]\),
- \(\text{TMP}\) – trans-membrane pressure \([P]\).

\[
J = \frac{\rho_p}{A_m} \left[ \frac{m^3}{m^2 \cdot h} \right] \tag{12}
\]

\[
J = \frac{2.08 \cdot 10^{-8}}{5 \cdot 10^{-3}} = 4.16 \cdot 10^{-6} \left[ \frac{m^3}{m^2 \cdot h} \right]
\]

Trans-membrane pressure is a pressure difference between the inner side and the outer side of a membrane. The pressure in the inner side of a fiber is the highest in the beginning and the lowest in the end. Therefore, the average pressure needs to be accounted.

\[
\text{TMP} = P_{\text{aver}} - P_p \ [\text{Pa}] \tag{13}
\]
Where:

$\text{TMP} - \text{trans-membrane pressure}$,

$P_{\text{aver}} - \text{average pressure inside hollow fiber membrane [Pa]}$,

$P_p - \text{pressure in the permeate side of membrane [Pa]}$.

As indicated in the experimental setup [3.1.7.1], the permeate flow is subjected to the atmospheric pressure. Therefore, it does not play any role in trans-membrane pressure’s calculation. The equation (13) can be simplified:

$$\text{TMP} = P_{\text{aver}} \quad (14)$$

$P_{\text{aver}} - \text{the average pressure inside hollow fiber membrane can be calculated as follows:}$

$$P_{\text{aver}} = \frac{P_f + P_c}{2} \quad (15)$$

Where:

$P_f - \text{Pressure inside fiber at the feed side [Pa]}$,

$P_c - \text{Pressure inside fiber at the concentrate side [Pa]}$.

The $P_c$ is monitored and the $P_f$ is unknown. The $P_f$ can be calculated:

$$P_f = P_c + \Delta P \quad (16)$$

(14), (15) and (16) give:

$$\text{TMP} = P_c + \frac{\Delta P}{2} \quad (17)$$

Obtained TMP can be used in (11) to find the $K_w$.

The membrane permeability is a useful unit to compare the performance of the same membrane during different periods of time. Nevertheless, when the membrane modules are produced manually, they can have different original permeability. In cases where the performances of different membranes are to be compared, the relative permeability becomes useful. Relative permeability is a dimensionless unit, which is the membrane permeability ($K_w$) at a particular time divided by the initial permeability of the clean membrane ($K_{w0}$).
3.3.4. Fouling deposition factor (β)

The retained particles deposit on the membrane surface. The rate of the deposition can be measured by a deposition factor. Only a fraction of the cross-flow membrane module feed water is forced to pass through the membranes. This fraction of water depends on the recovery ($R$) at which the unit operates. In dead-end filtration all the particles bigger than the membrane’s pores will be retained while in the case of the cross-flow, only a fraction of the water passing through the membranes is affected. Depending on a nature of the particles, the particles from this fraction may or may not accumulate on the membrane surface.

The deposition factor was firstly proposed by Schippers et al. (1981) in a model to predict flux decline in reverse osmosis systems [35]. It was defined as the fraction of particles deposited, which are present in the water passing the reverse osmosis membrane.

Knowing the incoming and outflowing concentrations, and knowing that the studied particles do not pass the membrane (permeate concentration = 0), one of the expressions to calculate the particle deposition rate is:

$$\beta = \frac{1}{R} + \frac{C_c}{C_f} \left(1 - \frac{1}{R}\right)$$  \hspace{1cm} (18)

**Where:**

- $\beta$ – particle deposition factor,
- $R$ – filtration recovery factor,
- $C_c$ – concentration of the particles in the concentrate stream,
- $C_f$ – concentration of the particles in the feed.

$$R = \frac{Q_p}{Q_f} \leq 1$$

$\beta$ physical meanings [Figure 21]:

If $\beta$=0 – none of the particles deposit on the membrane.
If $\beta=1$ – all of the particles from the water fraction that has been filtered deposit on the membrane.

If $0<\beta<1$ – a fraction of the particles from the water that has been filtered deposited on the membrane.

There may also be less usual values of $\beta$:

If $\beta<0$ – it means either that the particles are scouring or particle size distribution has changed.

$\beta>1$ – it means either that particles from the unrecovered part are retained in the vessel or particle size distribution has changed.
3.4. Analytical methods

3.4.1. TEP measurement

3.4.1.1. Spectrophotometric method

TEP has been measured by the spectrophotometric method developed by Passow and Alldredge (1995) [15] with the latest modifications made by L.O. Villacorte (PhD researcher at UNESCO-IHE) as follows:

1. 0.4 µm filter was rinsed with 20 ml milli-Q water.
2. Water sample was filtered through 0.4 µm polycarbonate filters using 0.2 bar vacuum.
3. The filtrate was filtered again through 0.1 µm polycarbonate filters using 0.2 bar vacuum.
4. The filters were rinsed by filtering 5 ml of milli-Q water at 0.2 bar vacuum.
5. Accumulated TEP on the filters were stained by 1 ml of Alcian blue solution (0.025% (m/v) and pre-filtered through 0.05µm polycarbonate filters).
6. After 10 seconds reaction time, 0.2 bar of vacuum was applied to remove excess dye.
7. The filter was flushed by 5 ml of ultra pure water (milli-Q) with 0.2 bar vacuum.
8. The filters were transferred to 50 ml beaker, soaked in 6 ml sulphuric acid (80%) and continually mixed on a shaker for 2 hours.
9. After 2 hours, the absorbance was measured at 787 nm using a spectrophotometer (Shimadzu UV-2501PC).
10. Absorbance corrections for stain adsorption on filter and effect of turbidity were also measured to calculate TEP levels in abs/(cm·l).

No calibration was performed for the method which means that the units of the TEP is in abs/(cm·l).

