Lappeenranta University of Technology

School of Engineering Sciences

Chemical Processes R&D

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Enzyme recovery with microfiltration: effect of spacer size and improvement of membrane cleaning

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Examiners: prof. Mika Mänttäri

M.Sc. Timo Vilpponen

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Mikrosuodatus entsyymien talteenotossa: virtausohjaimen koon vaikutus ja membraanien pesuprotokollan parantaminen

Diplomityö

2018

77 sivua, 38 kuvaa, 9 taulukkoa

Tarkastajat: Prof. Mika Mänttäri

FM Timo Vilpponen

Hakusanat: Mikrosuodatus, spacer, virtausohjain, entsyymi, likaantuminen, membraanin pesu, solujäte,

Työn tarkoituksena oli tutkia mikrosuodatuslaitteiston toimivuutta entsyymien talteenotossa eri virtausohjaimilla (spacer) varustetuilla membraanielementeillä, kehittää laitteiston pesuprotokollaa sekä tehdä alustava arvio mikrosuodatuksen tuottamasta jätemäärästä. Kirjallisuusosa sisältää katsauksen mikrosuodatuksen perusteisiin entsyymien tuotannossa, painottuen membraanin likaantumiseen ja pesuun, sekä mikrobiologisen jätteen käsittely- ja kierrätysmahdollisuuksiin.

Laboratoriokokein tehtiin alustava seulonta pesukemikaaleille ja entsyymeille kalvojen puhdistusta varten. Laboratoriotulosten perusteella erilaisia pesuprotokollia testattiin pilot-laitteistolla. Tavoitteena kalvon tehokas puhdistus sekä veden, kemikaalien ja ajan käytön minimointi. Pilot-kokeilla seurattiin myös erikokoisten spacereiden ja eri ajoparametrien vaikutusta entsyymin talteenottoon sekä jätemassan ja -veden määrää sekä laatua.

Laboratoriokokeiden perusteella yrityksen omasta tuotannosta saatavat entsyymit eivät ole yhtä tehokkaita membraanin pesussa kuin kaupalliset pesuaineet ja kemikaalit. Käytetyssä pesu sekvenssissä oli kolme vaihetta emäksinen ja entsymaattinen sekä pesu hypokloriitilla. Muokatulla pesuprotokollalla membraanien vuo palautui hyvin. Pilot-kokeiden perusteella pesukemikaalien ohella laitteiston huuhtelulla on suuri merkitys pesun onnistumiseen.

Koeajoissa käytettiin erikokoisia spacereitä kalvoelementeissä. Pienemmällä spacer koolla elementin aktiivinen suodatus pinta-ala sekä virtausnopeus kasvavat. Tämän myötä laitteen kapasiteetti kasvaa spacer kokoa pienenettäessä. Kuitenkin entsyymien läpimeno membraanista on heikompaa pienemmällä spacer koolla. Tämä saattaa johtua elementin suuremmasta painehäviöstä ja muutoksista virtausprofiilissa. Spacer koko ei vaikuta kriittisesti laitteiston peseytyvyyteen. Kokeiden perusteella spacer koon pienentäminen ei ole tällä hetkellä kannattavaa. Optimoimalla suodatusta paremmin pienemmälle spacerille sopivaksi pinta-alan kasvusta saavutettu kapasiteetin lisäys voidaan saada hyödynnettyä.

Membraaniprosessi entsyymien talteenotossa tuottaa enemmän jätevesiä esimerkiksi rumpusuodatukseen verrattuna. Uusia keinoja jäteveden käsittelyyn ja hyödyntämiseen sivutuotteina tarvitaan, jotta prosessin taloudellisuus paranee.

Abstract

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Enzyme recovery with microfiltration: effect of spacer size and improvement of membrane cleaning

Master's thesis

2018

77 pages, 38 figures, 9 tables

Examiners: Prof. Mika Mänttäri

M.Sc. Timo Vilpponen

Key words: Microfiltration, spacer, Enzymes, fouling, membrane cleaning, microbial waste,

Purpose of this master's thesis work was to improve enzyme recovery and cleaning protocol of microfiltration process at Genencors plant in Jämsänkoski. Also, waste streams produced were evaluated. Literature review of this work is focused on basics of microfiltration and especially on fouling and cleaning of membranes. Also, novel techniques and solutions for cleaning of membranes fouled with enzyme fermentation broth were studied. Lastly treatment and reuse possibilities of microbial waste produced are discussed in literature review.

In laboratory experiments screening for different cleaning solutions and enzymes for membrane cleaning was performed. Pilot scale filtration unit was used to study enzyme recovery on microfiltration and to develop cleaning protocol based on laboratory experiments. Different spacer sizes and operation parameters were tested to enhance enzyme recovery. Evaluation of waste streams and waste quality was made based on filtrations

Flux recovery with developed cleaning protocol was good. Caustic, enzymatic and bleaching/disinfection steps were used with commercial cleaning agents and NaClO. Commercial cleaning chemicals were more successful than enzymes in cleaning experiments Based on pilot data one critical factor for cleaning is also amount and intensity of rinsing with preferably hot water.

Smaller spacer size increases active membrane area and cross-flow of unit. This increases unit capacity during filtration but at the same time enzyme passage and specific flux is lower. Increased capacity cannot compensate decreased separation effectivity and amount of enzyme recovered is lower with smaller spacers. This could be due changes in flow hydrodynamics and increased pressure loss in the element. Also, optimal filtration parameters and feed properties could be different for smaller spacer size.

Membrane technology-based process in enzyme recovery produces lot of waste waters compared to traditional filtration methods. Waste streams have high COD and P concentration. Main waste streams are UF permeate from product concentration and MF retentate (cellular waste slurry). To enhance process economics new ways to treat and utilize waste streams are needed.

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Symbols and abbreviations

A	Membrane permeability coefficient
J	Flux trough membrane
K	Kozeny-Carman coefficient
R	membrane/fouling layer resistance
S	Internal surface area of membrane pores
abs	Absorbance
ds	Dry solids content
r	pore radius
x	membrane thickness
ε	Membrane porosity
λ	Wave length
τ	Membrane pore tortuosity
μ	Dynamic viscosity
ASC	Alkali-treated calcium-silicate composite
ATD	Anti-telescoping device
CIP	Cleaning in place
COD	Chemical oxygen demand
CP	Concentration polarization
CSC	Calcium-silicate composite
DP	Pressure difference across membrane element
DV	Diavolume
FRE	Flow resisting element
MF	Microfiltration
MTE	Mechanical thermal expression
NF	Nanofiltration
PR	Pathogen related proteins
RO	Reverse osmosis
RVDF	Rotary vacuum-drum filter
TAMP	Thermally assisted mechanical dewatering process
TL	Thaumatin like proteins
TMP	Trans membrane pressure

UF	Ultrafiltration

Literature review

1 Introduction

Enzymes are important biocatalyst contributing to daily life of all humans. Industrially produced enzymes are used in variety of applications from food additives to microbial control and to enhance different chemical production processes. Enzymes are expected to produce answers and solutions to some of world's biggest problems such as replacement of antibiotics, food shortage and waste treatment. Enzyme production is vastly growing in biotechnical industry.

Industrial production of enzymes consists three main parts: enzyme production, enzyme recovery and product formulating. In production parts micro-organisms are grown to produce enzymes in fermentation process. After fermentation enzymes, must be separated from growth medium and producer cells. This is traditionally done with centrifugation or filtration processes. Product formulation contains adjustments to enzyme solution pH, concentration etc. in order to improve product stability and other parameters important to customers.

Membrane technologies, especially microfiltration, are interesting option for enzyme recovery. Use of microfiltration in recovery of enzymes from fermentation broth has gained interest over centrifugal and other filtration methods. Microfiltration can be effective and economical alternative for traditional separation methods. Main challenges in utilization of microfiltration are finding optimum process conditions for maximum recovery and smooth operation. Other big challenge is flux loss and membrane fouling during operation. Fouling reduces efficiency of recovery and membrane lifetime. It also increases cost as regular cleaning and maintenance is needed.

This thesis work focuses on utilization of microfiltration in enzyme recovery at Genencors Jämsänkoski site and in general. Genencors plant in Jämsänkoski is part of DowDuponts industrial bioscience division and important enzyme producer in Finland. Increase in enzyme demand and applications leads to need of increasing production capacity and efficiency of production techniques in all plants.

Main purposes of work are to enhance cleaning procedure for microfiltration membranes used and evaluate potential capacity increase by changing to smaller spacer size in

membrane elements used. Smaller spacer size increases active membrane area in element. Comparison for elements was made in pilot filtrations for various products.

Cleaning procedure should be able to reach adequate level of cleaning and flux recovery for membranes after enzyme filtration with optimized time, chemicals and water consumption. For this goal laboratory screenings for suitable cleaning agents and pilot experiments with cleaning procedures were made.

Other topics covered are improving enzyme recovery by adjusting filtration parameters and evaluation of amount, properties and treatment methods of cellular waste produced. Optimization of filtration conditions to maximize enzyme yield and quality is very important. On microfiltration feed and flow conditions can have effect on flux and selectivity trough the membrane and fouling tendency of the sheets. Pilot unit is used to test different conditions to find suitable operating parameters for different products.

Microfiltration of fermentation broth produces quite much wastes, cellular biomass and waste waters. Treatment of cellular biomass left from production can be expensive as waste volumes grow. In ideal case waste, could be utilized for example in energy production or in another process. Utilizing waste streams would help to make process more economical. In this work waste treatment is considered in literature review and with evaluation of volume of waste streams and waste qualities to present suitable options for this part of process.

Other topics in literature part focus on microfiltration technology in general, membrane fouling and cleaning. Case for cleaning membranes fouled by enzyme fermentation broth in Genencor is covered already in literature work aiming to find some new chemical or enzymatic cleaning methods and other novel methods for membrane cleaning. Findings are tested in experimental part. Graphical expression of different goals for this work is presented in figure 1.

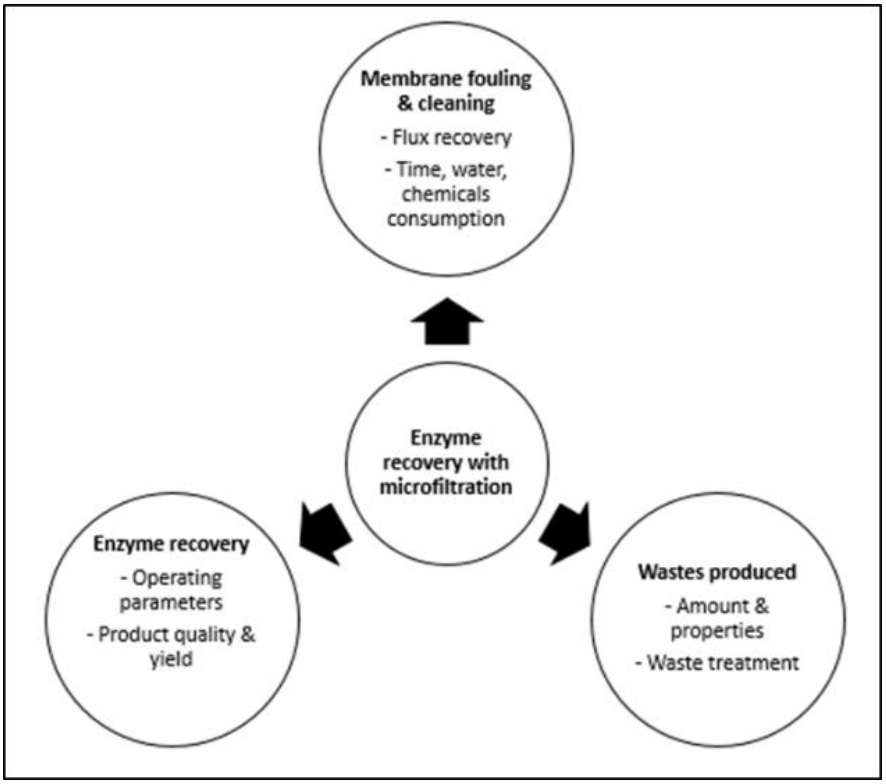

Figure 1 Different focus areas of this work.

2 Enzyme production

Enzymes are biocatalyst used in variety of applications in food, chemicals and other industries. Currently more than 5000 different enzymes are known and about 200 enzymes of microbial origin are used commercially. Biocatalyst are typically used in production of their natural products and derives. For example, use of cellulases in decomposing cellulose in pulp and paper industry. Different applications and enzymes are being developed at the increasing rate. (Yoo;Feng;Kim;& Yagonia, 2017)

Enzyme production starts with screening for producers of wanted enzyme suitable for applications. Variety of micro-organisms in nature produce different enzymes. Once suitable enzyme and producer are found they often undergo lot of testing. Micro-organism is often genetically modified to maximize yield of wanted enzyme. (Ratledge & Kristiansen, 2006)

Industrial production of enzymes is performed by fermentation process. Cultivation of micro-organism is started in laboratory and special inoculation tanks (seed fermentation). When enough micro-organisms are present in the growth medium they are transferred into large scale fermenter. In fermenter micro-organisms are fed with nutrients and often oxygen (in case of aerobic fermentation). During fermentation stage micro-organisms produce

enzymes. Enzyme production can be intra- or extracellular. Fermentation stage can take several weeks. Temperature, pH, oxygen and nutrition control are key for efficient fermentation process. (Ratledge & Kristiansen, 2006)

After fermentation enzymes are separated from cell biomass and growth medium. Separation can be done with filtration, flocculation, centrifugation or with some combination of those. In case of intracellular production of enzyme, cells must be destroyed to release enzymes into liquid medium. This can be done for example with some mechanical force applied to cells or ultrasound. After enzyme recovery solution is usually concentrated, typically with membrane applications or evaporation. (Ratledge & Kristiansen, 2006)

Final stage in industrial production of enzymes is formulation of enzyme product. This can include additional purification of enzyme, adjustment of enzyme concentration by dilution and often different kinds of additives. Most important goals for formulation is to ensure stability & safety of enzyme product and conservation of enzyme activity. Also, physical form of product can be altered to math requirements of different end users. (Ratledge & Kristiansen, 2006) Typical production process of industrial enzymes is presented in Figure 2.

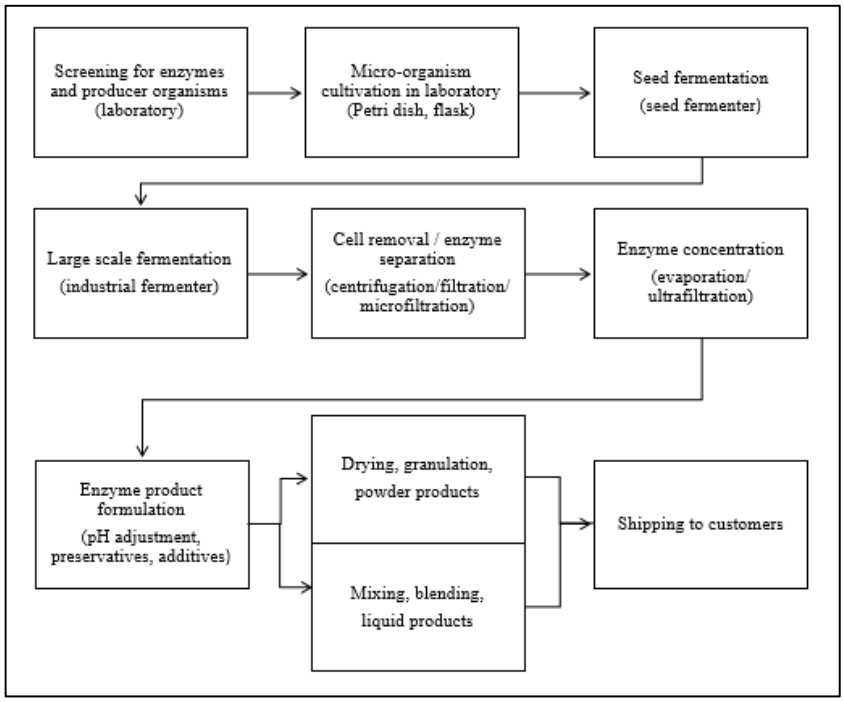

Figure 2 Typical process flow for industrial enzyme production. Top row cell/micro-organism growth, middle enzyme production, bottom product handling. (Ratledge & Kristiansen, 2006)

3 Membrane filtrations

By definition of Mulder (1996) membranes are selective barriers between two phases. Membranes are widely used in various separation processes. Membranes can be classified based on their function, pore size, material or structure. All membranes function in principle by some driving force (for example pressure or concentration difference) pushing Solvent and some particles depending on their size and other properties trough the membrane from feed to permeate side. (Mulder, 1996)

This work is focused on microfiltration membrane separation process. Microfiltration is used for separation of relatively large particles on scale of membrane technology and shares most resemblance with convectional filtration techniques. Separation on microfiltration is based on particle size and driving force of the separation is pressure difference. (Mulder, 1996) Pressure driven membrane separation process is presented in Figure 3.

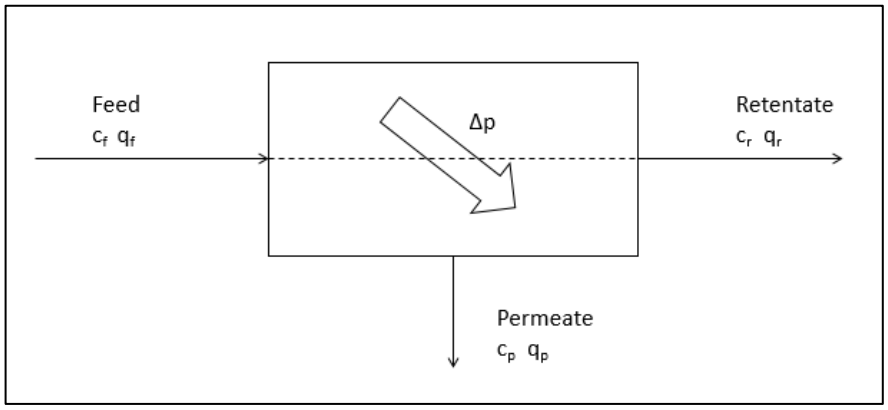

Figure 3 Schematic drawing of microfiltration process. *(Mulder, 1996)*

Flux trough the membrane for pressure driven processes such as microfiltration can be described with Equation 1. (Mulder, 1996)

$$J = A * \Delta p \tag{1}$$

Where *J* is the flux, *A* is the permeability constant covering factors of membrane and feed properties and *Δp* is the pressure difference across the membrane. For membrane with constant cylindrical pores The Hagen-Poiseuille Equation (2) can be used to describe the flux. (Mulder, 1996)

$$J = \frac{\varepsilon * r^2}{8 * \mu * \tau} * \frac{\Delta p}{\Delta x} \tag{2}$$

Where ε is porosity of membrane, *r* pore radius, μ dynamic viscosity of solution, τ tortuosity of pores and Δ*x* membrane thickness. Other commonly used description is Kozeny-Carman Equation 3 it assumes membranes formed by packing of symmetrical spheres. (Mulder, 1996)

$$J = \frac{\varepsilon^3}{K * \mu * S^2 * (1-\varepsilon)^2} * \frac{\Delta p}{\Delta x} \tag{3}$$

Where *K* is the Kozeny-Carman coefficient describing shape and tortuosity of pores and *S* internal surface area of the pores. Equations 1 and 2 are commonly used to describe flux through membranes even though structure of membranes is usually different and rarely contains symmetrical or even sized pores. (Mulder, 1996) From Equations 1-3 can be seen that most important parameters affecting microfiltration process are membrane properties and trans membrane pressure difference used in operation. Flux can also be improved by changing viscosity of the feed.

There are many different materials used for membrane sheets. Membrane are often made from polymeric materials such as polysulfone, polyethersulfone or inorganic materials, for example ceramics, sintered stainless steel and graphite. Selection of membrane material depends on process conditions (pH and temperature tolerance), feed solution (interactions between membrane material and feed particles) and economical aspects. (Gabelman, 2017)

3.1 Membrane modules

To increase filtration area per unit volume different kinds of modules are developed for membrane processes. Purpose of modules is to pack membrane sheets into smaller and more economical shape. Different module configurations can also be used to change the flow dynamics. Suitable module for each separation process is selected considering effectivity of separation, type of separation, ease of process operation, maintenance & cleaning and economic aspects. (Mulder, 1996)

Most common module configurations are hollow fiber, plate and frame, spiral wound and tubular membrane modules. Plate and frame modules are closest to traditional and laboratory cross flow filtrations. Plate and frame modules contain parallel membrane sheets with feed and permeate flow channels on each sides of the membrane. (Mulder, 1996)

Several improvements to plate and frame module have been made. For example, introducing rotors into each frame to create rotational cross flow. This enhances turbulence and shear forces in membrane surface decreasing its fouling potential and improving separation. (Metso Paper, 2006)

Hollow fiber module is constructed from several thin membrane tubes (fibers) packed into tight bundle. Permeate can enter fibers form outside bulk solution or on the opposite from fibers to outside space. (Gabelman, 2017) Tubular membranes work basically similar way but are not self-supporting and tubes are larger than with hollow fiber module. Figure 4 presents basic concept of hollow fiber module. (Gabelman, 2017) (Mulder, 1996)

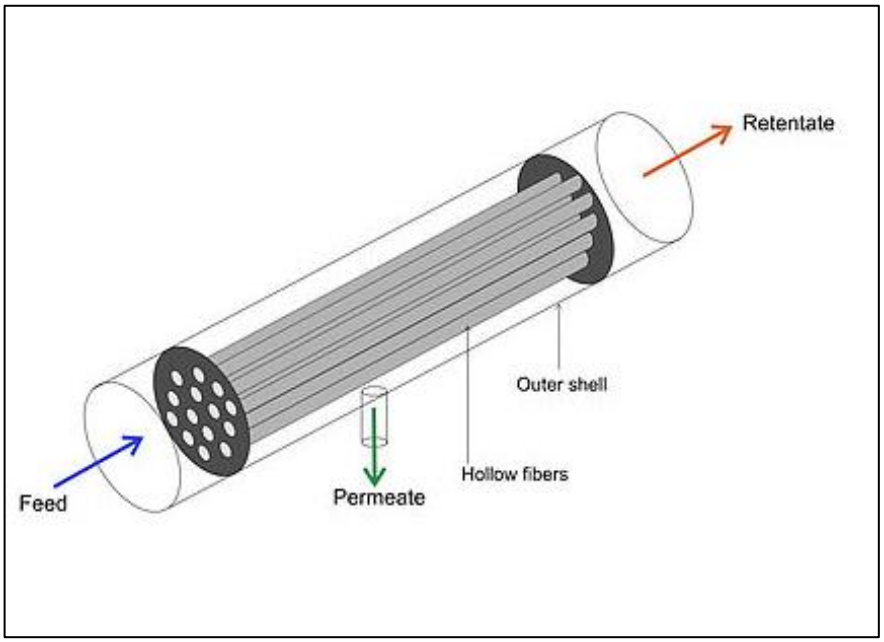

Figure 4 Hollow fiber membrane module configuration. (Shantanu, 2018)

Spiral wound modules are nowadays most commonly used membrane module. They consist of flat membranes wrapped around central pipe. Two membrane sheets separated by support structure are wrapped to spiral in the module housing tube. Permeate flows through membranes to central pipe in the channels generated. Feed is fed into housing from other end into space between membrane rounds. Module allows use of many different feed spacers on outer surface of membranes to change flow dynamics. Configuration of spiral wound membrane is presented in Figure 5. (Gabelman, 2017) (Mulder, 1996)

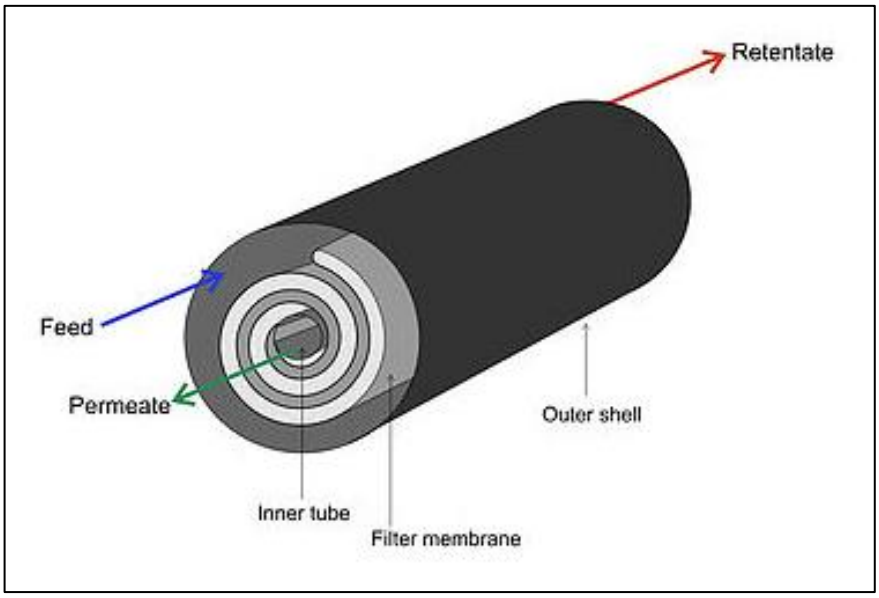

Figure 5 Spiral wound membrane module configuration. (Shantanu, 2018)

Some main advantages and disadvantages of different module configurations are presented in Table 1.

Table 1 Comparison between most typical membrane modules. (Gabelman, 2017)

Module	Advantages	Disadvantages
Hollow fiber	- High filtration area per unit volume - Possibility for backwashing	- Easily fouled - Plugging of fibers - Not suitable for high viscosities or undissolved solid contents
Plate and frame	- High flowrates with low circulation rates - Can be used for high viscosities and undissolved solids	- Low filtration area per unit volume - High costs
Spiral wound	- High filtration area per unit - Low energy consumption - Established technology (lower costs)	- Complex design - Not suitable for fibers - Flow channels can be plugged - Not recommended for high viscosities and undissolved solids
Tubular	- Easy to clean - Good flow hydrodynamics - Can handle solids and high viscosities	- Low packing density and high costs per unit area - High energy consumption

4 Membrane fouling

Performance of membrane filtration declines over period of time. This is due membrane fouling and concentration polarization phenomena. Fouling is often limiting factor in applying membrane technology into separation processes as it reduces separation effectivity and membrane lifetime. Replacing or cleaning membrane units can be expensive and regular

maintenance increases amount of dead times in processes. (Mulder, 1996) Figure *6* presents effect of fouling in membrane performance with time.

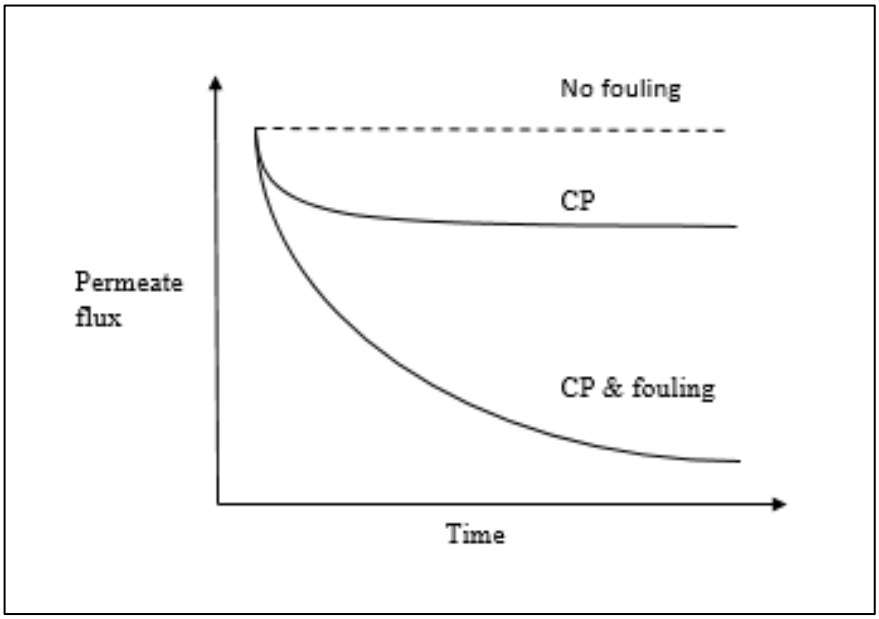

Figure 6 Effect of membrane fouling to permeate flux during membrane operation. *(Mulder, 1996)*

Effect of membrane fouling in flux can be presented by resistance in series model with Darcy's law. Model is expressed with Equations 4 and 5. (Guo;Ngo;& Li, 2012)

$$J = \frac{\Delta P}{\mu * R_{tot}} \tag{4}$$

$$R_{tot} = R_m + R_c + R_f + R_{cp} \tag{5}$$

Where R_{tot} is total resistance of membrane filtration, R_m resistance of membrane itself, R_c resistance of cake formed, R_f resistance caused by internal fouling (pore blocking and adsorption) and R_{cp} is resistance caused by concentration polarization. (Guo;Ngo;& Li, 2012)

4.1 Fouling mechanisms

Fouling is very complex phenomena and it is dependent on filtration conditions, membrane properties and feed solution. Fouling mechanisms can be roughly divided into four categories: pore blocking, adsorption, cake forming and biofilm formation. Also, some sources consider concentration polarization as one different type of fouling. (Mulder, 1996) Fouling mechanisms are presented in Figure 7.

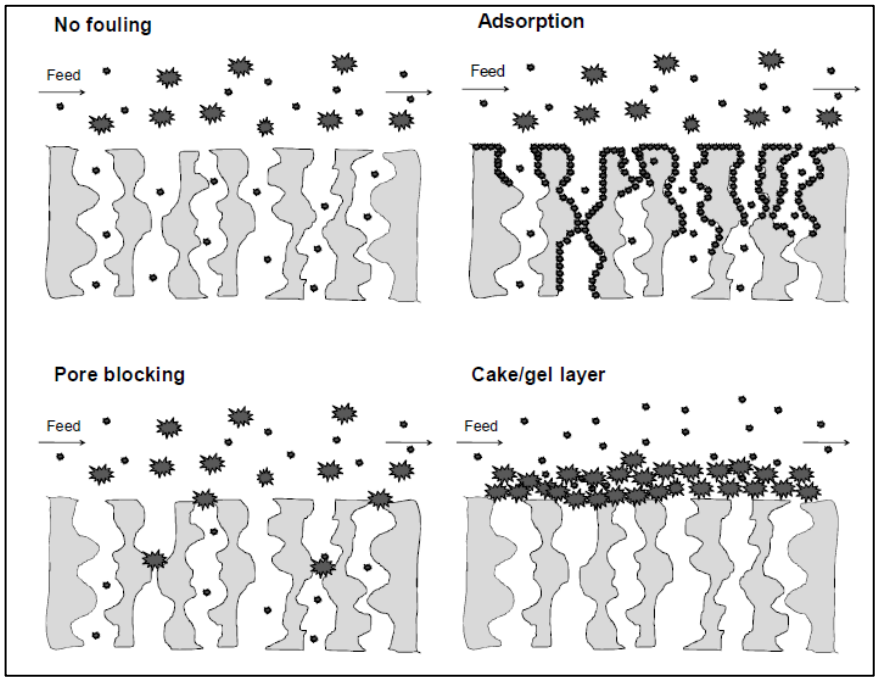

Figure 7 Different fouling mechanisms. (Puro, 2011)

Pore blocking happens when larger particles are pushed into membrane pores physically blocking hole channel. Particles can also start building up on membrane surface forming additional cake layer. Smaller particles can be adsorbed into membrane pores and surface chemically attaching themselves to membrane. (Shi;Tal;Hankins;& Gitis, 2014)

Microorganisms present in feed stream can be attached to membrane surface by electro kinetic or hydrophobic forces. If feed stream contains enough nutrients organisms can grow on membrane surface and create tight biofilm onto surface. (Nguyen;Roddick;& Fan, 2012) Some major foulant types and mechanism related to them are presented in Table 2.

Table 2 Examples of foulants and fouling mechanisms. (Shi;Tal;Hankins;& Gitis, 2014)

Foulant	**Main fouling mechanism**
Large suspended particles	Cake formation, module channels blocking
Colloidal particles	Cake formation, pore blocking
Macromolecules	Cake/gel formation, adsorption
Cations	Precipitation on membrane, facilitation of macromolecules
Biological substances	Biofilm formation, pore blocking, cake formation

In concentration polarization (CP) phenomena concentration of particles in the feed close to membrane surface increases compared to their concentration in the bulk solution. Higher concentration leads to back diffusion from membrane surface to the bulk solution and interferes with separation process. CP can also enhance other forms of fouling as possible foulant concentration near the membrane surface increases. (Guo;Ngo;& Li, 2012) Most severe form of CP is formation of gel like layer onto membrane surface (Shi;Tal;Hankins;& Gitis, 2014).

4.2 Factors affecting membrane fouling

Membrane fouling is affected by multiple different factors in feed and operating parameters of process. For example, feed pH, concentration, temperature and operating pressure & flowrate. Fouling is also dependent on membrane type and component being separated. Typically, experimental work is needed to find factors for each case individually. (Koo;Mohammad;Suja;& Talib, 2012)

4.2.1 Operating parameters

Crossflow velocity of process can have big effect on fouling tendency of operation and type of fouling occurring. Usually increasing crossflow velocity reduces fouling. Increased crossflow velocity increases turbulence and shear forces in the flow making it more difficult for particles to get trapped to membrane pores or surface. (Choi;Zhang;Dionysiou;Oerther;& Sorial, 2005)

Applied pressure also has clear effect on fouling of membrane. Difference in applied pressure can change entire fouling mechanism as well as effect on scale of fouling problem. Very high pressure can lead to pore blocking and internal fouling becoming dominant mechanism over cake formation in lower pressures. Pressure also effects on cake tightness and removability from membrane surface. (Velasco;Ouammou;Calvo;& Hernández, 2003)

4.2.2 Feed properties

Feed pH effects charge of feed compounds and thus into attraction between membrane surface and potential foulants. If charges are opposite attraction is higher and fouling potential increases. Potential cakes or foulant layers are also tighter in these cases. (Velasco;Ouammou;Calvo;& Hernández, 2003)

Temperature of the feed is other important factor affecting fouling potential of feed solution. Changes in temperature effect on feed viscosity and sometimes properties of feed particles

(for example denaturation of proteins at high temperatures) or membrane surface. (Koo;Mohammad;Suja;& Talib, 2012)

4.3 Prevention of fouling

Prevention of membrane fouling begins with choosing correct membrane type and module design for operation and feed solution. Module design can be used to increase shear rate and turbulence on flow near membrane surface. Both can be used in reducing fouling. Shear rate can be increased by pumping feed solution in higher flow rate or design of flow channels. Turbulence can be promoted by feed spacers and mixers. Membrane surface chemistry has big effect on fouling. Choosing correct membrane material or modification of membrane surface can help to prevent attachment of foulants into membrane. (Nguyen;Roddick;& Fan, 2012)

Also, pretreatment of feed can be useful. Removal of some potential foulants beforehand can increase membrane operation time and make cleaning easier. Larger particles can be removed by prefiltration or by centrifugation for example. Also, treatment with antimicrobial agents can be used to prevent biofouling. (Nguyen;Roddick;& Fan, 2012)

5 Membrane cleaning

Membrane fouling is usually impossible to neglect only by preventive methods discussed earlier. Some fouling is unavoidable during operation and regular maintenance and cleaning of membranes is required to maintain high filtration performance. Several different methods are used depending membrane and fouling types. (Mulder, 1996) Choice of method depends on interactions between foulants and membrane surface. Suitable cleaning method weakens the attachment of foulants to membrane and enables foulant removal and thus flux recovery. Cleaning methods can be divided into physical and chemical methods. (Nguyen;Roddick;& Fan, 2012) Mulder (1996) presents four classifications: hydraulic, mechanical, chemical and electric cleaning. Typically, different methods are combined in cleaning sequence to obtain maximum effect.