3.4.1.2. Liquid Chromatography Organic carbon detection (LC-OCD) method

Since there is no precise method to measure TEP and their colloidal precursors, LC-OCD was used to have a close estimation of TEP content in the most important batch of TEP stock solution in terms of biopolymers carbon. For this purpose, water samples were sent to Doc Labor Dr. Huber Laboratory (Karlsruhe, Germany). In the LC-OCD method, the relative responses of organic carbon, ultraviolet and organic nitrogen at different retention time were
measured with an online organic carbon detector (OCD), UV detector (UVD) and organic nitrogen detector (OND). As a result, five fractions of natural organic matter were identified based on their sizes or molecular weight (MW) from high to low MW; biopolymers (BP), humic substances (HS), building blocks (BB), low MW acids (LMA), and low MW neutrals (N). Each fraction resulted in a peak in LC-OCD chromatogram as can be seen in [Annex 3]. The chromatogram results were processed on the basis of area integration using CHROMCalc software (DOC-LABOR, Karlsruhe). Since TEP are large macromolecules, LC-OCD analysis was performed without 0.45μm pre-filtration. Although biopolymers concentration is not a direct measure of TEP, in this study it is assumed that the concentration of TEP and their precursors is equal to biopolymer concentration.

3.4.2. E. coli plate count

A culture of *E. coli* strain ATCC 25922 (1.4·10⁹ CFU/ml) prior to the experiments has been kept in 4°C. It has been also made sure that these bacteria survive in the artificial sea water for a period of 10 hours (duration of one experiment). In the beginning of each *E. coli* experiment the solution of the bacteria has been infused into the feed water to make the proper concentration. The samples of the feed water as well as the samples of the concentrate water have been taken according to the set plan [Figure 15].

Immediately after each sample has been taken, it has been diluted and plated (dispersed into the agar plates [Annex 4]). Since the experimental water contained millions of the cells per millilitre, serial dilutions have been made in sterile PBS. Two different dilutions of each sample, that were projected to result in as much as possible but not more than ~300 colonies per plate, have been plated either in duplicate or triplicate. Immediately after it, the plates have been placed into 37°C incubator and kept overnight. The colonies have been counted the next day.

3.4.3. Membrane autopsy

After the biofouling experiments, the tubes containing hollow fibers have been frozen, cut into pieces, placed into the beakers with milli-Q water, tightly closed and sonicated. After the fouling in sonification bath has detached and dissolved, the solution water has been used for the TOC and ATP analysis.
3.4.3.1. **TOC**

TOC measurement was performed using the Shimadzu TOC-VCPN total organic carbon analyser based on 680°C combustion catalytic oxidation method.

3.4.3.2. **ATP**

Water samples were sent to Het Waterlaboratorium (Haarlem) for ATP measurement.

3.4.3.3. **Zeta potential analysis**

The zeta potential measurements show how thick is the layer of charge around the objects that are being measured. To measure the zeta potential of the microspheres, the device Zetasizer Nano ZS was applied (autotitration method).

3.4.3.4. **Light microscopy analysis**

Microscopic observation was performed using an Olympus BX51 microscope with digital camera.
4. Results and discussion

The biggest part of the experiments conducted in membrane fouling simulator (MFS) were considered to be unproductive. For that reason the results in this section are presented in different order than they have been described in the experimental methods section. First results presented are all the results from the cross-flow ultrafiltration system; next are results from the MFS.

4.1. Cross-flow filtration in UF hollow-fiber membrane

4.1.1. Particle deposition

4.1.1.1. Polystyrene microspheres

Different solutions of polystyrene particles were prepared and the relationship of the particle concentration with corresponding turbidity (calibration curve) was measured. The obtained results are represented by the dots in Figure 22. The equation of this dependency has been found which later has been used to translate the turbidity of the feed and the concentrate waters into actual concentration of particles throughout the experiments.

![Turbidity of the ASW with different polystyrene particle concentrations](image)

Figure 22. Polystyrene microspheres concentration and ASW turbidity dependency. FNU - Formazin nephelometric unit (by ISO 7072). The unit expresses turbidity, not the absorbance, therefore the wavelength of the machine is just of a normal visible light.
The range of about 10 million particles/ml appeared to be optimal for the turbidity translation into particle concentration measurements. Performing experiments with lower concentrations of particles, small changes in turbidity values could possibly not be detected by the used device. Using bigger concentrations would create non-realistic turbidity concentrations of the feed water in UF and RO systems. Based on this, 10 million particles/ml was selected for the feed water of the polystyrene deposition experiments.

As described in section 3.2.1, to support or contradict that the TEP enhances particulate fouling, three types of experiments were performed (only TEP, only particles, TEP with particles). Before each test, the hollow fiber membrane units were prepared and flushed with the artificial sea water for at least 24 hours. During the experiments containing particles, the flux parameter was maintained constant. The turbidity of the feed and the concentrate waters were systematically measured and, using the formulas described in section 3.3.4, the particle deposition factor was calculated. Generally, one of the two outcomes was expected:

- Based on the definition of the TEP, it would create a sticky layer on the surface of the membrane and enhance the deposition of particles;
- TEP would not play any role in particulate fouling.

Surprisingly, it was found that instead of enhancing the undesired deposition of particles, the TEP extensively reduced it. The results of polystyrene particle deposition factor when no TEP and when 0.5 mgC/l of the TEP in the feed waters was involved are presented in Figure 23.

![Figure 23. Polystyrene deposition factor with and without TEP](image-url)
It can be clearly seen that when the feed water contains no TEP, the particle deposition factors vary around 0.7 (i.e. about 70% of the particles from the filtered fraction of the feedwater accumulate on the membrane and the rest 30% flush away by the lateral flow). When the feed water besides the particles contains the TEP, the particle deposition factors vary around zero (no particles deposit).

The changes in pressure parameters have also been monitored and converted into membrane feed channel pressure drop and membrane permeability. Both of these parameters did not change enough in the period of 10 hours to distinguish the difference in membrane performances during the short-term experiments (Figure 24, Figure 25).

Figure 24. Feed channel pressure drop. The difference between 2 nearest points is often as big as between the first and the last point.