5.1 Physical cleaning

Physical cleaning involves hydraulic, pneumatic and mechanical processes or many times combinations of those. Also, novel methods as electronic or magnetic fields can be applied. Especially hydrodynamic cleaning methods such as backflushing, flow relaxation and higher

cross-flow operation have become standard part of membrane operations. (Nguyen;Roddick;& Fan, 2012)

According to Qaisrani & Samhaber (2011) backflushing/backpulsing is effective in reducing cake deposition in membrane surface and effect of concentration polarization, but less effective against internal fouling of membrane. In backflushing operation of filtration unit is reversed and permeate forced back across the membrane to the feed side. Reversed flow pushes particles of the membrane surface. Principles of process are presented in Figure 8. Effect of backflush is dependent on pulse duration, pulse amplitude (pressure peak of reverse operation) and intervals of cleaning. (Shugman;Aldrich;Sanderson;& McLachlan, 2013)

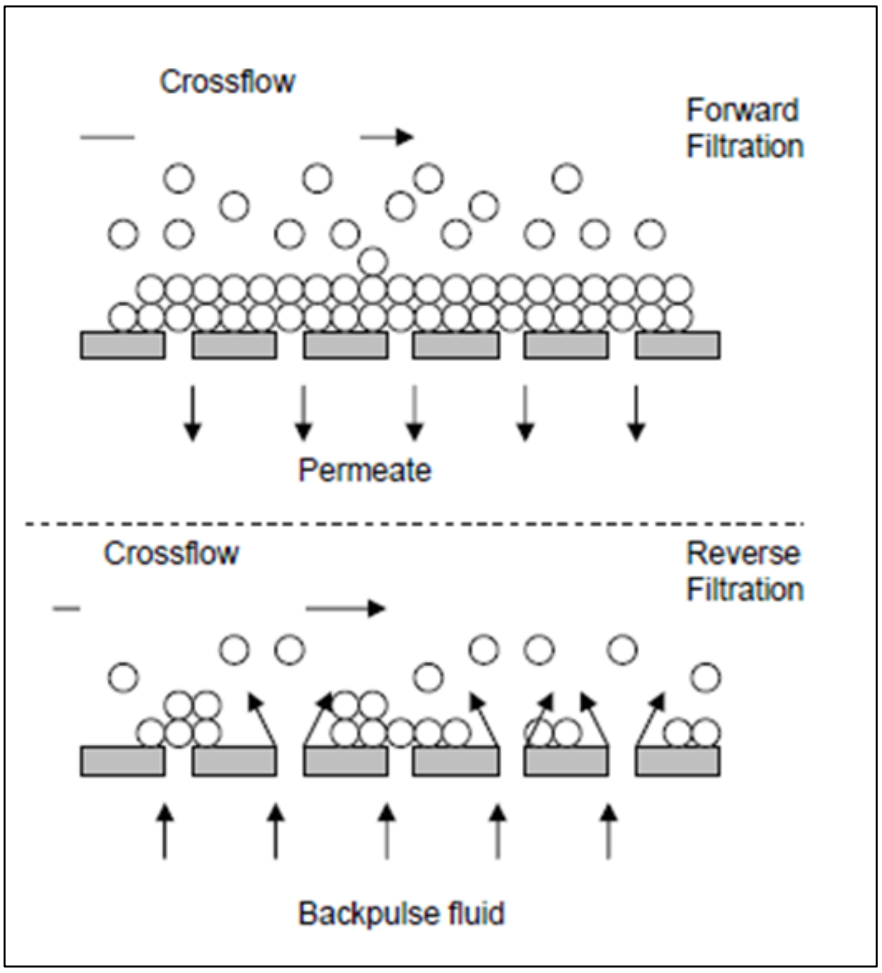

Figure 8 Basic concept of utilizing backflushing in membrane cleaning (Shugman;Aldrich;Sanderson;& McLachlan, 2013)

Other main hydraulic cleaning methods are flow relaxation by decreasing TMP and temporary operation with higher cross-flow velocities. Releasing pressure (flow relaxation) and changes in flow turbulence by changing flow velocity help reducing cake/gel layer

forming and concentration polarization phenomena but are weaker for removing adsorbed or pore blocking particles. (Shon;Smith;Vigneswaran;& Ngo, 2007) Often these methods are used periodically during operation and not so much in cleaning cycle. Effectively designed procedure can be used to increase membrane lifespan and operation time between cleaning periods. (Shon;Smith;Vigneswaran;& Ngo, 2007)

Pneumatic methods include air bubbling, air sparging, air lifting and air sourcing. Methods are typically beneficiated from low maintenance cost, easy integration to membrane system and reduction of chemicals needed. However, effectiveness of methods is often limited, and air pumping costs can be high. (Nguyen;Roddick;& Fan, 2012) Qaisrani & Samhaber (2011) found air bubbling effective in reducing cake deposition and recovering permeate flux. Even better results were obtained using air bubbling in combination with the backflushing.

In some cases, oversized sponge balls are applied in cleaning of tubular membranes. They can scrape foulants of the membrane surface. Method is time consuming and may damage membrane surface. Applications of mechanical cleaning in membrane technology are rare. (Qaisrani & Samhaber, 2011)

Popovic et al. (2010) found that utilizing ultrasound in membrane cleaning can increase effect of chemical cleaning of protein foulants. Ultrasound works either displacing foulants from membrane pores and/or enhancing effect of chemicals. This could be due increased contact between reagents and foulant. (Popovic´;Djuric´;Milanovic´;Tekic´;& Lukic´, 2010) Ultrasonic waves create acoustic field that causes cavitation and thus breaking cake layer, disturb microbial cells and detach foulants from membrane surface. However, application of ultrasound can damage membrane pores. Also, it could be difficult to implement to industrial or even pilot scale. (Nguyen;Roddick;& Fan, 2012)

Electric fields can be used in removing charged particles from membrane surface. Charged particles and molecules will move into direction of electric field. Method can be used to enhance other cleaning processes or even during the filtration operation to reduce fouling. Method requires using of specific electric conducting membranes and complex modules. (Mulder, 1996)

5.2 Chemical cleaning

Various types of chemicals may be utilized in membrane cleaning. Some typical cleaning chemicals are for example caustic, acidic, surfactants, complexing, oxidants/disinfectants

and many different enzymes. Also, multiple commercial cleaning blends combining different chemicals are available. (Nguyen;Roddick;& Fan, 2012) Chemicals in cleaning can modify foulants and fouling layer and alter their interactions with membrane surface. Chemicals can also work by dissolving or displacing foulants. Some examples and main functions of different types of chemical cleaning agents are presented in Table 3. (Shi;Tal;Hankins;& Gitis, 2014)

Table 3 Main types of chemical cleaning agents. (Shi;Tal;Hankins;& Gitis, 2014)

Cleaner type	**Example chemical**	**Function in cleaning**
Caustic	NaOH, KOH	pH regulation, hydrolysis, alteration of surface charges
Acidic	HCl, H_2SO_4	pH regulation, hydrolysis, dissolution of inorganics
Surfactant	SDS	Dispersion of deposits
Complexing	EDTA	Complexion with metals, removal of mineral deposits
Oxidant	H_2O_2, NaClO	Oxidation of organics, disinfection
Enzyme	Proteases, Lipases	Degrading of proteins and other specific targets

Chemical cleaning can affect membrane surface as well as foulants. Harsh cleaning conditions can degrade especially polymeric membranes and cause serious problems for filtration process. (Shi;Tal;Hankins;& Gitis, 2014)

Effects on foulant layer are not always positive. If wrong type or excessive volumes of chemicals are used problem may become worse than before cleaning. For example, Cai & Liu 2016 found that chemical cleaning with NaClO enhanced biofouling potential of membrane bioreactor. Membrane part was cleaned at first but NaClO caused cell lysis releasing extra cellular substances that could increase fouling of membrane. Also, living cells had more tendency to attach on surface after treatment. NaClO caused cell lysis and triggered defensive response from bacteria releasing different polysaccharides and proteins enhancing membrane fouling. (Cai & Liu, 2016)

Similar issues can be faced also with other cleaning agents. Low pH during acid cleaning can lower net charge of organic foulants leading to more sever fouling. Use of surfactants

can cause foaming inside membrane module and their residuals can be difficult to remove from membrane unit. (Kim;Zhu;Herzberg;Walker;& Jassby, 2018) Also, if cleaning is not complete residuals of cell debris in membrane surface can act as landing spots for foulants and increase membranes fouling potential in following filtration cycles (Parkar;Flint;& Brooks, 2004).

5.3 Cleaning sequences

Usually cleaning of membrane systems is done with multiple cleaning steps. Different steps are needed to recover flux as much as possible. Cleaning sequence should be designed carefully to minimize water and chemicals consumption and process dead times. During cleaning cycle, process is not producing any valuable products, so time of cleaning cycle should be minimized. If interval between cleaning is too long or cleaning is not completed, the membrane performance and life time can be drastically weakened. (Shi;Tal;Hankins;& Gitis, 2014)

Optimization of cleaning time for different steps is also important from perspective of cleaning efficiency of single step. Too short time of cleaning might not be complete but on the other hand too long duration of cleaning can cause damages on the membrane. If cleaning solution is recycled for too long periods of time re-fouling with already removed particles could cause weakening of cleaning results (Field;Hughes;Cui;& Tirlapur, 2008). Petrus et al. (2008) studied cleaning of ultrafiltration membranes with enzymatic solution. In their work, good cleaning of membranes was obtained but if cleaning was continued too long or with too high enzyme concentration fouling of membranes by enzyme adsorption began during cleaning experiments. (Petrus;Chen;& Norazman, 2008)

Typically, first stage of cleaning is to remove all process feed and product from system and rinse it with water. This is usually followed by mechanical cleaning to displace cake and loose particles from membrane surface and pores. Most common methods are backflushes and changes in flow conditions to disturb fouling layer. Flow velocity is usually higher and TMP lower than during normal operation. Higher flow rate increases turbulence and lower pressure can relax cake layer. Lower TMP is used because increasing TMP could push particles deeper into membrane pores. (Shi;Tal;Hankins;& Gitis, 2014)

After removing loose particles usually at least one chemical cleaning step is needed to recover flux. Typically, several chemical cleaning steps are used with different types of

solutions with water rinses between them. Sequence is depended on types of foulants present and process conditions. (Shi;Tal;Hankins;& Gitis, 2014)

Physical and chemical cleaning stages can also be used simultaneously. This can reduce cleaning time and combined methods can have bigger effect than standard cleaning. For example, chemically enhanced backflushing and combining chemical soaks with air sparging are promising options. However, these might need more complex installations and increase costs of cleaning operation. (Shi;Tal;Hankins;& Gitis, 2014)

5.4 Design and optimization of cleaning procedure

Design and optimization of cleaning protocol for membrane process requires knowledge of foulant and experimental work. Cleaning methods and chemicals used must be selected properly and their dosages and operation parameters optimized carefully. Extensive laboratory and pilot testing is usually necessary. (Shi;Tal;Hankins;& Gitis, 2014) Choice of suitable cleaning method for targeted foulant is often based on trial and error (Mohammadi;Madaeni;& Moghadam, 2002).

Probably simplest method for finding suitable cleaning agents are quantitative tests with many different chemicals and commercial blends. Hijnen et al. (2012) performed experiment with 27 cleaning agents to clean biofouling of NF membranes in laboratory scale. They used knowledge of foulant type to produce uniform biofilm samples for screening with chemicals. Biofilm and chemical samples were mixed in beakers and after experiment remaining biofilm concentration analyzed. Similar test was used for multiple step protocols. Results obtained were validated in pilot unit. (Hijnen, ym., 2012)

After finding suitable cleaning agents' optimization of operating conditions and parameters of individual chemical and mechanical cleaning steps follows. Parameters considered are for example temperature, pH and concentration of chemical cleaning and pressure and duration of backpulses. (Chen;Kim;& Ting, 2003) Many statistical approaches can be applied to this part. For example, statistical factorial design or Box-Behnken design can be very useful. Chen et al. (2003) successfully used factorial method to optimize physical and chemical cleaning conditions for UF and RO membranes used in municipal wastewater treatment.

Problem with methods presented is number of laboratory experiments needed meaning increasing consumption of time and materials in design stage. With prior knowledge of foulant type, processes already installed and reports from literature, design process could be

streamlined to reduce time between laboratory, pilot and industrial implementations of protocol.

6 Microfiltration in enzyme production

Microfiltration is relatively coarse membrane separation process. It works mainly by size exclusion with macropores. Separation capability of microfiltration is from 0.1 to 10 µm. For microfiltration, chemical properties of feed solutions are not as important in separation process than with nanofiltration or reverse osmosis process, but they can still effect on membrane operation, for example by adsorption of particles onto membrane surface. (Gabelman, 2017)

Microfiltration is widely used for recovery of proteins. Microfiltration is proven to be effective in recovery of extracellular products, but challenges with flux decline and membrane fouling remain significant. (Saxena;Tripathi;Kumar;& Shahi, 2009), (Charcosset, 2006), (Frenander & Jönsson, 1996)

Cross-flow microfiltration has many advantages over traditional separation methods such as centrifugation and convectional filtration methods in enzyme recovery. It is generally more effective, giving higher enzyme yields and activities, it's easier to clean and maintain and economically beneficial. (Keefe & Dubbin, 2005) Centrifugation increases temperature of feed potentially causing protein denaturation and it is not effective in separation of low density products. Also, centrifuges require lot of maintenance. Dead end filtration is often problematic due cake formation and chemical precipitation due chemicals necessary. (Reinehr, ym., 2017) Microfiltration systems are easier to clean than dead end filtrations and cleaning is more economical as cleaning in place (CIP) is possible. Microfiltration also eliminates need of filter aids that are often necessary in drum filtration. This decreases costs and environmental impacts of process. (Keefe & Dubbin, 2005)

6.1 Methods to enhance microfiltration process

Permeate flux can be increased by altering different parameters in feed flow. Most commonly altered parameters are feed pH, temperature and composition or process parameters such as trans membrane pressure difference (TMP) and flow hydrodynamics. Increasing temperature decreases liquid viscosity. Increased viscosity typically leads to lower flux. Altering pH can influence particle interactions with membrane surface. But

temperature and pH might also, have an effect on fouling tendency and membrane durability. (Gabelman, 2017)

Increasing TMP and flow velocity increase flux until certain point has reached after that the flux remains stable or might decrease because of concentration or gel layer formed close to membrane surface. (Gabelman, 2017) Flow hydrodynamics can also be altered using different turbulence promoters or flow restrictions. These spacers disturb flow increasing its turbulence which effects on mass transfer and decreases effect of concentration layer. (Mulder, 1996)

Bacchin et al (2006) presented review of so called critical flux phenomena. Critical flux is theoretical maximum stable permeate flux without fouling occurring on membrane process. If parameters are then altered (for example increase of pressure or temperature leading to higher flux) fouling will begin and flux will decline over period of time. Evaluation of critical flux is used in finding optimal process parameters for membrane separation. (Bacchin;Aimar;& Field, 2006)

Different pre-treatment methods of feed can be used for membrane separation not only to reduce fouling, but also to improve separation efficiency. Coagulation/flocculation pre-treatment can be used to enhance filtration. Coagulation/flocculation changes feeds particle concentration, size and dimensions in order to improve its filterability. Many different methods such as polymeric flocculants and hydrolyzed metal salts can be used as coagulation agents. (Wang;Liu;& Li, 2013) Kim et al. (2001) successfully tested commercial flocculants in microfiltration of yeast suspensions. Optimal flocculant concentrations and stirring velocity & time for flocculation were found. Use of flocculants improved permeate flux trough membrane and decreased membrane fouling. (Kim;Akeprathumchai;& Wickramasinghe, 2001)

7 Fouling problem at Genencor and possible solutions

Membrane fouling during recovery of enzymes from fermentation broth could be due to different reasons. Fouling could be caused by different biopolymers interacting with membrane surface and pores rather than growth of biofilm. There are multiple different biological substances present in fermentation broths in addition to microbial cells and wanted enzymes. These biopolymers, particulate matter in fermentation media and soluble

microbial byproducts have big effect on membrane fouling. Problem in identification of fouling problem is synergistic effects of all different compounds. (Kujundzic, ym., 2010)

Main foulant in Genencors microfiltration process has been identified as *Trichoderma reesei* cells and its residuals. Enzymes being separated are extracted from cultivated *T. reesei.* Used membranes removed from filtration units clearly show fouling layer at the membrane surface. This could be due biofilm growth of *T. reesei*, but more likely is cake and gel layer formed by cells and their residuals. Fermentation stage should use all nutrients from solution which would limit growth of fungi during filtration process.

Different additives for fermentation process could have part in the fouling problem. For example, some antifoaming agents can cause issues in microfiltration. Also, enzyme proteins could be adsorbed to membrane pores increasing fouling. Previously done tests for foulant propose that also cellulose and hemicellulose are present at the fouling layer.