Figure 25. The change in relative membrane permeability. The difference between 2 nearest points is often as big as between the first and the last point.
After the experiments feeding different waters into a clean membrane were completed, the effect of particulate fouling on a TEP pre-fouled membrane was tested. This time, the TEP-free water with particles were supplied into the membrane that was initially coated with TEP and the particle deposition factor throughout the experiment was monitored. The results of this test are presented in the graph below:

![Graph showing particle deposition factor over time](image)

**Figure 26.** Polystyrene particle deposition on a clean and on a pre-fouled membrane. The results of the 1st hour ($\beta>1$) could be neglected because in the very beginning the system might have been unstable and cause this error.

The different approach to test the effect of TEP on particulate fouling resulted in different outcome. When the feed water was free from the TEP but the membrane was TEP-fouled initially, the particles deposited on the membrane more in comparison to when the membrane was clean. This effect was bigger at the beginning of the experiment. After the period of 4-5 hours the particle deposition factors have reached about 0.7 and leveled out. The explanation of this is that when the TEP was on the surface of the membrane (but not in the water) it had very little or no effect on the particle deposition rate.

4.1.1.2. **Carboxyl-group coated microspheres (1µm)**

The Polybead® Carboxylate Red dyed microspheres were of the same size as the previously used ones, but the particles were of different color, so the calibration curve (particle concentration – solution turbidity dependency) was re-determined [Figure 27]. The feed water with 10 million particles/ml has been decided to be used for these tests as well.
Using the same experimental and computational methods, the data of the particle deposition factors have been obtained. The results of the three different types of experiments are presented in Figure 28.

This time the particle deposition factors varied around zero barely reaching bigger absolute values than 0.2. The results of the $\beta$ indicate that throughout the experiments the particles principally did not deposit on the membrane. This is possibly due to the negative charges of carboxylated particles which might have led to higher repulsion between the particles and the membrane surface and possibly the TEP which are also negatively-charged.
4.1.2. Zeta potential measurements of the microspheres

Zeta potential measurements of the 3 out of 4 types pf particle experiments have been performed:

- Polystyrene particles in ASW
- Polystyrene particles with 0.5 mg C/l TEP in ASW
- Carboxylated particles in ASW

The analyser was not capable of accurately measuring the zeta potential in the salty water. The measurements have been performed in 25°C in different pH. The results are provided in [Annex 5]. When the experiments are performed, the zeta potential dependency on pH graph is expected to be a smooth graph going up where at some pH (between 2 and 10) the zeta potential of the particles becomes neutral.

The zeta potential of the particles are most likely negative because the results were negative. On the other hand the values are very small (0 to -12 mV). At the actual pH (pH of the ASW = 8) the zeta potential values varied from -6 to -9 mV. Only when the values are very negative (more than -40 mV) or very positive (more than +40 mV) the particles can be considered as stable. When the absolute value of zeta potential never reaches more than 30 mV (in our case they don’t reach more than 10 mV), the particles are not stable. When the particles are not stable they can change the charge easily. In general zeta potential measurements in sea water matrix are inaccurate and the test is inconclusive.

4.1.3. Bacterial deposition

In this section, the experiments of the early bacterial fouling are discussed. A common laboratory grown strain E. coli (ATCC 25922) was infused into the feed water and the measurements of bacterial concentration were made. Prior to the experiments, it was made sure that the cells survive in the artificial sea water for the period of 1 experiment (10 hours) and that the concentration of the cells remain rather stable. The membranes were pre-flushed with the artificial sea water for a period of at least 1 day. To avoid any possible effect of bacterial growth or decay in the ASW in time, the samples of the feed and the concentrate waters have been taken simultaneously, immediately diluted and plated. Only the difference of the bacterial concentration in the feed and the concentrate waters at virtually the same time of measurement was important, not the change of actual bacterial concentration inside the feed water in time. The actual values of bacterial concentrations in water are provided in [Annex 6].

The next day, colony forming units were counted. To illustrate the cell stickiness on the membrane, the same expression (cell deposition factor) was applied. Running the system with
small water recovery (R), the deposition factor (β) is especially sensitive to measuring errors. Therefore, an attempt to plate samples with concentrations of about 50 colony forming units per plate have created too big variation between the samples and resulted in unreliable results. The results of this experiment became only accurate when replicating samples with at least 300 colonies per plate were made.

![Deposition factor of e.coli](image)

**Figure 29.** E. coli ATCC 25922 deposition factor with, without TEP and on a TEP pre-fouled membrane

The graph in Figure 29 shows the deposition factor of E. coli when no TEP was involved in the process; when the TEP was present only in the feed water; and when initially pre-fouled membrane with TEP was used. When no TEP was involved, the cell deposition factor on the membrane through the experiment was on the average -0.06. This implies that the bacteria on the clean membrane basically did not deposit or they initially deposited but eventually scoured out lateral flow. When the TEP was introduced into the feed water and when the membrane was pre-fouled with the TEP, the average deposition factors throughout the experiments were 0.67 and 0.56 respectively. Overall results imply that the presence of the TEP can enhance the bacteria to stick on the membrane throughout its early operation process (10 hours).

The *E.coli* did not attach on the clean membrane within the period of 10 hours. This is basically because the membrane materials are designed in a way to prevent microorganisms to stick. Nevertheless with the presence of the TEP the surface of the membrane became covered with it and promoted the attachment of *E.coli*. 
4.1.4. TEP-enhanced (bio) fouling

The experiments in this section were conducted to illustrate the effect of TEP on a (bio)fouling development within the period of 3 weeks. The key preference of setting up these experiments was not to re-establish the exact conditions in the water treatment plants but to solely distinguish the effect of TEP, focusing only on variations of major operational parameters (e.g. flux and feed channel pressure drop). The experimental setup also aimed to clarify the role the TEP plays, namely whether it is being used solely as a sticky matrix or as the bacterial food source as well.

In the previously described experiments (Section 4.1.2.), the known concentration of bacterial cells was infused into the feed water. It was done with the intention to track the differences of the \textit{E.coli} cell concentrations in the feed and the concentrate waters and derive the cell deposition. In current setup, the intention was to verify the rate of bacterial growth by monitoring the decline in membrane performance and analyzing biofilm accumulation by membrane autopsies. Therefore, the artificial sea water was inoculated with 10 volumetric percent of real sea water from the North Sea. This provided the membrane feed with microbial culture that would be able to reproduce in the saline conditions for unlimited time.

The known concentration of laboratory cultured TEP was infused for the TEP-containing experiments. The feed water solutions were gently mixed and manually vacuum-filtered with 5 µm pore size filters to imitate cartridge filtration pre-treatment step in the real water treatment plants. Where relevant, the needed concentrations of nutrients after the vacuum filtration were infused (NaCH$_3$COO for the carbon, NaNO$_3$ for the nitrogen and NaH$_2$PO$_4$ for the phosphorus). Because of the limitation of experimental resources, recycle flow approach was applied (20 litres solution for each experiment of which the concentrate and the permeate waters were constantly mixed with the feed water bulk). In order to prevent the eventual depletion of nutrients when the same water is being recycled, the initial concentrations of C, N and P were re-injected every 7 days.

The TEP are non-stable materials, the properties of which depend on the species by which it is produced and environmental factors that affect those species. Once extracted, the physical-chemical properties of the TEP also change in time. Therefore, the TEP that has been extracted from the same bulk of diatoms at the same time has been chosen to be used for the experiments. It has been also decided to eliminate the factor of possible TEP aging and run the experiments simultaneously. For this reason a system of four UF hollow-fiber filtration units were designed and constructed. Applying this system the full performance of up to four membrane units could be monitored simultaneously with only one digital scale and two sets of digital pressure sensors with only some extra operational work [Annex 7].
The membranes were prepared and pre-flushed with artificial sea water for at least one day. Once started, the system was controlled maintaining the constant flux in the membrane units and regularly taking pressure readings for a period of 3 weeks. The performance of the membranes in terms of the change in membrane permeability is presented in the figure below. (Note: the first 4 experiments have been run simultaneously, the additional experiment $C+P+N+extraC$ has been performed after. Membrane autopsies of the additional experiment were not performed due to time limitations).

![Figure 30. The change in relative membrane permeability of the long-term biofouling experiments](image)

The initial feed channel pressure drop of the membrane units were $67\pm10$ mPa. The initial permeability of the membranes – $46\pm6$ l/h·m$^2$·Pa. The value for permeability provided by manufacturer was $100$ l/h·m$^2$·Pa. Nevertheless, the permeability does not depend linearly due to the applied pressure and this unit becomes smaller when the small laboratory-scale pressures are applied. To eliminate the effect of the difference in permeability in between the clean membrane units, the results are provided starting from a relative permeability – 1.

From the graph above it can be observed that TEP enhances biological fouling. When the TEP is present in the feed water and sufficient nutritional conditions for the microorganisms are maintained, the membrane performance declines abruptly. In the period of 3 weeks the membrane permeability of the experiment (green curve) decreased by 81% and the filtration...
process could not continue. Whereas in the experiments where the feed water contained only TEP (black curve), the permeability decreased by 28% and when only the basic additional nutrients (blue curve) - by 44%.

Fouling is a very complex phenomenon requiring many levels of investigation together with multifaceted membrane autopsies. Nevertheless, from the change of the membrane performances over time, the following interpretations can be made:

- Comparing the TEP+C+P+N with TEP and C+P+N

The TEP has produced extra biofouling. This is due to the fact that during the last week of experiment the decline in membrane permeability of TEP+C+P+N was higher than of the TEP and the C+P+N taken together. The faster growth could be because the presence of the TEP has provided more of available nutrients (extra carbon content); or that the TEP has created favoring conditions for microbial growth in another way (stickiness, shelter).

- Comparing the TEP+C+P+N with C+P+N+extraC

In the experiment C+P+N+extraC the carbon content of the TEP (0.5 mg C/l) has been replaced with the carbon from acetate. Unlike other nutrients, this extra carbon was injected only once in the beginning and has not been restored. If the sharp decrease in membrane permeability of TEP+C+P+N was due to the fact that the microbes grew faster because they consumed the TEP, the membrane performance decrease of C+P+N+extraC should be just as big or even bigger, because the carbon from acetate is easier bio-available. The results show that the C+P+N+extraC did not decrease as abruptly as TEP+C+P+N which implies that the filtration performance decrease in TEP+C+P+N was not due to the nutritional content of TEP. However, it has to be noted that the C+P+N+extraC experiment was performed several weeks after the TEP+C+P+N experiment, so the condition inoculated seawater might have changed.

- Comparing the C+P+N with C+P+N+extraC

The decrease in membrane permeability of the C+P+N was almost the same as of C+P+N+extraC. This implies that there was no carbon shortage throughout the C+P+N experiment. If the carbon shortage had been present, the permeability decrease of C+P+N+extraC would have been more severe.
Comparing the TEP with the TEP+P+N

In the experiment where no additional nutrients were added, extensive biofouling could not develop (the TEP line shows relatively small linear decrease). Nevertheless, when the extra nitrogen and phosphorus were introduced, the microorganisms only needed additional carbon to grow. The decrease in membrane permeability of the experiment TEP+P+N during the first 5 days was extensive and later it leveled out. It can imply that when no carbon but the rest of the basic nutrients were available, the carbon content could have been consumed from the TEP and the biofilm grew until it was available.

The membrane performances could have also been evaluated in a more abstract way – feed channel pressure drop. This expression is a pressure difference between the two ends of a membrane and it is the only way the fouling is evaluated in MFS. The figure below provides the graphs of the changes in feed channel pressure drops of the same set of experiments.

![Feed channel pressure drop](image)

Figure 31. The change in feed channel pressure drop of the long-term biofouling experiments

After 18 days, the feed channel pressure drop of experiment TEP+C+P+N grew up to 6 times its original value and the experiment collapsed (sudden decrease in feed channel pressure drop...
occurred due to a compression of the fiber). In all the rest of experiments, the feed channel pressure drop has relatively slightly increased.