7.1 Fungal cell wall composition

Fungal cell wall is complex structure containing especially proteins and polysaccharides such as chitin, glucans and glycoproteins. Cell wall is dynamic organism under constant remodeling. Main purposes of cell wall are maintaining fungi shape, protect it from outside influences and relay messages and signals for fungi. Fungal cell wall is vastly different from other cell types. (Selitrennikoff, 2001) In Figure 9 basic composition of fungal cell wall is presented.

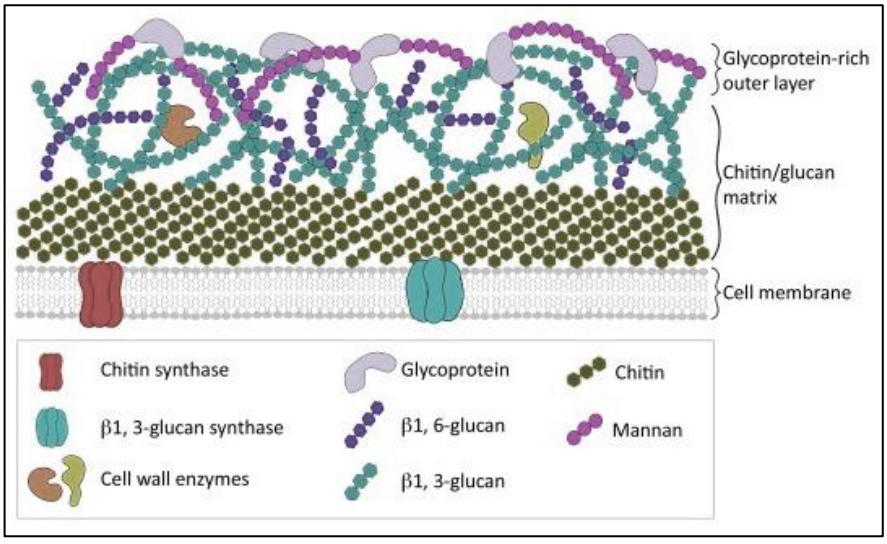

Figure 9 Basic structure of fungal cell wall. (Geoghegan;Steinberg;& Gurr, 2017)

For filamentous fungi, such as *T. reesei* cell wall consist about 10-20 % chitin (in yeasts typically 1-2%), 50-60 % glucans (mainly 1,3-β-glucan) and 20-30 % proteins (Bowman & Free, 2006). Chitin (a long linear homopolymer of beta-1,4-linked N-acetylglucosamine) is responsible for cell walls structural integrity. Glucans are polysaccharides forming most of the cell wall. They create branched matrix that provides cell with mechanical strength. Glucans also bind other cell wall components together with covalent bonds. (Bowman & Free, 2006)

Glycoproteins attached to cell wall are very important for fungi cells functions. They relay signals and messages to cells, control cell wall synthesis and remodeling. Many kinds of glycoproteins present are for example mannoproteins and galactomannans. (Bowman & Free, 2006)

7.2. Cleaning of membranes at Genencor

There are multiple enzymes that could be utilized in membrane cleaning at Genencor. Many enzymes have ability to degenerate compounds in fungal cell wall and other antifungal activities. These could help in removing foulant from membrane surface. Also, commercial cleaning blends and various different chemicals could be used.

7.2.1 Enzymatic cleaning of fungi based foulant

Cellulose and hemicellulose found in the foulant can be degenerated by various enzymes known as cellulases and xylanases. Different cellulases and xylanases are widely used in enzymatic biomass conversion. (Farinas, ym., 2010)

Cellulases degenerate cellulose to oligosaccharides and cellobiose and further into glucose (Nguyen;Freund;Kasanjian;& Berlemont, 2018). Xylan is one major form of hemicellulose and xylanases are enzymes capable of degenerating it. Xylanases typically randomly cleave the glycosidic links in the polysaccharide structure of xylan. (Moreira & Filho, 2016)

Chitin is important part of fungal cells which could present large part of foulant at Genencors microfiltration sheets. Chitinases are large family of enzymes degenerating chitin. Chitinases are present in fungi where they are used in cell wall degeneration for cell growth and to be utilized against competitive fungi. Chitinases are also present in plants that can use them for protection against fungi. Chitinases work mainly by hydrolyzing chitin chain. (Langner & Göhre, 2016) Chitinases have shown antifungal activity against *Trichoderma reesei* (Selitrennikoff, 2001).

β-Glucanases are present in many plants and contribute to plants defense against the fungal pathogens along with its other purposes in plant. β-Glucanases degenerate glucans present in fungal cell wall causing weakening it and making it more vulnerable for cell lysis. Often β-Glucanases work in combination with Chitinases and other antifungal enzymes. (Balasubramanian;Vashisht;Cletus;& Sakthivel, 2012)

Mannans and galactomannans in the cell wall can be degenerated by mannanases and mannosidases (Huang, ym., 2013).

Various other enzymes with antifungal activities have been identified from different sources. Many plants produce antifungal proteins to defend themselves against attacks. These are called pathogen related (PR) proteins. Selitrennikoff (2001) gave 13 classes of PR: s and more have been found since. Antifungal mechanisms for many of these proteins are still unknown. (Selitrennikoff, 2001), (Wong, ym., 2010)

For example, thaumatin like proteins (TLP) belong to PR group-5. They resemble well known thaumatin protein in amino acid sequence even though their properties and functions are very different. Many TLP: s, such as osmotin and zeamatin, can bind into cell walls β-glucan and disturb cell wall synthesis and prevent proper cell wall assembly. (Liu;Sturrock;& Ekramoddoullah, 2010)

Saponins are another large family of antifungal proteins extracted from plants. They can be recovered from many onion species among other plants. Saponins can form complexes with sterols in fungal cell membrane. These leads to increase of membrane permeability and leakage of cell material causing cell lysis. (Lanzotti;Romano;Lanzuise;Bonanomi;& Scala, 2012)

There are enormous number of different antifungal proteins being extracted and research to discover more and understand their mechanisms is going on. Many of these could be utilized in operations needing antimicrobial agents in the future. Other than presented enzymes for example some antimicrobial peptides (defensins), protease inhibitors and many others could be useful in membrane cleaning from biological fouling as well as other antimicrobial applications. (Wong, ym., 2010)

When utilizing enzymes in membrane cleaning it is important to consider process conditions with enzymes used. Enzymatic activity correlates with pH and temperature. Optimal values

of parameters should be used to increase activity and cleaning efficiency. Wrong pH and temperature can decrease efficiency or denaturate enzymes completely. (Farinas, ym., 2010)

7.2.2 Chemical cleaning of fungi based foulant

Multiple different brands and compositions of chemical cleaning solutions are available. Solutions are often blends of alkalis or acids with surfactants, complexing agents, oxidants and/or disinfectants. Sometimes also enzymes are included. (Nguyen;Roddick;& Fan, 2012) Information and knowledge available from manufacturer as well as laboratory experiments, should be used when selecting commercial solutions.

Li et al. (2005) studied cleaning of ultrafiltration membranes fouled by glutamic acid fermentation broth containing cells, proteins, antifoaming agent and some fermentation by products, much like feed ferment at Genencors process. They found that sodium dodecyl sulfate (SDS) (surfactant) and hydrogen peroxide (oxidant/disinfectant) both gave competitive results in membrane cleaning. SDS achieved 94,3 % and H_2O_2 82,0 % flux recovery rate. Cleaning speed of H_2O_2 was faster than with other solutions. (Li;Li;Fu;Wickramasinghe;& Chen, 2005)

Surfactants affect to interactions between foulants and membrane surface. Surfactants can remove foulants due electrostatic or hydrophobic actions. Surfactants can also affect to foulants surface tension making it more vulnerable to other cleaning agents. (Masse;Puig-Bargués;Mondor;Deschênes;& Talbot, 2015)

Oxidants such as hydrogen peroxide and peracetic acid (PAA) are strong disinfectants showing also antifungal activity. Commonly mixture of H_2O_2, PAA, acetic acid and water is used. Acetic acid and H_2O_2 in the mixture help keeping it stable. PAA is much stronger oxidant than H_2O_2. Oxidants release active oxygen which oxidizes proteins and enzymes in cell walls thus interfering with cells vital actions. (Kitis, 2004)

Xu and Liu (2011) showed that chemical uncoupler 2,4-dinitrophenol (DNP) could be used in inhibition and detachment of biofilms from membrane surfaces. DNP can carry protons through cell walls and thus inhibit cells ATP synthesis. This prevents microbes from attaching to membrane surface and forming of biofilm. DNP was also shown to detach already formed biofilm from membrane surface. (Xu & Liu, 2011)

Salt solutions can be utilized in cleaning membranes fouled by proteins. Salts can increase proteins solubility (salting in) and affect their recovery from membrane pores. (Corbatón-Báguena;Álvarez-Blanco;Vincent-Vela;& Lora-García, 2015) Saline solutions can also affect interactions in fouling layer by causing concentration difference between bulk solution and fouling layer. Also, ion-exchange reactions between salt ions and foulant molecules can break the foulant layer. (Corbatón-Báguena;Álvarez-Blanco;& Vincent-Vela, 2014)

8 Waste treatment

Main waste streams are cell waste from enzyme separation and process waters used elsewhere for example from product concentration, rinsing and wash waters. Cell waste contains solid biomass from fermentation process. Mainly used yeast and fungal cells and their residuals and some nutrients used in fermentation medium. Cell waste is slurry with high water content. Currently waste is sent elsewhere for treatment. Utilization of slurry in other processes locally or by finding different users for it could help to improve economic efficiency of the process. Waste could be used for example in energy production. Waste waters are led to water treatment plant nearby in co-operation with another factory.

8.1 Dewatering of cell waste

To make cell waste more applicable for potential users it should often be dewatered. Dewatering can also lower the transportation costs of the waste as its volume is reduced. Several equipment and processes for dewatering are available.

8.1.1 Mechanical dewatering

Typical mechanical processes are different types of filtrations, presses and centrifuges. In Table 4 comparison of basic processes for mechanical dewatering is presented. Gravity based thickening or sedimentation can sometimes be useful first step in dewatering but cannot usually remove enough water and other dewatering methods are needed. Vacuum and pressure filters are traditionally used. Pressure filters are effective, but they often need lot of operation and are applicable only in batch mode. Vacuum belt and drum filters are less effective but also need less supervision and can be operated continuously. (Bajpai, 2015)

Different kinds of filter presses can be used to obtain higher dry solids content than just filtration. Plate and frame or chamber presses are most common filter press configurations. Filter cloth is attached to frames that set-up chambers. Chambers are then filled with slurry and under pressure water passes through filter cloth in chamber walls. Inside the chamber

cake is formed. After separation chamber opens and cake can be removed. (Chen W. , 2013) Separation can be enhanced with flexible filter cloths which can be inflated to squeeze the filtration cake even more. Other variation is tube press where inside the chamber is bladder that can be inflated to press cake towards the filter cloth. (Metso Corporation, 2015) Chamber presses can cause difficulties with cleaning of chambers and they are also quite complex to operate. (Bajpai, 2015)

Other common press types are belt, screw and rotary presses. Belt presses consist two filter belts facing each other. Filtration cake is pressed between them to squeeze out the water. Pressure increases towards end of the belts due belts moving closer together and rollers applied. Typical belt filter press is presented in Figure 10. Down side of belt presses is need for belt washing and often flocculants are needed for feed. (Wakeman, 2007)

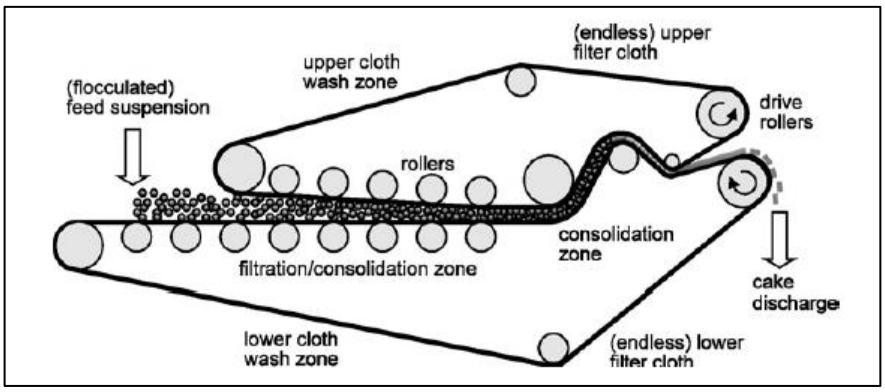

Figure 10 Typical configuration of belt filter press. (Wakeman, 2007)

Screw press is based on screw conveyor which diameter increases. Conveyor is inside pipe that is covered with filter cloth. As sludge is conveyed to top of pipe pressure increases due decreasing of space in unit. Cake is formed, and water squeezed out. (Yan, ym., 2014) Operating principle of screw press is presented in Figure 11. Rotary press consists of channel between two rotating filtration elements. Sludge travels around the channel and during it water is separated. Pressure is created with restriction of channel outlet and friction caused by slow rotation of filtration elements. (FOURNIER INDUSTRIES INC., 2017)

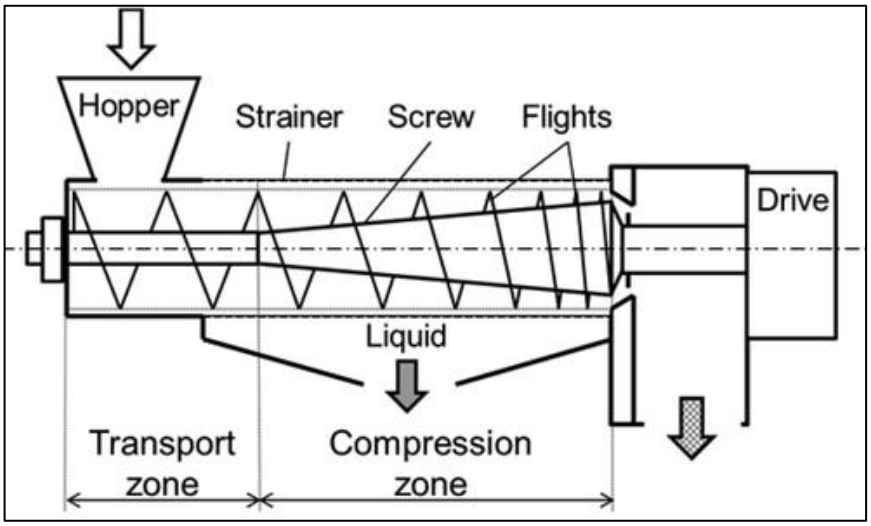

Figure 11 Operating principle of screw press. (Yan, ym., 2014)

Centrifuges are another important type of mechanical dewatering equipment. Dewatering in centrifuges is based on centrifugal forces caused by high speed spinning of equipment. Forces separate compounds based on their weight. Several different models are available, most important ones being disc, bowl and decanter centrifuges. (Bajpai, 2015) Decanter centrifuge can be operated continuously. It instantly applies high g-forces to feed solids. Screw conveyor is used inside the centrifuge bowl to carry separated solids to outlet. Concept of decanter centrifuge is presented in Figure 12. (Wakeman, 2007)

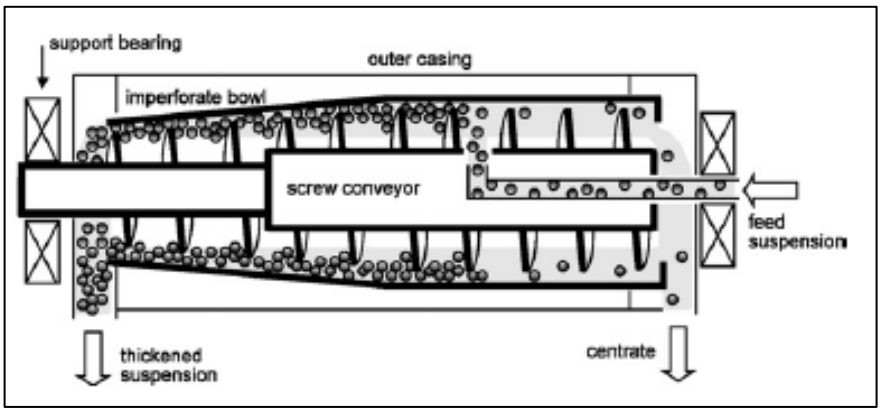

Figure 12 Basic concept of decanter centrifuge. (Wakeman, 2007)

Koza et al. 2017 found gravity screening followed by filtration centrifuge effective for dewatering fungal biomass producing filter cake with 30 % dry solids with short operation time. Procedure was found to be more effective than belt filtration and filter press used in their research. (Koza;Norton;& van Leeuwen, 2017) In filtration centrifuges rotating basket is equipped with filter cloth. Separation of particles is affected by filtration but also

centrifugal forces. This can lead to larger particles forming coarse pre-coat layer and putting liquid flowing through the cake also under centrifugal force in addition to pressure caused by liquid head. Centrifugal filters are effective but expensive equipment, sometimes referred as “Rolls Royces” of solid-liquid separation. (Svarovsky, 2000)

Table 4 Properties of different mechanical dewatering equipment, combined from: (Bajpai, 2015) (Wakeman, 2007)

Equipment	Achievable dry solids content, %	Power consumption, kWh/t	Operational notes
Gravity thickener	5-10	0-10	- Large footprint - Simple operation
Vacuum filtration	20-30	20-30	- Simple, continuous operation - Often pre-treatment or flocculants needed
Chamber press	35-50	30-90	- Typically, batch mode - Complex operation and cleaning - For high capacities, large installations needed
Belt press	25-40	20-30	- Continuous, relatively simple operation - Belt washing important - Often flocculants needed - Larger footprint
screw/rotary press	20-40	20-30	- Continuous, simple operation - Small footprint
Centrifuges	25-35	30-60	- Complexity in operation & cleaning - Batch or continuous operation