The membrane performance tests are not sufficient to state anything about the growth of the fouling due to bacterial utilization of the TEP. However, they clearly show that TEP enhanced biofouling and that the enhancement in this case was mainly not due to the nutritional value of TEP.

After 22 days, the membrane tubes were disconnected and immediately frozen for the membrane autopsies. The frozen membranes were cut (30 cm of each side – the front, the middle and the end), put into 180 ml beakers with milli-Q water, shaken and sonicated. Extracted biofilm/foulants dissolved in solutions were used for the analysis of TOC and ATP. The values of the TOC and ATP were calculated per membrane area and are presented in Figure 32 and Figure 33.

![Figure 32. Total organic carbon (TOC) per membrane area test of the long term biofouling experiments (2 duplicates each measurement)](image)

Due to the carbon mass balance, organic carbon in a close system can go from one form to another without losing its total quantity. As expected, the TOC of the TEP+C+P+N (100 µg C/cm²) equals to the summation of TEP (50 µg C/cm²) and the C+P+N (50 µg C/cm²). It can be also observed that the organic carbon foulant quantity is rather similar along each membrane. To discuss whether the growth of bacterial biomass has been enhanced due to the addition of the TEP, ATP analysis is presented below:
The ATP analysis illustrates that the presence of TEP enhanced higher bacterial activity on the membranes in comparison to when it was just with dissolved nutrients.

The result that the TEP together with the nutrients had a higher fouling effect in comparison to what would be just a summation of the TEP and the nutrients can be seen in both graphs of membrane performances [Figure 30, Figure 31] and the graph of ATP [Figure 33]. Nevertheless the graph of TOC [Figure 32] does not match the ATP graph, but it does not contradict the proposed conclusion. This is because of the following reasons:

1. The TOC analysis measures all of the dissolved organic carbon, not only the carbon of bacteria. The dissolved carbon pool consists of the carbon from sea water, the carbon from TEP and additional carbon from nutrients. The carbon from bacteria makes up only a tiny amount. Therefore the difference of bacterial concentration between the experiments should not be reflected in TOC graph so clearly as in the ATP graph.

2. The ATP does not measure the amount of bacteria, but it measures how active the bacteria is. The results show that in the experiment TEP+C+P+N the bacteria was more active. TOC is not synonymous to ATP and the bacterial activity could not be reflected in the TOC graph.
4.2. The “TEP paradox” explained

Fouling is a complex phenomenon, the four forms of which often develop simultaneously and are intertwined. The fact that in the described experiments, the presence of TEP enhanced (bio)logical fouling but reduced the particulate fouling will be named the TEP paradox.

To explain the TEP paradox many potential conclusions were considered. One sound hypothesis was that in the particulate fouling experiments, the TEP could have repelled the particles because of its charge (negatively charged TEP covers the surface of the membrane and pushes the negatively charged particles away all the way to the concentrate side). However, this hypothesis was eventually rejected. If the repulsion force due to the same charge type was responsible, the membrane pre-covered with TEP would have forced away the polystyrene particles as well as (or even more than) the membrane that was being fouled with the TEP and the particles simultaneously. It has been observed that when was that when the membranes were pre-fouled with TEP, the TEP had only a slight effect on enhancing the fouling with polystyrene. On the other hand, when the TEP was present in the feed water it considerably reduced the deposition of particles.

The explanation was found after taking into account theoretical back transport velocities of the filtering particles and testing the water samples of the experiments under a light microscope.

4.2.1 Back-transport velocity

4.2.1.1. Particulate (colloidal) backtransport mechanisms

The main objective of this section is to explain the factors that controlled particle deposition in the cross-flow UF setups under applied operational conditions.

When the water containing particles or colloids is filtered in a cross flow mode, the particles that are rejected accumulate close to the membrane surface. Accumulated particles become subjected to two forces of opposing directions: (1) convective forces toward the membrane surface due to water permeation and (2) backward shear forces caused by tangential flow due to cross flow filtration.

The backtransport velocity is a particle movement away from the membrane surface and it is caused by three main mechanisms (applicable for particulate/colloidal fouling):
• **Backtransport velocity due to Brownian diffusion** represents back diffusion due to random movement of particles through their interaction with water molecules. This mechanism can be used successfully for macromolecules (<0.001 µm). However, this model fails to predict flux decline caused by colloidal solutions (0.001-1 µm) [38].

• **Backtransport velocity due to shear induced diffusion** - the individual interaction between particles caused by random movements from the streamlines in tangential flow [39];

• **Backtransport velocity due to inertial lift forces** cause lateral migration of large particles back to the bulk fluid under high tangential flow rate [Figure 34].

![Figure 34. schematic illustration of the different forces imposed on particles in cross flow filtration systems](image)

Total backtransport velocity is the sum of the three above mentioned velocities. According to these models, backtransport velocity of the colloids and particles in hollow fiber UF membranes is mainly a function of a particle size and the cross flow velocity (but there are many more of
the less significant parameters [Annex 8]). Whenever backtransport velocity of a particle exceeds the permeate flux rate, particles move away from the membrane towards the bulk solution.

The backtransport velocities have been studied in UNECO-IHE by researcher Muna Gharaibeh (2012). The graphical demonstration of the total backtransport velocities of the TEP particles in the UF system used in this thesis is illustrated in the [Figure 35]. For the particles of different materials this chart slightly differs, but considering polystyrene particles are relatively buoyant like TEP, this example is adequate to explain the trend of the backtransport velocity dependency on a particle size.

Figure 35. effect of cross flow velocity and particle diameter on backtransport velocity [11]

The yellow curve (CFV = 0.15 m/s) indicates all the hydrodynamic conditions that were maintained in every UF experiment of this thesis. It can be seen from the graph that the particles of the sizes approximately between 0.1 and 1.0 μm have the lowest backtransport velocities and therefore tend to remain on a membrane surface the most. The smaller and the bigger particles have exponentially bigger back-transport velocities. During the cross-flow filtration process, most of the larger particles will not reach the membrane mainly due to lateral migration.
4.2.1.2. The case of the microspheres

The feed waters of the experiments containing particles have been investigated under a light microscope. In the images in Figure 36 and 37, the concentrate effluent of the polystyrene particle experiments with and without the TEP are displayed. The figures below are not the average ones, but the ones that the most clearly illustrate the phenomenon and with the best resolution. More of the images are in [Annex 8]
Figure 36. The polystyrene microspheres in ASW with the presence of the TEP from the concentrate water under the light microscope.

Figure 37. The polystyrene microspheres in ASW from the concentrate water under the light microscope.
The presence of the TEP in the feed water led to aggregation of the polystyrene particles into large flocks with diameters up to 40 µm. These flocks [Figure 37] have a much higher backtransport velocity that prevents them from getting too close to the membrane surface. The formed aggregates are caught by the cross flow stream and end up into concentrated water effluent. This also explains the occurrence of particulate fouling when the membrane was pre-fouled with TEP. The TEP that was already stuck on the membrane could not aggregate the particles in the way the free-floating TEP did. The particles stick together only due to the TEP that is in the water but not due to the TEP that already covers the membrane surface.

The case of carboxylated particles was different. A slight electronegative nature of the membrane material repels also electronegative carboxyl groups. These particles naturally did not deposit on the membranes and therefore were not further studied in other setups. The significant and remarkable effect on fouling with the neutral polystyrene particles was further investigated in MFS.

4.3. Membrane fouling simulator

Some of the fouling experiments were conducted using the membrane fouling simulator. The outcomes of the experiments are described in the following sections.

4.3.1. Membrane deposition of particles

The fouling of polystyrene microspheres was studied in MFS. MFS has a much wider feed channel cross sectional area compared to a tiny hollow fiber membranes and therefore requires a much higher feed flow rate to maintain the same (0.15 m/s) flow. Since experimental materials (the microspheres, ASW) were limited, it has been decided to apply the recycle flow scheme and recirulate the effluent of the MFS directing it back into the influent tank. The experiments could therefore run longer and the turbidity of the effluent samples was measured when possible (i.e. during the day time). The results are graphically represented in [Figure 38].
In this case, the particle deposition factor ($\beta$) could not be calculated because water recovery in the MFS is zero as there was no permeation process but it is rather a flow-through system. The change in relative particle concentration (concentration of particles in effluent water at given moment divided by the initial concentration) was applied to express the results. The experiments support the data collected in UF hollow fiber membranes and extend the trend to a longer perspective. When the TEP is absent, the spacer of the RO membrane in the MFS fouls with the particles more in comparison to when TEP is involved.

The pressure parameters were measured and the change in feed channel pressure drop is presented in a graph below.

Figure 38. the change in relative particle concentration in MFS in time

Figure 39. change of feed channel pressure drop in MFS
Unlike in UF hollow fiber membranes, the pressure drop trend is apparent and precise. Pressure data corresponds to turbidity measurements. In this aspect, the MFS is more useful for particulate fouling experiments, because in a certain tests where turbidity cannot be measured, the MFS pressure data is supposed to reflect the particle deposition.

Moreover, because of a high colour contrast between the membrane surface and the particles, the extent of particle deposition could also be visually seen by visual observation through the sight window of the MFS [Figure 40].

4.3.2. TEP-enhanced (bio)fouling studies

4.3.2.1. TEP from algal bloom impacted waters

Real sea water collected from the Jacobahaven UF-RO demonstration water treatment plant (Zeeland) during the algal bloom period was tested in the MFS. Inside the Jacobahaven plant the water company Evides has been doing the algal concentration assessments inside water that they intake for the treatment. They use the method of measuring the chlorophyll in the water and make conclusions weather the algae is there. Based on the data from Evides, on May 22 of 2012 there was one of the highest chlorophyll concentration recorded during the spring season (4 – 10 µg chlorophyll/l). In general in the year 2012 the chlorophyll concentration in the water was not very high (in the year 2009 it reached 60 µg chlorophyll/l).

The aim was to illustrate the effect of this plant’s ultra-filtration pre-treatment step to the fouling development in the RO membranes and (if these tests were successful) to compare this effect with the non-algal bloom season.
The raw sea water was collected from the influent of Jacobahaven plant after the strainer pre-treatment step and manually filtered with the 5 µm pore size vacuum filter. The effluent of the UF pre-treatment was also sampled. The 20 litre solutions were spiked with the basic nutrients (C, N, P) and fed into MFS with the water recycling approach. To prevent the nutrient run-off when applying recycling, the nutrients were restored every 7 days.

Unfortunately, all of the MFS long term biofouling tests were unsuccessful. The data of pressure transmitter was not adequate to indicate any logical trend. This may have happened because of one or both of these MFS characteristics:

- The length of the membrane channel in MFS is too short (20 cm) to reflect biofouling in RO spiral-wounds; and/or the the channel was too narrow (1 cm) in which the wall-effect might have greatly affected the hydrodynamic conditions.
- The absence of the permeate flow highly distorts the development of biofouling.

Figure 41. The feed channel pressure drop - 5 um filtered raw sea water in MFS

Figure 42. The feed channel pressure drop - UF filtered sea water in MFS
4.3.2.2. TEP from algal cultures

Finally, the MFS was exploited for the long term bio-fouling experiments with the TEP obtained from the lab-grown algal culture. Two experiments were selected (from the TEP; C+P+N; TEP+C+P+N, TEP+P+N and C+P+N+extraC) that were wanted to be compared the most. These were the C+P+N and the TEP+C+P+N. It was unexpected that the MFS experiments will give the results; therefore, the concentration of the TEP as well as of the nutrients were doubled in comparison to the same experiments in UF hollow fiber membranes.