8.1.2 Drying and other methods

Water in the sludge can be divided to free and bound water. Free water can be easily separated with mechanical dewatering, but bound water is trickier. Bound water can be attached particle surface by adsorption or other forces or it can be trapped into microorganisms and particle flocs. (Ruiz-Hernando;Simón;Labanda;& Llorens, 2014) Typically, mechanical dewatering techniques can achieve about 30 % of dry solids because of intracellular water (Koza;Norton;& van Leeuwen, 2017). Result lowers waste volume

considerably but is still low for utilizing waste in energy production or in other applications. Moisture still left in waste lowers its energy value significantly. Due this mechanical dewatering is often followed by drying. More recently different mechanisms are combined to dewatering processes to assist mechanical dewatering. (Mahmoud;Arlabosse;& Fernandez, 2011)

Sludge drying in separated dryers is effective, but often consumes lot of energy. Often some waste heat could be utilized in dryers. If suitable heat source is available dryers are good choice. Typically, rotary, fluidized bed and multiple heart dryers are used in sludge drying. (Bajpai, 2015) Due high energy consumption of drying process lot of recent development is focused on enhancing mechanical dewatering processes. These enhancements include thermal, ultrasound, electric fields and many different chemicals used as flocculants etc. (Ruiz-Hernando;Simón;Labanda;& Llorens, 2014)

One well known method is to implement heat source into mechanical dewatering process. Thermally assisted mechanical dewatering process (TAMP) usually works with moderate heating (~80 °C) added into walls of mechanical dewatering apparatus with relatively low pressure, but also direct heating can be used. Other often used method besides TAMP is called mechanical thermal expression (MTE). It includes pre-heating of slurry to process temperature which is over the boiling point of water. During the process backpressure is used to prevent evaporation. After mechanical process pressure is released and flash evaporation occurs releasing water. (Mahmoud;Arlabosse;& Fernandez, 2011)

Chemical polymers are commonly used as flocculants to enhance efficiency of mechanical dewatering processes. Different chemicals can also be used to free bound water from microbes but especially from particle surfaces. Treatment with oxidants or alkali chemicals are discovered to be useful. (Jin;Zhang;& Zheng, 2015) Jin et al (2015) found that amount of free water in sewage sludge increased significantly thus enhancing dewaterability of sludge, when treated with K_2FeO_4 and $KMnO_4$ oxidants. Mo et al (2015) presented rapid Fenton treatment technique for sewage sludge treatment. Iron-catalyzed decomposition of H_2O_2 in acidic solutions produces hydroxyl radicals. Radicals can degenerate sludge compounds binding water and therefore improve sludge dewaterability. (Mo;Huang;Dai;Liang;& Sun, 2015)

Utilization of ultrasound in dewatering process is one viable option. Ultrasound can disturb microbes containing intracellular water. Ultrasound treatment can release up to 30 % of bound water from microbes to free water which is easy to remove by mechanical dewatering methods. (Bajpai, 2015) Sonification can also help to disintegrate flocs and particles in sludge increasing amount of free water. If ultrasonic treatment is too long or frequency of ultrasound too high advances can be lost. Long sonification creates more but smaller flocs increasing total area of compounds and thus their effect on water binding. (Jin;Zhang;& Zheng, 2015)

Also, electric fields can be applied to enhance mechanical dewatering. Mahmoud et al (2011) present that electric field assisted process can increase dewatering ability of pressure based mechanical dewatering system. Mechanism behind this is probably electro-osmosis. It creates charged double layers around the sludge particles decreasing binding of bulk water to sludge particles. (Mahmoud;Olivier;Vaxelaire;& Hoadley, 2011)

8.2 Inactivation of microbes

As microbes used in fermentation and production of enzymes are genetically modified they should be inactivated (killed) before reuse or disposal of waste. Typical methods for microbe inactivation include thermal, chemical and other more novel technologies.

Thermal treatments for pasteurization (killing pathogenic and most of other microbes) and sterilization (killing all microbes) are known since 1800's from food preservation. (Micali;Fiorino;& Parisi, 2016) Main difference between techniques is higher temperature used in sterilization. Pasteurization can be done in temperatures 60 to 75 °C but sterilization typically requires temperature over 100 °C. Also, treatment duration is critical for effectiveness of process. (Micali;Fiorino;& Parisi, 2016) In some cases, dewatering, drying and heat treatment for microbe control can be combined. FKC Co. have patented system combining pretreatment of sludge with rotary screen and heated screw press for dewatering and pasteurization of feed. (FKC Co., Ltd, 2018)

Other important parameter affecting microbial growth is pH. Increasing or lowering pH with acids or bases (typically acetic acid and NaOH) can lead to inactivation of microbes sensitive to pH changes. (Ratledge & Kristiansen, 2006)

Disinfection of waste sludge can also be done using different chemical methods. Traditionally oxidative chemicals such as hydrogen peroxide and chlorides (for example

NaClO or free chlorine gas). Chlorination can cause inactivation of microbes in many different mechanisms for example oxidation, enzyme inhibition and damaging cell membrane. Other oxidative reagents form hydroxyl radicals with water causing oxidation and thus inactivation of microbes. (Fatta-Kassinos;Dionysiou;& Kummerer, 2016)

Also, other than oxidizing chemicals can be used. For example, aldehydes, isothiazolones and some surfactants. Chemicals can disturb micro-organisms metabolism. Problem with chemicals is often their toxicity and possible environmental issues, production of toxic by-products and corrosivity (especially with oxidants). Also, cost and dosage of chemicals consumed needs to be considered. (Aquaprox, 2009)

In addition to thermal treatments and chemicals some other methods for microbial control are available. These methods include for example use of pressurization, ultrasound and UV-radiation. (Butz & Tauscher, 2002)

8.3 Waste water treatment

With different dewatering methods used lot of waste water is produced. Even if main proportion of solid materials is removed and possible microorganisms inactivated amount and quality of waste water could cause problems. Water could contain high concentrations of phosphor, nitrogen and different organic compounds. This waste water could need additional treatment before it can be led to centralized water treatment plants.

8.3.1 Phosphorus removal

High amounts of phosphorus in waste water can lead to serious environmental problems. Phosphorus is used in fertilizers and in waste waters it causes eutrophication. This leads to increasing growth of unwanted algae and related algae toxins dangerous for humans and environment. (Jiang;Amano;& Machida, 2017) Phosphorus is limiting pollutant for Genencors waste water treatment agreement with UPM:s water treatment facilities. Recovery of phosphorus from waste water could have also economic benefits if phosphorus could be reused in process or sold to other industries.

Removal of phosphorus from waste waters is often done by biological method or with chemical precipitation. Recovery of phosphorus in different salts with low water solubility is one common technique. Precipitants containing Mg^{2+} and Ca^{2+} ions such as MgO, CaO, $MgCl_2$ and $CaCl_2$ can be used to form struvite (Magnesium-ammonium-phosphate) and various calcium-phosphates. Precipitation is however sensitive process depending on for

example pH, temperature, COD and suspended solids contents. (Egle;Rechberger;& Zessner, 2015)

Also, adsorption is promising option for phosphorus recovery. Jiang et al. (2017) studied use of calcium-silicate composite (CSC) and alkali-treated calcium-silicate composite (ASC) in phosphate recovery from water. Both were successful in recovery with adsorption capacities of 70 mg/g and 120 mg/g. ASC:s were highly selective for phosphate over other ions and process performed well over pH range from 3.0 to 13.0. Phosphates were desorbed easily with 2% citric acid solution and were at reusable quality for fertilizers. (Jiang;Amano;& Machida, 2017)

Various other treatment technologies are already available and being studied. Choosing suitable method requires much information about waste water quality and composition. Recover is technically possible and large scale applications exists. Challenge is in choosing correct method for each case and economical aspects of implementing processes. (Egle;Rechberger;& Zessner, 2015)

8.4 Potential applications for wastes

There are numerous potential applications for waste biomass. Traditionally biomass can be converted directly to energy and heat. More novel applications include conversion of biomass to different biofuels or extracting specialty chemicals from it. There are multiple ways to use and gain economic advantages of waste compared to landfilling it. (Demirbas, 2001)

Probably most common way to utilize waste in energy production is direct combustion process. This could be done locally or at any power plants nearby. In direct combustion process biomass is converted to heat and energy by directly burning it in combustion chamber. Electricity and heat are then recovered with help of steam cycle. Typically, efficiencies of electricity production from biomass vary in range of 20-40 %. Higher water content of sludge decreases effectivity of combustion process. Often biomass is burned together with coal and other wastes in central power plants. (Demirbas, 2001) For example, in Figure 13 on-site incineration of sewage sludge process from Outotec is presented. Process utilizes fluidized bed incinerator after sludge dewatering and drying. (Outotec, 2016)

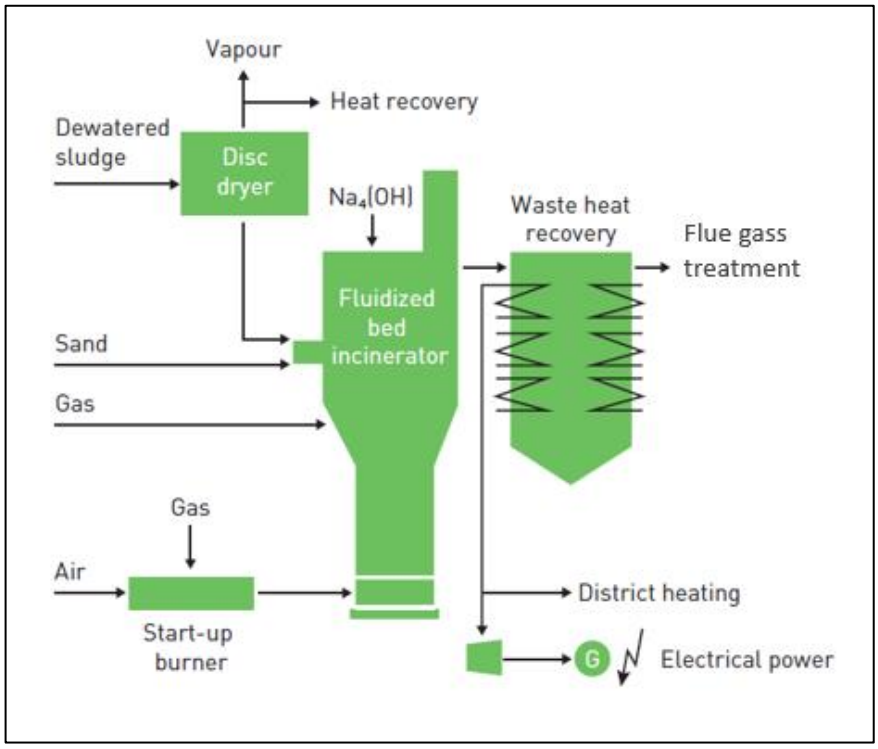

Figure 13 Outotec sludge incineration process. (Outotec, 2016)

Currently waste sludge from Genencor is transported for biogas production facility. Biogas can then be used in production of energy and fuels. Biogas production is based on anaerobic digestion of wastes. In first stage polymers and monomers present in waste are converted into acetate and hydrogen. At the second stage microbes produce methane from acetate, hydrogen and CO_2. (Weiland, 2010)

Production of biofuels and specialty chemicals from biomass sources is currently gaining lot of interest. New ways to produce greener fuels and find replacements for oil is important. Many biorefinery projects to convert biomass sources into biofuels and chemicals are ongoing and area is under intensive research. (Sheldon, 2011) These could produce potential applications and end users for fungal biomass waste produced with fermentation process.

Biomass could be utilized in removing of heavy metals from waste waters. Dead fungal biomass could be used as bioadsorbent to remove heavy metal ions from aqueous solutions. Uptake of metal ions can be metabolically independent process (adsorption, complexation or chelation of metal ions into cell wall compounds) which allows utilization of waste and by-products of microbial biomass. (Ahluwalia & Goyal, 2007) Rahman et al (2014) studied effectivity and kinetics of *Trichoderma sp* in bioadsorption of heavy metal ions (Cd_2^+, Ni_2^+

and Cr_3^+). *Trichoderma sp* biomass was found to be effective in removal of ions from industrial waste water with removal rate up to 100 %. (Rahman;Shahadat;& Won, 2014)

Waste water produced in in enzyme production rich in nutrients such as phosphorus and nitrogen. It could be utilized as water in agriculture. EU has strict regulations for reuse of waste water and value is not high. It could be more beneficial to extract phosphorus and use it directly. Phosphorus is important raw material for fertilizers for example. Phosphate rock is also nonrenewable resource. Technologies for phosphor recovery are already available and relatively simple. (Egle;Rechberger;& Zessner, 2015)

Experimental

9 Materials and methods

9.1 Membranes used

Membranes used in laboratory and pilot experiments were spiral wound microfiltration elements from common membrane vendor. Membranes consisted of a semipermeable polyethersulfone (PES) layer and polyolefin backing material. Two different spacer sizes were used.

Typical operating pressure given by manufacturer was 2,1-8,3 bar and maximum pressure for membrane was 9,7 bar. Maximum operating temperature was 80 °C and pH between 2,0 and 10,0 on continuous operation. For cleaning allowed pH was 1.8 - 11.0 and temperature max 50 °C with chlorine and 80 °C for cleaning not involving chlorine. Membrane is also sensitive for cationic surfactants or polymers and could be irreversibly fouled if exposed to them according to membrane manufacturer.

In addition to new membrane elements previously fouled membranes used in enzyme recovery were obtained from another production. Sheets were fouled during similar separation process as in pilot experiments made in this thesis. Sheets were used in cleaning experiments at laboratory and with the pilot unit. Sheets were stored and transported to Finland before tests which could affect membrane condition and results of experiments. Fouled membrane element used in experiments is presented in Figure 14.

Figure 14 Fouled membrane element from another production facility.

9.2 Analyzing methods

Membrane samples from enzyme and chemicals cleaning experiments were inspected with *Zeizz Discovery VIZ* stereomicroscope to find and see changes in fouling layer on membrane surface.

Supernatant samples collected in cleaning experiments were analyzed for their dry solids content (%) and absorbance over wave lengths of 200-500 nm. *Shimadsu UV-1800* spectrophotometer was used in analyzing absorbances from supernatant samples. Solids content was measured with *Metter Toledo* analyzer. Method is based on drying of the samples.

Activity of enzyme samples obtained from pilot runs were performed with automatic *Konelab analyser* or by manual analyzing methods for different enzymes based on controlled enzyme reactions with substrates causing changes in absorbance. Analyses were performed by Jämsänkoski laboratory staff.

Konelab was used also to measure phosphate content of waste water samples. COD content of waste water samples was measured by analyze kit from HACH containing readymade analyze, control and standard reagents. *HACH Lange HT 200* COD-reactor and *HACH Lange DR2800* spectrophotometer were used.

9.3 Screening for membrane cleansers at laboratory scale

Screening for different enzyme and commercial chemical cleaning solutions was performed. Chemicals and enzyme solutions were tested in two parallel experiments. In first experiment series, previously fouled membrane was soaked in cleaning solution and in second series cleaning solution was dosed with foulant slurry.

Some enzymes produced at Jämsänkoski were tested for enzymatic cleaning of membranes. Pieces of membrane were dipped into 60 ml, 50/50 V-% enzyme concentrate and water solutions. For reference, also pure water was used for one sample. Solutions pH was adjusted for optimal enzyme activity with acetic acid and NaOH. Solutions with membrane sheets were incubated for 16 h in 45 °C temperature with mixing of 70 rpm in incubation cabin (*Infors AG, CH-4103 Bottmingen*). Solutions used are presented in Table 5 and procedure in Figure 15.

Table 5 Test solutions.

Test solution/enzyme	sample number
Water	1
Enzyme mixture	2
Cellulase A	3
Betaglukanase	4
Cellulase B	5
Xylanase A	6
Cellulase C	7
Xylanase B	8

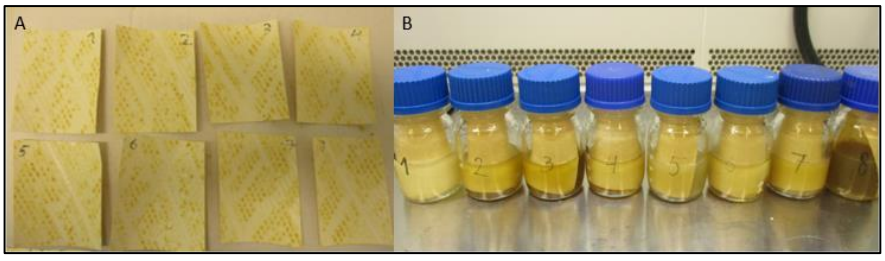

Figure 15 Experimental procedure for membrane dipping experiments.

Membranes were studied visually with help of stereomicroscope to observe changes in membrane surface and foulant layer before and after incubation period. After incubation membranes were gently rinsed with water before inspection. When taken to room temperature membranes tend to dry out quickly resulting scaling of the surface layer of membrane and foulant. Membranes were kept moistured during inspection and preparations of experiment with water.

For test 2 foulant was scraped of membrane surface and added to water to create 50 w-% foulant slurry/mass. Test solution of 40 g was created with foulant mass, enzyme concentrate and water. Mixture was mixed carefully. Solution pH was adjusted with acetic acid and NaOH and active enzyme was added to the solution so that there was 10 w-% of active enzyme protein per foulant mass added. Experimental procedure is presented in Figure 16.

10 ml of solution was centrifuged with 4000xG for 3 min (*Herafuu multifuge X3R)*. Supernatant was collected as "0" sample for analyses. Then solution was incubated as in test 1 in 45°C with mixing of 70 rpm for 16 h. After incubation, final sample of 10 ml was collected and centrifuged as before. Supernatant was retained for analyses.