Creating non-real conditions with very high concentrations and running the MFS for a period of 1 month, the proof that the TEP enhances the biological fouling was achieved (Figure 44 and 45).
4.4. TEP from algal bloom impacted waters

To demonstrate the significance of the UF pre-treatment step to minimise biofouling in the RO system in Jacobahaven plant, some experiments were also performed using UF hollow fiber membranes.

The raw sea water was collected from after the strainer pre-treatment step and manually filtered with the 5 µm pore size vacuum filter. The effluent of the UF pre-treatment was also sampled. The 20 litre solutions were spiked with the basic nutrients (C, N, P) and fed into UF hollow fiber membrane units with the water recycling approach. To prevent the nutrient run-off, the nutrients have been restored every 7 days.

Even though it is not rational to test the water that has already passed the UF membranes (Jacobahaven plant) in the UF setup (UNESCO-IHE laboratory), the UF membranes used in the laboratory had much tighter pores (7 kDa compared to 150 kDa in the plant). The results of experiments are provided in Figure 46.
The UF pre-treatment had indeed a significant effect on the biofouling prevention. The TEP containing water collected in May 22, made 6 times higher decline in UF 7 kDa membrane performance than the water pre-treated with 150 kDa UF. Moreover, the linear trend in permeability decline of UF pre-treated water indicates organic fouling while the curved polynomial trend of the water containing the TEP from the raw water indicates the biological fouling.
5. Conclusions (and recommendations)

- If present in the feed water, TEP reduce the deposition of suspended solid non-sticky particles (polysterene and carboxyl group coated polystyrene) in cross-flow filtration membranes. TEP causes aggregation of these particles. Large aggregates have bigger backtransport velocity that prevent them from reaching the membrane surface. At points farther from the membrane surface, the cross flow velocity is always higher due to the lower effect of wall friction. This higher flow captures the aggregated flocks, leading to lateral migration of particles all the way to the concentrate effluent. The TEP that have already deposited on the membrane have no effect on the particle aggregation but rather enhance the deposition of suspended particles to the membrane. Only the free-floating TEP can promote the gathering of the particles into the flocks.

- The short term bacterial deposition experiments that have been performed solely with the *E.coli* cells, and the long term biofouling experiments that have been performed with natural sea water verify that the TEP enhance bacterial attachment to the membrane. Bacterial properties assist them to use the TEP as a sticky matrix. The consumption of the TEP may or may not be responsible for the biological fouling, but the physical properties of the TEP certainly assist the deposition of microbes.

- In order to minimise biological fouling in RO membranes enhanced by TEP, the use of UF pre-treatment proves to be a very effective approach.

- Membrane fouling simulator is a useful device for studying particulate fouling. It has a glass for real-time observation and the pressure sensor connected to MFS reflects the particles deposition rate. Nevertheless, due to its very limited length and the lack of the permeate flow it does not reflect the long term biofouling in real spiral wound membranes. The long hollow fiber membranes with permeate flow are much more beneficial for biofouling tests.

- The TEP can be possibly applied for commercial purposes. By aggregating solid particles the TEP can promote the sedimentation in still-standing waters. There is a huge room for studying the potential TEP application as a natural biological coagulant.
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Figure 31. The change in feed channel pressure drop of the long-term biofouling experiments

Figure 32. Total organic carbon (TOC) per membrane area test of the long term biofouling experiments

Figure 33. Adenosine triphosphate (ATP) per membrane area test of the long term biofouling experiments

Figure 34. Schematic illustration of the different forces imposed on particles in cross flow filtration systems

Figure 35. Effect of cross flow velocity and particle diameter on backtransport velocity [11]

Figure 36. The polystyrene microspheres in ASW with the presence of the TEP under the light microscope

Figure 37. The polystyrene microspheres in ASW under the light microscope

Figure 38. The change in relative particle concentration in MFS in time

Figure 39. Change of feed channel pressure drop in MFS

Figure 40. The spacer view of the experiment without TEP (top) and with TEP (bottom) after 10 hours

Figure 41. The feed channel pressure drop - 5 um filtered raw sea water in MFS

Figure 42. The feed channel pressure drop - UF filtered sea water in MFS

Figure 43. The feed channel pressure drop - 5 um and UF filtered raw sea water in MFS

Figure 44. Feed channel pressure drop - long term biofouling experiments in MFS

Figure 45. The spacer view at the end of the experiments C+P+N (top) and TEP+C+P+N at the end of experiment

Figure 46. The effect on membrane permeability filtering algal bloom impacted water and UF treated algal bloom impacted water

Figure 47. Abiotic formation of TEP in aquatic systems
Annexes

Annex 1. The salt recepie of the artificial sea water (ASW)

<table>
<thead>
<tr>
<th>Component Name</th>
<th>Reagent</th>
<th>mg/L for 1 L</th>
<th>mg/L for 5 L</th>
<th>mg/L for 10 L</th>
<th>mg/L for 20 L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Carbonate</td>
<td>Na2CO3</td>
<td>2</td>
<td>0.002</td>
<td>0.010</td>
<td>0.019</td>
</tr>
<tr>
<td>Potassium Bromide</td>
<td>KBr</td>
<td>8</td>
<td>0.008</td>
<td>0.041</td>
<td>0.082</td>
</tr>
<tr>
<td>Sodium Hydrogen Carbonate</td>
<td>NaHCO3</td>
<td>213</td>
<td>0.21</td>
<td>1.07</td>
<td>2.13</td>
</tr>
<tr>
<td>Potassium Chloride</td>
<td>KCl</td>
<td>739</td>
<td>0.74</td>
<td>3.69</td>
<td>7.39</td>
</tr>
<tr>
<td>Calcium Chloride dihydrate</td>
<td>CaCl2.2H2O</td>
<td>1.540</td>
<td>1.54</td>
<td>7.70</td>
<td>15.40</td>
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<tr>
<td>Sodium Sulfate</td>
<td>Na2SO4</td>
<td>3.993</td>
<td>3.99</td>
<td>19.97</td>
<td>39.93</td>
</tr>
<tr>
<td>Magnesium Chloride hexahydrate</td>
<td>MgCl2.6H2O</td>
<td>10.873</td>
<td>10.87</td>
<td>54.36</td>
<td>108.73</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>NaCl</td>
<td>23.668</td>
<td>23.67</td>
<td>118.34</td>
<td>236.68</td>
</tr>
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</table>

Annex 2. Components of the media used in cultured TEP

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount (per 1L of MQ)</th>
<th>Compound</th>
<th>Amount (per 1L of MQ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>24.55g</td>
<td>Na2 EDTA</td>
<td>4.16g</td>
</tr>
<tr>
<td>MgCl2.6H2O</td>
<td>9.82g</td>
<td>FeCl3.6H2O</td>
<td>3.15g</td>
</tr>
<tr>
<td>CaCl2.2H2O</td>
<td>0.53g</td>
<td>CuSO4.5H2O</td>
<td>0.01g</td>
</tr>
<tr>
<td>Na2SO4</td>
<td>3.21g</td>
<td>ZnSO4.7H2O</td>
<td>0.022g</td>
</tr>
<tr>
<td>K2SO4</td>
<td>0.85g</td>
<td>CoCl2.6H2O</td>
<td>0.01g</td>
</tr>
<tr>
<td>NaHCO3</td>
<td>50.4g</td>
<td>MnCl2.4H2O</td>
<td>0.18g</td>
</tr>
<tr>
<td>AlCl3.6H2O</td>
<td>0.0241g</td>
<td>Na2Mo4.2H2O</td>
<td>0.006g</td>
</tr>
<tr>
<td>Vitamin B1</td>
<td>0.1g</td>
<td>Vitamin B12</td>
<td>0.0005g</td>
</tr>
<tr>
<td>Biotin</td>
<td>0.0005g</td>
<td>Na2SiO3.9H2O</td>
<td>30g</td>
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</tbody>
</table>
Annex 3. Example of LC-OCD measurement for cultured TEP stock
Annex 4. The coliform agar composition

ChromoCult® Coliform Agar (Cat.No. 1.10426.0500) was used for the growing of the E.coli colonies. The receipe in grams per litre is:
Peptones 3.0; sodium chloride 5.0; sodium dihydrogen phosphate 2.2; disodium hydrogen phosphate 2.7; sodium pyruvate 1.0; tryptophan 1.0; Agar-agar 10.0; Sorbitol 1.0; Tergitol®7 0.15; chromogenic mixture 0.4.

Annex 5. The zeta potential measurement results (autotitration)

![Zeta potential of polystyrene particles in ASW](image1)

![Zeta potential of carboxylated particles in ASW](image2)
The meaning of zeta potential values [40]:

<table>
<thead>
<tr>
<th>Zeta potential [mV]</th>
<th>Stability behavior of the colloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>from 0 to ±5</td>
<td>Rapid coagulation or flocculation</td>
</tr>
<tr>
<td>from ±10 to ±30</td>
<td>Incipient instability</td>
</tr>
<tr>
<td>from ±30 to ±40</td>
<td>Moderate stability</td>
</tr>
<tr>
<td>from ±40 to ±60</td>
<td>Good stability</td>
</tr>
<tr>
<td>more than ±61</td>
<td>Excellent stability</td>
</tr>
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</table>
Annex 6. The values of *E.coli* concentration

<table>
<thead>
<tr>
<th>duration h</th>
<th>No TEP</th>
<th>TEP</th>
<th>TEP prefouled membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mln cells/1 ml</td>
<td>Cc/Cf</td>
<td>beta</td>
</tr>
<tr>
<td>0</td>
<td>5,928</td>
<td>5,502</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>5,88</td>
<td>6,408</td>
<td>1,090</td>
</tr>
<tr>
<td>1</td>
<td>4,86</td>
<td>5,25</td>
<td>1,080</td>
</tr>
<tr>
<td>2</td>
<td>4,302</td>
<td>5,193</td>
<td>1,207</td>
</tr>
<tr>
<td>4</td>
<td>4,158</td>
<td>4,92</td>
<td>1,163</td>
</tr>
<tr>
<td>6</td>
<td>4,158</td>
<td>5,058</td>
<td>1,216</td>
</tr>
<tr>
<td>8</td>
<td>5,004</td>
<td>5,58</td>
<td>1,115</td>
</tr>
<tr>
<td>10</td>
<td>3,406</td>
<td>4,194</td>
<td>1,214</td>
</tr>
</tbody>
</table>

The table above illustrates the real *E.coli* concentrations throughout the experiments that have been described in this report (in the feed, in the concentrate waters and their changes in time). The data is based on a colony forming units.
Annex 7. The “4 X UF” setup empowering simultaneous run of 4 filtration experiments
Annex 8. The factors determining backtransport velocity

\[ \gamma_w \] — shear rate [s\(^{-1}\)]
\[ D_B \] — Brownian diffusion coefficient [m\(^2\)/s]
\[ \varphi_w \] — concentration polarization factor,
\[ \varphi_b \] — particles volume fraction at the membrane surf.
\[ \varphi_b \] — particles volume fraction in the bulk
\[ L \] — membrane length [m], (1 m)
\[ k \] — Boltzmann constant (1.38\times10\(^{-23}\) kg.m\(^2\)/s\(^2\))
\[ T \] — absolute temperature [K], (291 K)
\[ a \] — particle radius, assuming spherical particles [m]
\[ \eta_f \] — water dynamic viscosity [Pa.s], (0.001033 Pa.s)
\[ \tau_w \] — shear stress [Pa]
\[ f \] — Darcy friction factor
\[ \rho_i \] — water density [kg/m\(^3\)], (1030 kg/m\(^3\))
\[ U \] — cross flow velocity [m/s],
\[ Re \] — Reynolds number,
\[ D \] — membrane tube diameter [m], (0.0008 m)
Annex 9. The light microscopy images of 1µm polystyrene microspheres without TEP (left column) and with TEP (right column)