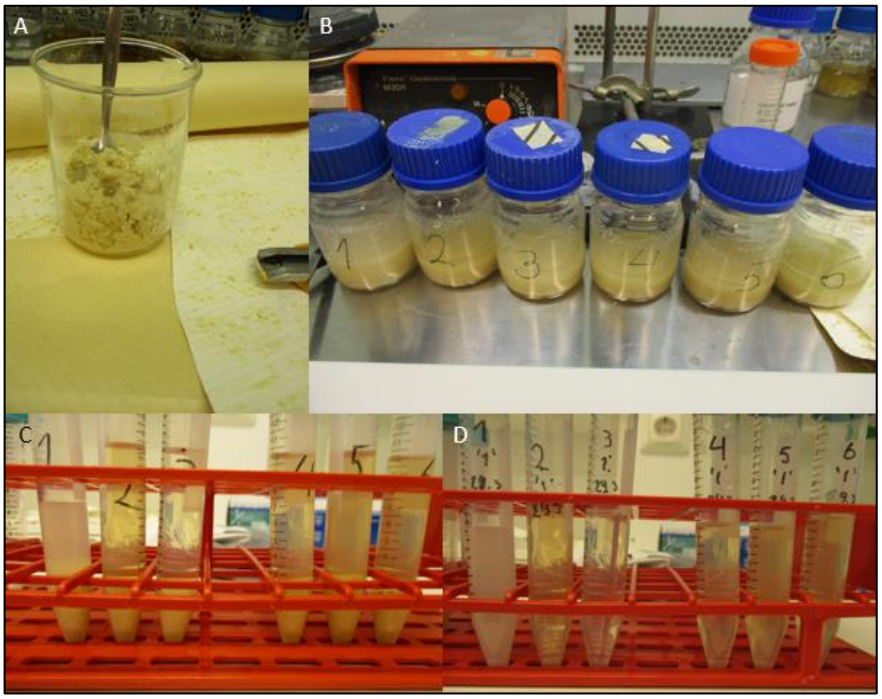

Figure 16 Experimental procedure for series 2. A) foulant mass 50 w-% collected foulant and water B) Prepared test solutions with foulant mass before incubation. C) Centrifuged samples after incubation D) final sample supernatants for analyses.

Experimental procedure for screening of different cleaning solutions and chemicals was similar to enzyme screenings. Chemical screening was done to cleaning solutions and chemicals currently used in some application in Genencor (cleansers not necessarily designed for membrane sheets). Chemicals and commercial solutions used are presented in Table 6. Sample preparation, collection (centrifugation 3min 4000xG) and incubation conditions (45 °C, 16 h and mixing of 70 rpm) were kept similar to enzyme screenings.

Table 6 Chemicals and commercial cleaning solutions used in chemical screening.

Cleaning solution		Description
1	Water	Water
2	A	Caustic cleaning solution for membranes
3	B	Enzymatic cleaning solution for membranes
4	C	Caustic cleaning enhancer
5	D	Oxidizing cleaning enhancer
6	E	Strong disinfectant
7	F	Chosen as acidic reference, used to clean precipitations at production

9.4 Pilot filtrations and pilot scale cleaning studies

9.4.1 Pilot unit

Pilot unit contained three loops each containing multiple spiral wound membrane elements. Loops could be run separately or all together. In addition to three microfiltration loops pilot has also its own ultrafiltration loop for concentrating product. Each loop contained its own flow and sample ports. Retentate and permeate flows were recycled and collected to feed tanks for MF and UF. Pilot unit is presented in Figure 17**Virhe. Viitteen lähdettä ei löytynyt.**.

Figure 17 Pilot unit used in experiments. Feed, retentate and permeate lines connected to separate tanks and pumps outside the picture.

9.4.2 Operating pilot

Pilot operation is started by connecting feed, concentrate and dilution pumps and tanks to unit with tubes. Feed tank is filled with ferment and dilution tank with fresh process water. After connections are secured manually valves are opened and unit is filled with ferment by starting feed pump. At this point, only retentate is removed form unit and recycled into feed tank.

Filtration is started by slowly and periodically starting circulation pumps in unit. Pressure difference in loops is increased to target value. TMP is set by opening permeate outlet valve slowly until correct TMP value is reached. Permeate is collected to permeate/concentrate tank. Fresh water from dilution tank is added into feed to replace permeate removed and maintain constant volume of feed.

After enough permeate is collected ultrafiltration loop is started to concentrate permeate. Ultrafiltration has its own feed and retentate recycling pumps. UF retentate (concentrate) is recycled back to concentrate tank and permeate to dilution tank to save amount of fresh water used. Flowsheet of the unit presenting recirculation streams is presented in Figure 18**Virhe. Viitteen lähdettä ei löytynyt.**.

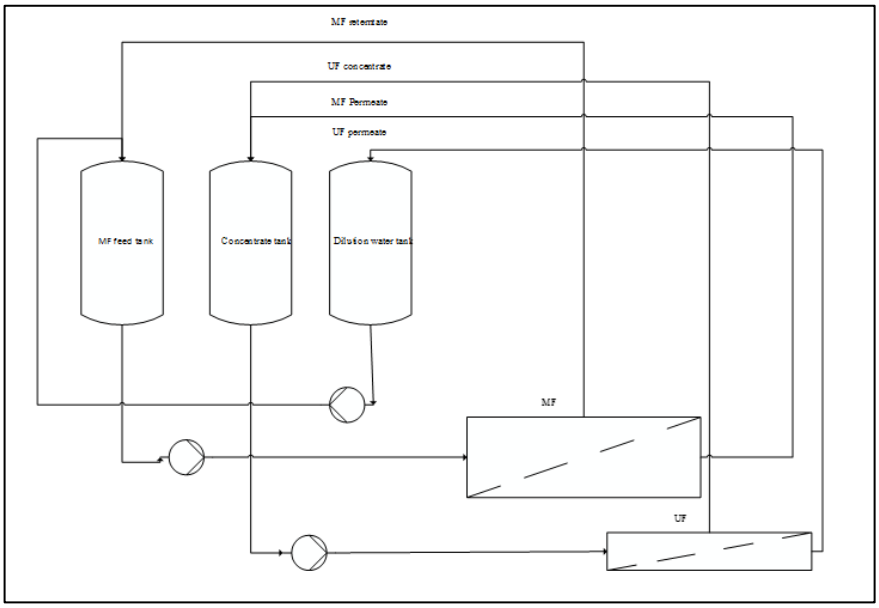

Figure 18 Rough flowsheet of the unit presenting recirculation streams of process.

Amount of dilution water used is measured to follow progress of filtration, typically 4 diavolumes (DV) of filtration is performed in pilot run. Temperature, pressures and feed, retentate and permeate properties are monitored and adjusted if necessary. Each loop contains its own heat exchanger to control temperature. Operation is ended by closing permeate recovery valve and shutting down pumps in reverse order.

9.4.3 Cleaning of unit

Washing of loops is done as CIP procedure by recycling cleaning solution in unit. Operation of unit is done similarly to filtrations, but both permeate and retentate are recycled to feed tank. Cleaning is performed with low TMP. During rinses unit is filled and drained with clean water.

9.4.4 Clean water flux measurement

Clean water flux (CWF) of units was followed to see changes in fouling layer and membrane performance. CWF was measured before and after each pilot run to see changes happened and to track success of cleaning protocol. CWF was measured by recycling clean water through the pilot system at temperature of 25 °C and with different TMP:s starting from 0 to 0,5 bar otherwise similarly to cleaning procedures. Automated instruments recorded permeate flow from unit and pump outputs of process. Flux recovery is good indicator for success of cleaning. Retentate pump output can be followed to see effects of foulant in

spacers. If pump output drops more mass is attached to membrane spacer or surface and resistance to flow is higher so pressure is increased with less work from the pump.

9.4.6 Pilot filtrations

In total six pilot experiments were made, five with ferment filtration and one for only cleaning tests with previously fouled membranes. First one with only one Loop and larger spacers to familiarize with unit and test cleaning protocol. Remaining four pilot filtrations were performed with two Loops and differing spacer sizes (small (S) and large (L)) for membrane elements. Goal of these experiments was to find out differences in membrane performance with different spacer sizes, test different operating parameters (TMP, feed properties etc.) and evaluate & optimize cleaning protocol. Pilot experiments made are listed in **Virhe. Viitteen lähdettä ei löytynyt.**.

Table 7 Pilot filtrations performed. Filtrations were performed with four different products (A-D) which have slightly different feed properties for filtration.

filtration / Product	**Goals**
1. A 7.5.18	Familirize with unit, test for cleaning protocol (only L spacer)
(Previously fouled membranes) 14.5-7.6.18	Test for cleaning chemicals (no ferment filtration, only cleaning tests for previously fouled membranes (L))
2. B 27.6.18	Comparison of different spacer sizes, run & feed parameters, evaluation & development of cleaning protocol, samples from waste streams
3. C 11.7.18	
4. D 23.7.18	
5. A 31.7.18	

10 Results and discussions

10.1 Results of laboratory scale enzyme and chemical cleaning experiments

Two experiment sets of cleanser screenings were conducted for enzymatic and chemical cleaning agents. Both sets contained two experiment series with dipping of membrane sheets

in cleaning solutions and test for foulant mass break down in cleaning solution. Results for enzyme screenings are presented in Figures 19-23 and for chemicals in Figures 24-27.

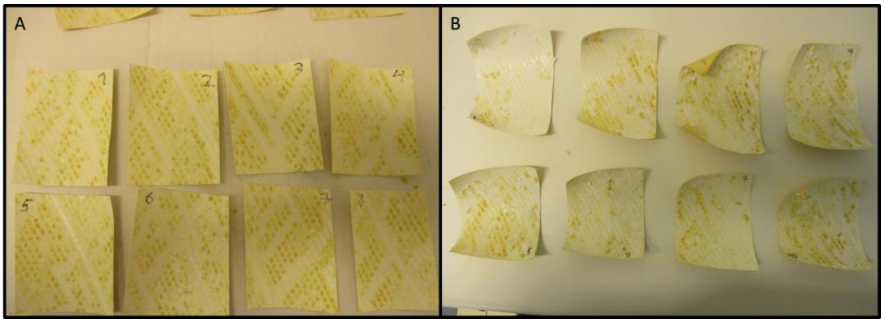

Figure 19 Membrane pieces before A) and after B) dipping experiment for enzyme solutions. Enzyme solutions from Table 5.

In Figure 19 membrane sheets before and after dipping experiment in enzyme solutions are presented. Solutions seem to have impact on membrane sheets, but total removal of foulant is not achieved. Figure 20 presents closer look to most promising solutions.

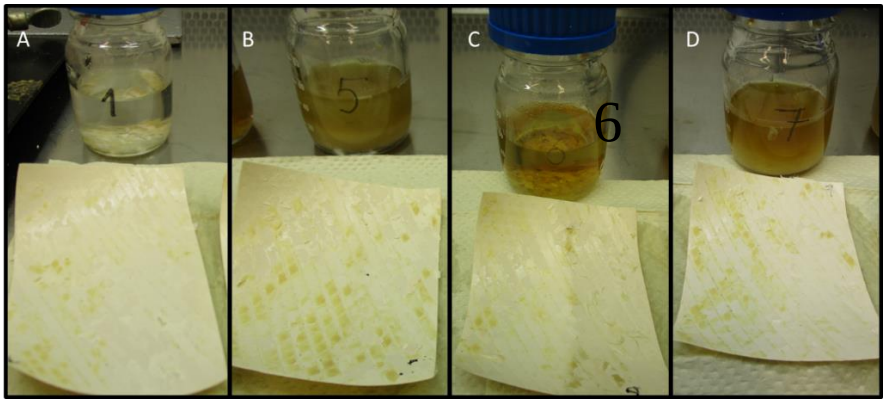

Figure 20 Solutions and membrane sheets with best removal of foulant during experiment A) Water B) Cellulase B C) Xylanase A D) Cellulase C

Most of the foulant is removed from part of membrane being in direct contact with solutions. Especially in sample bottles 1 and 6 some flakes of membrane surface and foulant can be seen. This could be due scaling of membrane surface due membrane drying during preparations for experiment or transport. Membranes are old which can affect results. In Figure 21Figure *21* stereomicroscope images of membrane surfaces are presented.

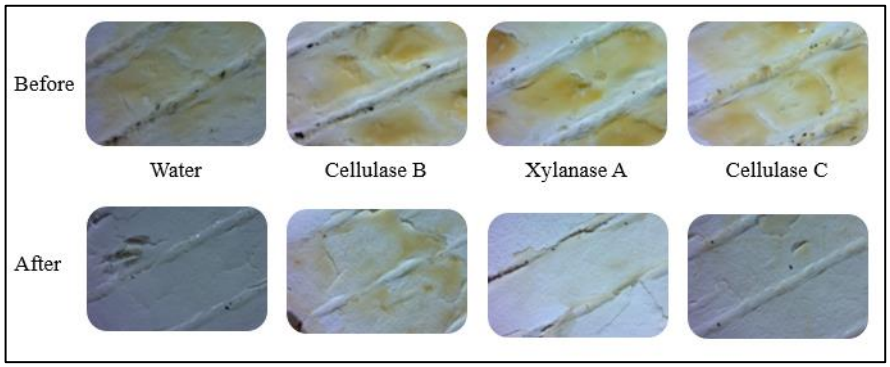

Figure 21 Stereomicroscopic images of membrane surfaces before and after experiment.

Breaking down of foulant mass during incubation in experiment 2 was followed by measuring absorbance and dry solids content of collected supernatant samples before and after incubation.

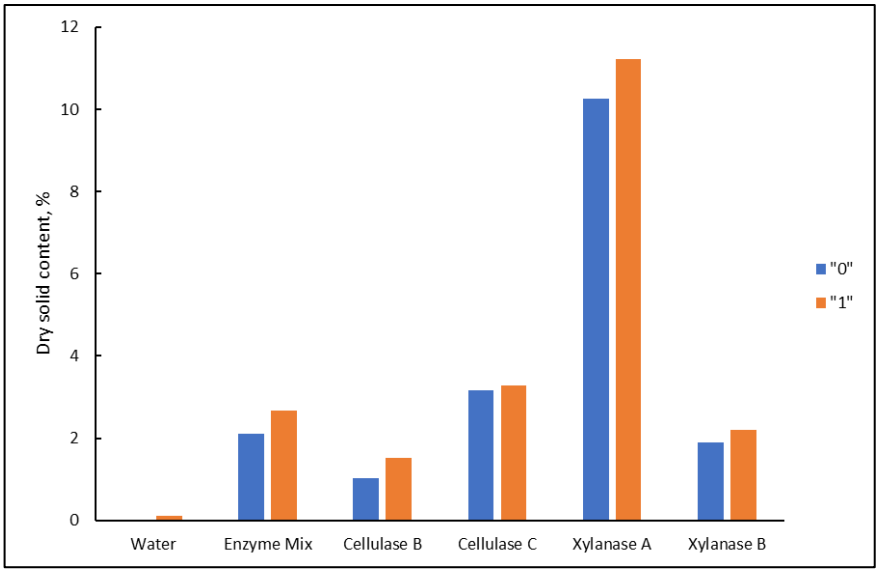

Figure 22 Dry solids contents of samples. “0” before and “1” after experiment.

Figure 22 presents dry solids content of supernatants collected before and after incubations. Dry solids content in samples increased little bit during incubation. This could indicate working of enzyme solutions as larger flocks of foulant are broken down into smaller pieces found in supernatant.

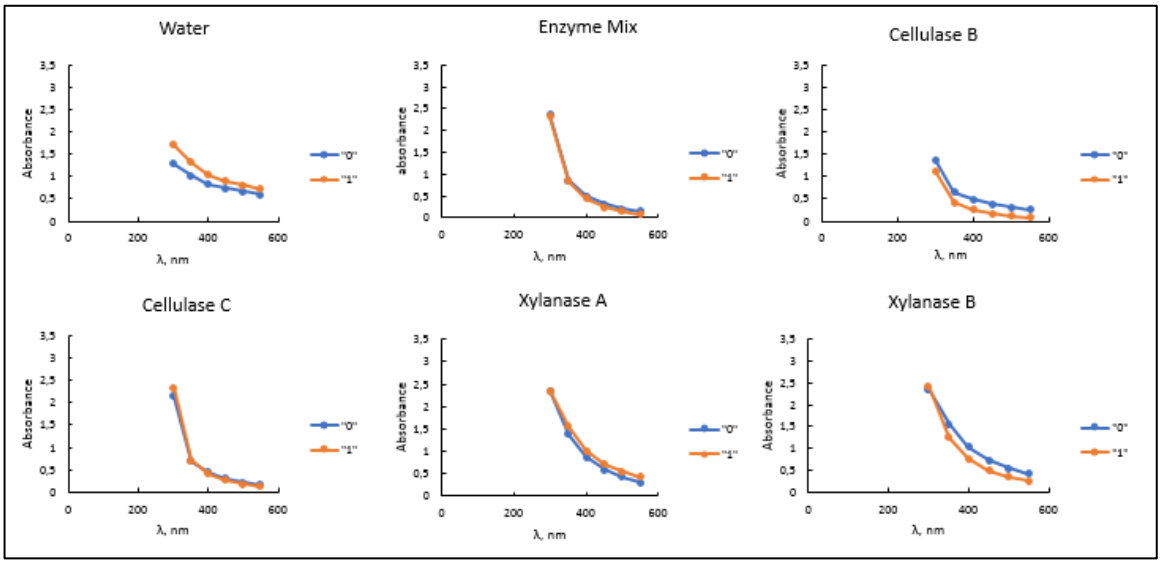

Figure 23 Changes in sample absorbances in experiment. “0” before and “1” after experiment.

Same phenomena could be seen in absorbances of water and Xylanase A solutions as their absorbance increases during experiment as shown in Figure 23. Changes are relatively small and in some samples absorbance is even lower after experiment.

Chemical cleaning solutions were tested in similar experiment than enzymes. Figures 24-27. present results for chemical cleanser screenings. Figure 24 presents membrane sheets before and after experiment. Some solutions seem to work very well, but many have virtually no effect. First solution is only water, but similar effects compared to first screening are not seen. In first experiment with enzyme solutions water was very effective. This raises more questions about effect of scaling of in enzyme screening.

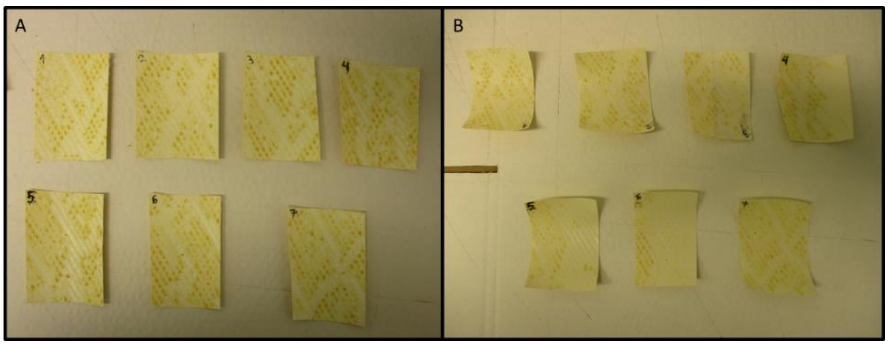

Figure 24 Membrane pieces before A) and after B) experiment with commercial cleaning solutions and chemicals. Chemical solutions from Table 6.

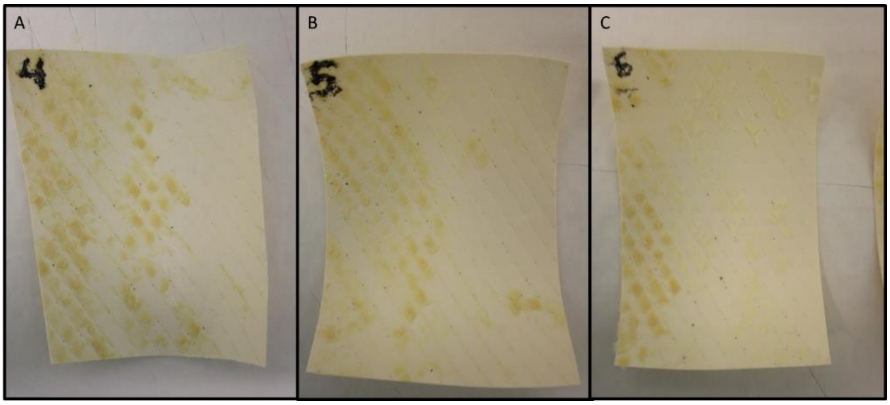

Figure 25 Most effective cleaning solutions in experiment. 4. solution 5. solution D 6. solution E

Membranes tested with solutions C and D seem very clean after experiment. Solution E seems to work on first sight, but foulants are only bleached instead of being removed from membrane surface as seen in Figure 25. Stereomicroscopic images of membrane surfaces are presented in Figure 26.

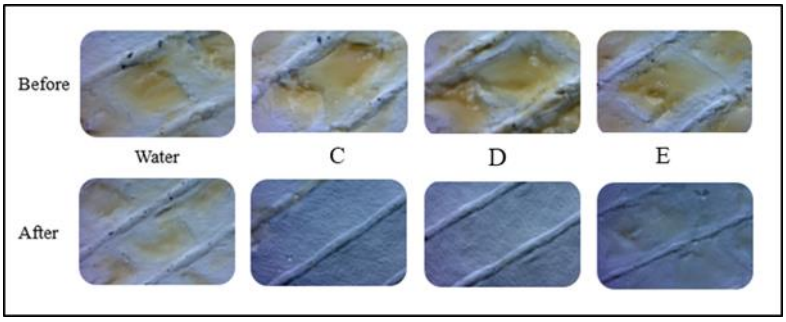

Figure 26 Stereomicroscopic images of most interesting results with commercial cleaning solutions and pure water

Test for foulant mass break down were also performed for chemical cleaning agents. Dry solid contents of all samples were below limits of measurement unit for trustworthy results. Absorbance measurements were more successful. Absorbances of supernatant samples are presented in Figure 27.

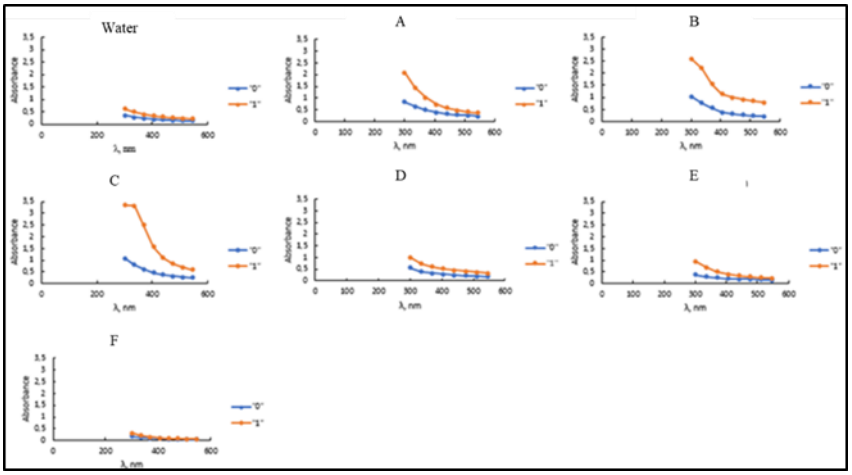

Figure 27 Changes in absorbances during experiment for commercial cleaning solutions and chemicals. “0” before and “1” after experiment.

For most samples absorbances did increase after incubation as expected if cleaning solution has effect. Biggest increases came with solutions A, B and C.

Caustic solutions A and C, enzymatic (protease) B and oxidative D were most effective in both experiments made. Acid cleaning wasn’t successful and solution E seemed to bleach foulant rather than remove it. From enzymes tested Cellulases B & C and Xylanase A had some success on dipping experiment but not with foulant dozing. This could be partly because of condition of membrane sheets after transportation and storing. Condition of membrane sheets also, prevented filtration tests in laboratory scale.

10.2 Results of pilot filtrations and cleaning experiments

10.2.1 Pilot filtrations and effect of spacer size on enzyme separation

First pilot filtration went smoothly without any difficulties. Flux increased slightly towards end of the run. This could be because of effect of feed scrubbing the surface of membrane. Previous filtration of loop was performed with yeast product that could have caused larger particles to form fouling layer that was not completely removed in previous wash (also CWF before experiment was lower than usual).

Four filtrations were performed in two loops with two different kinds of membrane elements. Different spacer size was used. In loop B small (S) spacers and in loop C large (L). Smaller spacer size increases membrane surface area and crossflow. Filtrations were performed to evaluate differences in recovery performance and cleanability of membranes after filtration.

Membranes in loop B (S) were brand new compared to membranes in loop C which were already used for 2,5 test filtrations.

This difference in membrane age could be seen especially during filtration with Product B which was first filtration with S elements. S membrane conditioning continued during filtration after membrane was exposed to filtration pressure. Flux in this filtration is clearly lower than in other filtrations. Also, CWF before filtration is much lower than after filtration. Conditioning opens membrane pores and removes excessive parts of membrane surface layer. Conditioning could play part in other filtration as well but its effect in later filtrations should be small.

Experiments were done in summer time which caused some problems for temperature control. Cooling capacity of pilot unit was not enough to keep temperature constant and it increased during filtrations causing increase of flux and decrease in TMP. Flux increase could also be caused by dilution of feed during filtration as enzymes recovered in permeate are replaced with dilution water lowering solids content of feed.

During second experiment with Product C (third filtration) UF feed pump started leaking during the filtration. Pump power was dropped to minimize leak which meant that UF was not able to keep up with MF filtration. This led to problem with MF permeate collection as tank was filled faster than UF could work. For last DV:s TMP was dropped to obtain lower flux and prevent over filling of permeate tank.

Pumps in pilot struggled to maintain DP (pressure difference across elements/loop) with S membranes. DP was lower during washes and during fourth filtration with Product D DP dropped also. D ferment has lower solids content than others. Pumps in pilot unit couldn’t increase DP back and flux dropped after start.

Final pilot filtration (5.) was performed again with Product A. filtration went smoothly and high fluxes were obtained. Also, DP was maintained well. After filtration product was concentrated and stabilized for further testing (granulation, product quality). In other test filtrations, final concentrate was disposed after experiment.

With S spacers ca. 25% more membrane area per element is obtained. This in theory leads to increase of capacity (total permeate flow of the unit). With S membrane total permeate flow increases but flux per membrane area decreases at the same time. This means that full

25% of capacity increase is not achieved. Relative change in permeate flow for different filtrations is presented in **Virhe. Viitteen lähdettä ei löytynyt.**.

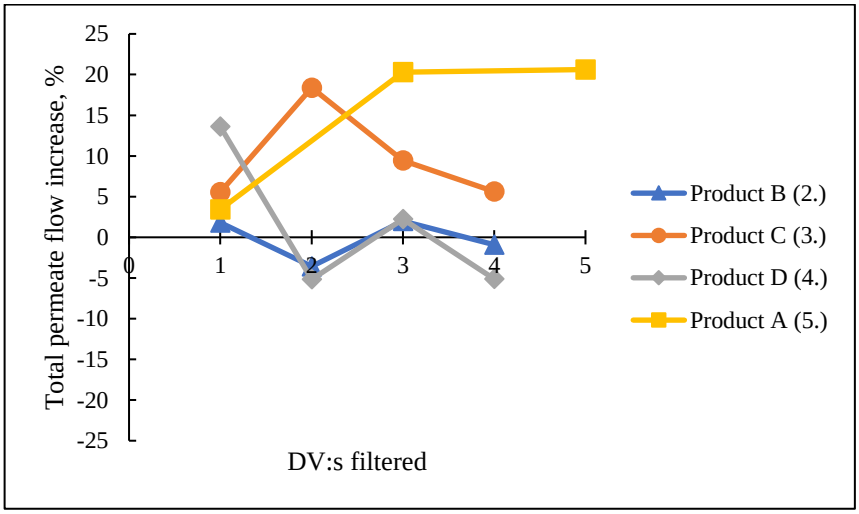

Figure 28 Relative change of total permeate flow between loop with S membranes and L membranes at different DV:s filtered.

For second filtration with Product B which was first filtration with S membranes no changes are observed. This is most likely due membrane conditioning. For third filtration with Product C effect is clear (increase of 5-20 %). Fourth filtration had problems with dropping of DP during filtration and that leads to lower permeate flow. On final filtration with Product A increase of 20% was obtained.

With S spacer elements membrane area in module is increased and it seems that capacity of unit increases even though actual flux per membrane area decreases. Also, trend is increasing as well as with CWF. All feeds have different properties which could affect filtration as well as variations in process conditions such as temperature and TMP.

Possible increase in total permeate flux is promising but much more important is recovery of actual active enzymes. Differences in activity passages (relation of activity in permeate compared to retentate during filtration) for different pilot filtrations are presented in **Virhe. Viitteen lähdettä ei löytynyt.**. Passage for each product is weaker with S spacers, on average 15-20 % lower. Lower passage means that even if capacity increases separation of enzymes is weaker with S spacers.

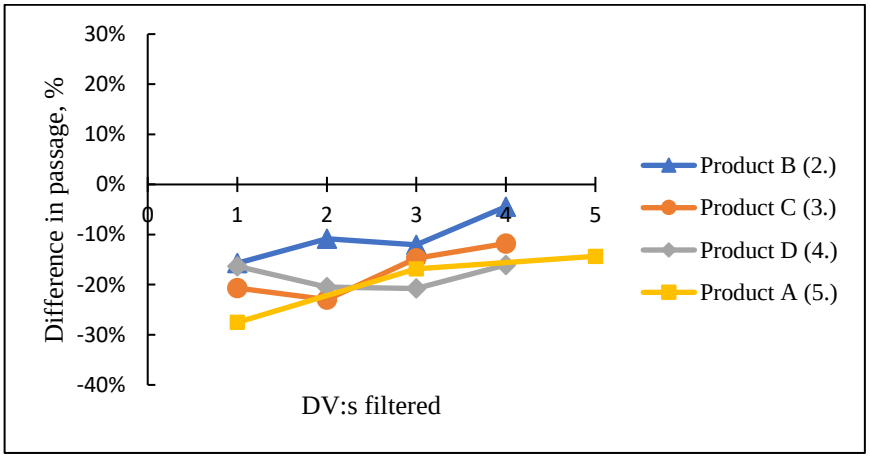

Figure 29 Differences in activity passage for different filtrations.

Activity/enzyme flux trough membrane is lower with S than L spacers. Increased total permeate flow can't compensate lower flux and passage as also enzyme flow is lower. **Virhe. Viitteen lähdettä ei löytynyt.** presents difference in enzyme flux and **Virhe. Viitteen lähdettä ei löytynyt.** presents difference in total enzyme flow from loops.

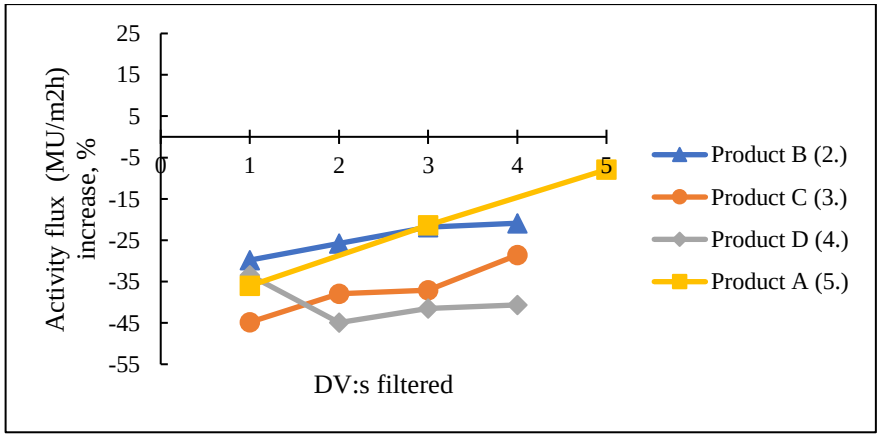

Figure 30 Change in activity flux (per membrane area) for different products with S and L membranes.

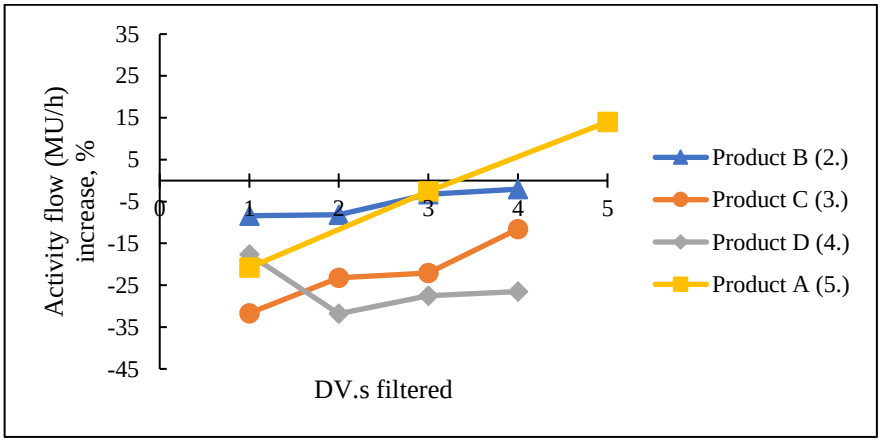

Figure 31 Change in activity flow (per Loop / elements) for different products with S and L membranes.

From **Virhe. Viitteen lähdettä ei löytynyt.** can be seen that even with increased capacity and permeate flow from S membranes amount of enzyme recovered is lower with S than L membranes in all but final filtration with Product A. This is due lower passage of enzyme and specific flux of enzymes per membrane area. Enzyme recovery is about 15-30 % lower in Product C and D filtrations and 5% lower in B filtration. In last filtration with Product A after 3 diavolumes of filtration increase in enzyme recovery is seen. At the end of filtration 13 % more enzyme is recovered with S membranes.

Role of spacers in spiral wound membranes is very important. Spacers provide space and structure of flow channels keeping membrane sheets separated. Spacers also work as static mixers for feed flow. Spacers promote mass transfer in between bulk flow and membrane surface layer preventing fouling and disturbing concentration polarization layer. (Saeed;Vuthaluru;Yang;& Vuthaluru, 2012)

In this case, smaller spacer size produces thinner flow channels in membrane element. Because of thinner channels more membrane rounds fit to same element dimensions thus increasing active membrane area of element. This increase of ca. 25 % in active membrane area increases total permeate flow/capacity of Loop, but enzyme fluxes and passages for each product are lower.

Decreasing of enzyme passage and flux per membrane area could be caused by changes in flow hydrodynamics due thinner feed channel and spacer size. Other changes to spacer geometry or membrane/element structure were not reported by membrane manufacturer. It seems that changes in membrane elements lead to decrease in turbulence of the flow. This

increases effect of CP causing decline in the flux and enzyme passage. Similar phenomenon was reported by Sablani et al (2002) for RO membranes. In their study flux (l/m^2h) decreased up to 50 % when decreasing spacer thickness from 0.1168 to 0.0508 cm, best production capacity (l/h) was found with 0.0711 cm spacer thickness. Study was made with sodium chloride solutions. (Sablani;Goosen;Al-Belushi;& Gerardos, 2002)

With smaller spacer size and feed channel height pressure loss in the elements typically increases as resistance to flow increases. (Bucs;Radu;Lavric;Vrouwenvelder;& Picioreanu, 2014). This was probably happening also during pilot runs as pumps struggled to keep DP up causing changes to flow profiles in the elements.

Other thing to consider is experimental set up. It could be possible that operating parameters used favor separation with L spacers. Adjustments or optimization of parameters such as TMP, DP, feed properties (DS, pH etc.) could help to decrease difference in performances between membranes. Other thing to note is difference between membrane condition. S membranes were brand new (compared to L with 2,5 previous filtrations) which could affect results especially in the first pilot run (B). Also, it has to be notes that passages in general are quite low and more work needs to be done for optimization of process and feed parameters.

10.2.2 Effect of new cleaning protocol after pilot filtrations

For cleaning, new cleaning protocol was used based on laboratory trials and knowledge from previous filtrations. Protocol contained first displacement with water then caustic wash with A, enzymatic wash with B and disinfection step with E solutions warm rinses with water were done between each washing step. Main changes in cleaning protocol were use of solution B instead of enzyme mix used previously in enzymatic step and increase in temperature of rinsing water between cleaning steps. Similar protocol with slight variances was used in all pilot tests.

Virhe. Viitteen lähdettä ei löytynyt. presents changes in membranes CWF at 0,5 bar TMP before and after filtration and cleaning. For L membrane, which was already been used results vary from increase of 15 % to drop of almost 20 %. Still all CWF:s are quite good and in range of sustainable operation (actual fluxes presented in **Virhe. Viitteen lähdettä ei löytynyt.**). This variation in cleaning success could be caused by many different factors. Membrane fouling and flux recovery is dependent on product, fermentation batch and

filtration conditions in addition of success of cleaning protocol. Slightly worrying is that trend seems to be downwards.

For S membranes cleaning is good and fluxes are recovered in all cases. Trend in CWF for S is increasing. This could be due membrane conditioning during filtrations (especially on first filtration with new membrane). Continuing flux increase could mean that membrane is not yet operating at full capacity which could affect the results. Based on pilot experiments S spacers doesn't increase membrane fouling tendency or cause risk with cleanability of membrane.

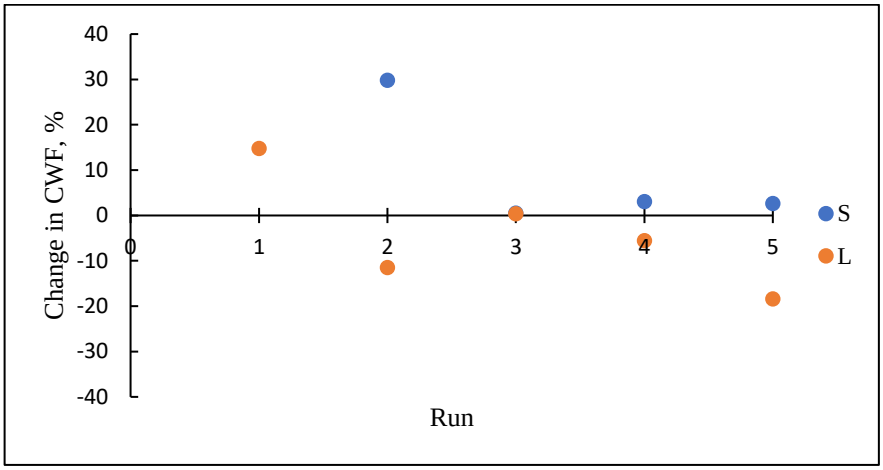

Figure 32 Changes in membranes CWF before and after filtration and cleaning at 0,5 bar TMP.

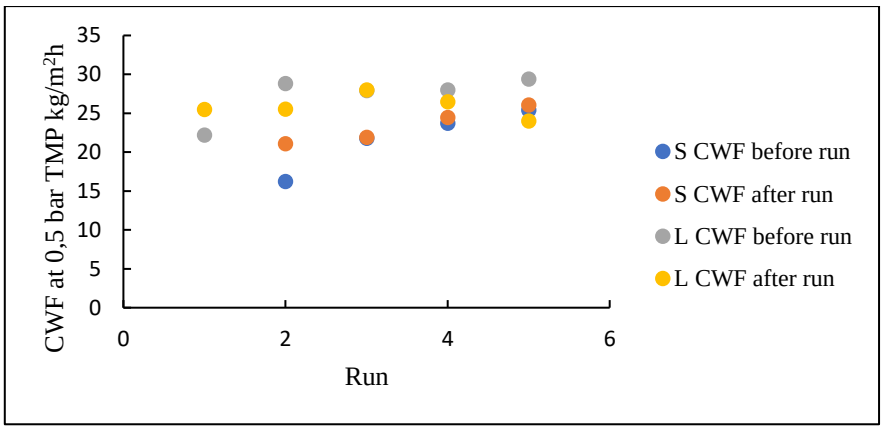

Figure 33 CWF before and after each pilot filtration at TMP of 0,5 bar.

Cleaning protocol used seemed to work quite well. There is some variation in CWF after cleaning, but overall fluxes are quite good. Solution B added to replace enzyme mix could be helping but also wash done without it (4.) was acceptable. In comparison to previous

cleanings massive development is not seen. Fluxes are bit higher, but similar variation of cleaning results continue. Rinsing and circulation times of cleaning steps seem to play important role as well as chemicals used.

10.2.3 Previously fouled membranes cleaning

Most promising cleaning agents from laboratory cleanings were tested with previously fouled membranes from another production facility. Membranes were used in production and their cleaning had already been tried before. Cleaning solutions C and D were chosen to test due promising results on laboratory experiment even though they are not designed for membrane operation. Enzymatic solution B performed in laboratory trials better than previously used enzyme mixture. Acidic and second caustic wash (A) were done later few weeks after first 3 steps as results with spacer fouling were not good after these steps. Cleaning procedure for sheet is presented in **Virhe. Viitteen lähdettä ei löytynyt.** and results from cleaning are presented in Figures 34 and 35.

Table 7 Cleaning protocol used for test cleaning of fouled membranes. Steps 4 and 5 performed 2 weeks later than first 3.

Cleaning step		**Agent**	**T, °C**	**pH**	**Circulation time, h**	**Rinse**
1	Caustic clean	C	45	~11	1	Warm 2 pcs
2	Enzymatic clean	B	45	8,11	1	Warm 2 pcs
3	Oxidative clean	D	45	6,80	1	Cold 2 pcs
4	Acid clean	F	30	3,03	1	Warm 2 pcs
5	Caustic clean	A	10	10,3	1	Warm 2 pcs

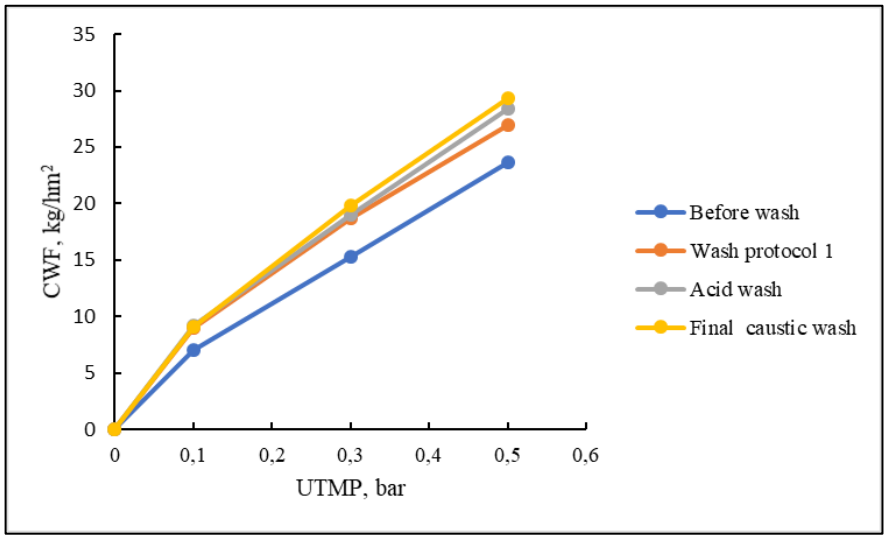

Figure 34 Clean water fluxes measured before, between and after cleaning steps. Wash protocol 1 first three cleaning steps performed.

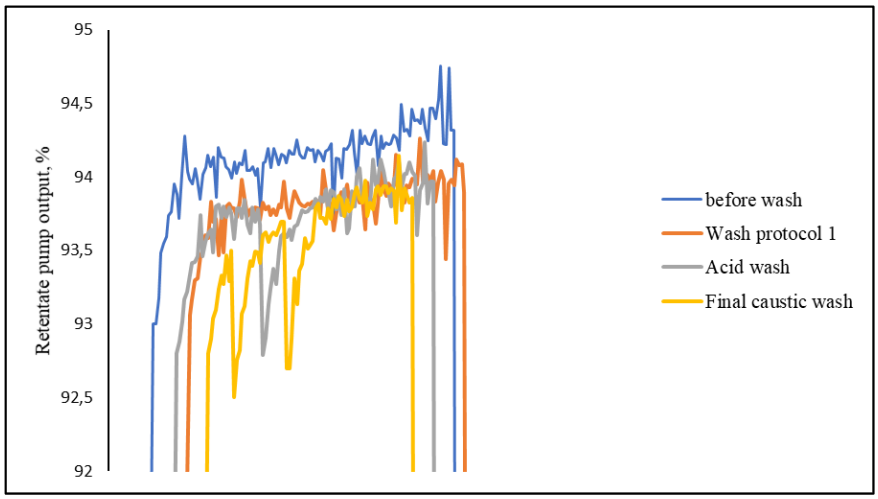

Figure 35 Retentate pump outputs before, between and after cleaning steps. Wash protocol 1 first three cleaning steps performed.

CWF in **Virhe. Viitteen lähdettä ei löytynyt.** increases after each wash with combined increase about 25 %. Problem with use of C and D solutions is that they are not designed for membranes and contain surfactants that may be harmful for membranes (anionic membranes and cationic surfactants). These surfactants can bound to membrane surface and increase fouling in the long run. Acids and A wash seem to help also. Output of retentate pump decreases with washing from average 94,1% to 93,6% after all wash steps. This indicates foulant stuck in membrane spacers. Membranes were removed and opened after cleaning

experiment. **Virhe. Viitteen lähdettä ei löytynyt.** showcases membrane surface after experiment.

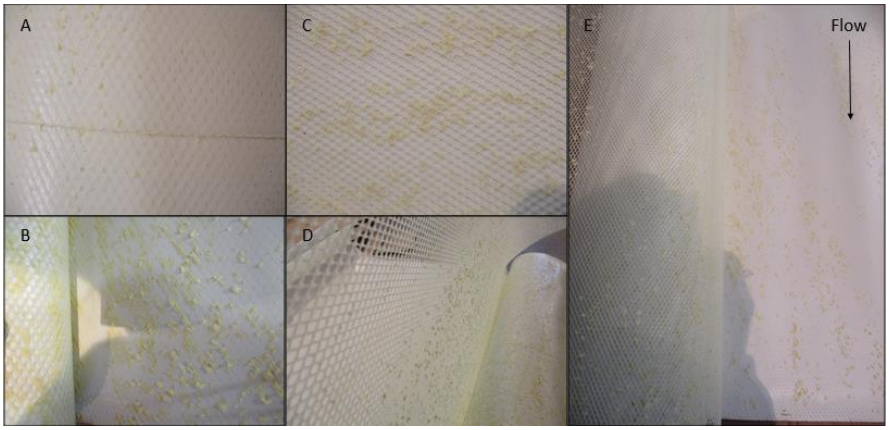

Figure 36 Membrane surface after cleaning steps.

Surface looks intact and is quite clean in many places especially in first element as seen in **Virhe. Viitteen lähdettä ei löytynyt.** but cleaning performance worsens to last element which is still very dirty. Foulant mass is loosened up and easy to remove from membranes but stuck in spacers. Cleaning chemicals seem loosen up the foulant but more intensive rinsing and possibly some other methods to increase stress to fouling layer are needed to remove foulant from membrane surface and spacers. In this first cleaning, negative effects, negative effects from C or D agents were not seen.

12 Waste streams

Waste streams produced by pilot unit were evaluated after each pilot filtration. Membrane based enzyme recovery process produces quite lot waste water (UF permeate after concentration of product and rinsing waters form washing of the unit). Other waste fraction produced is waste slurry/paste of used fermentation broth (MF retentate). Basic mass balance of unit is presented in Figure 37.

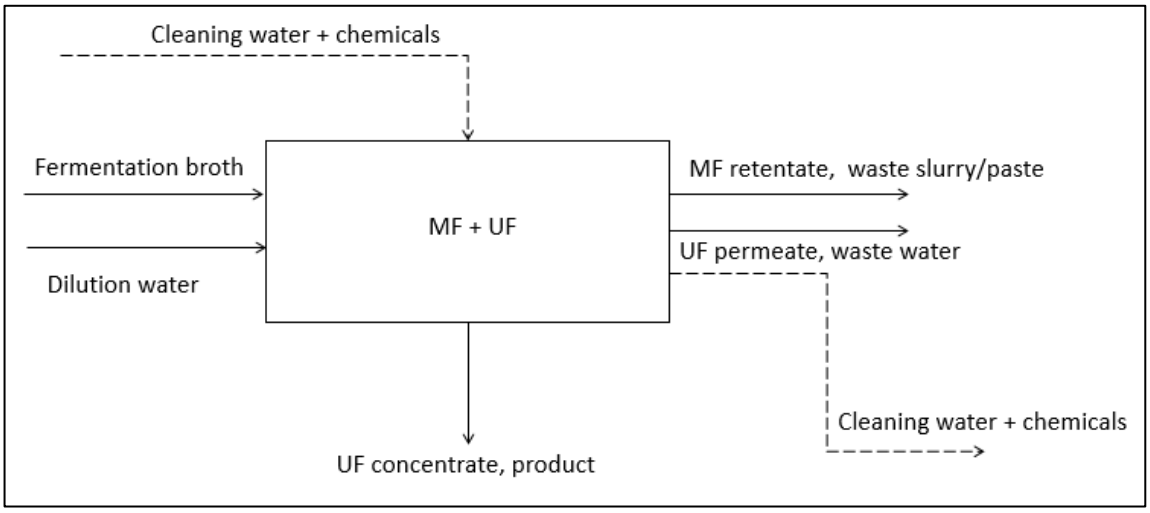

Figure 37 Basic mass balance of MF & UF unit during filtration (solid lines) and cleaning (dashed line).

Waste streams contain high concentrations of COD and P which would increase costs and limit waste water disposal options. To improve process economy new side products and end uses for wastes should be researched. Figure 38 presents one option for enhancing waste utilization. Waste slurry of MF could be dewatered and used for example in energy production. Recovery of phosphorus from waste waters could bring benefits as it could be sold for example to fertilizer production or agricultural end users.

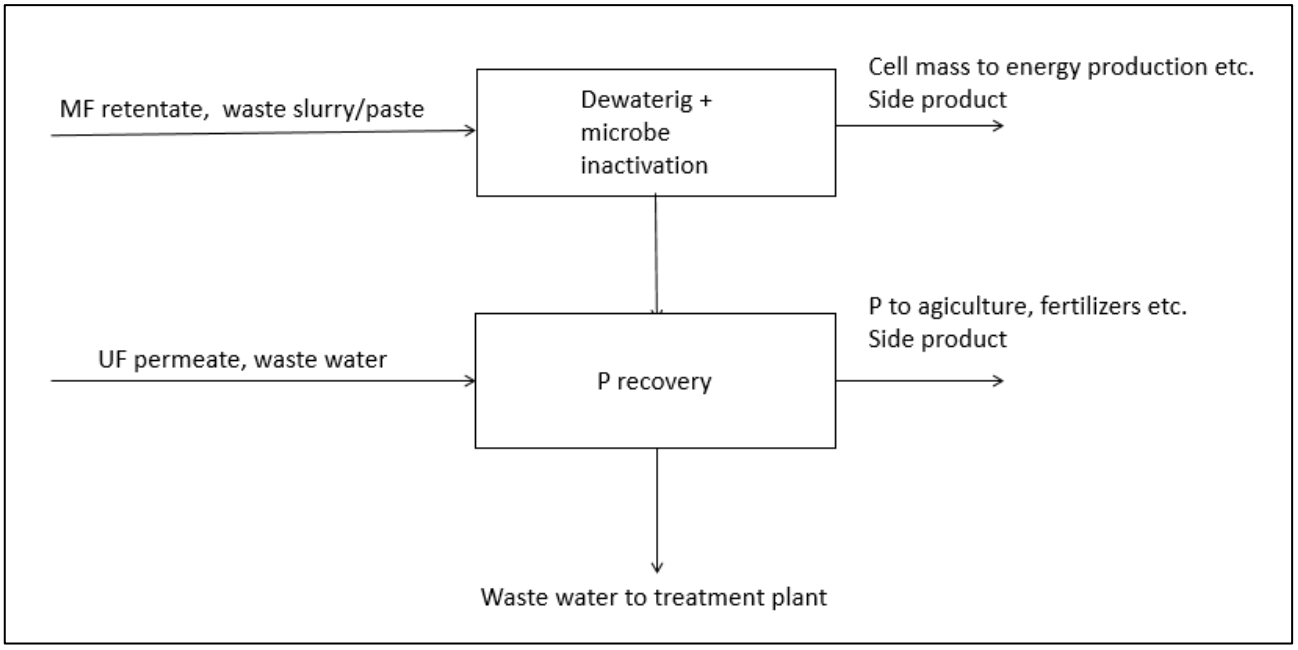

Figure 38 One possibility to improve process economy by collecting and reusing waste streams as side products.

13 Conclusions

Purpose of this thesis was to study ways to improve applicability of microfiltration for enzyme recovery. Focus areas in experimental part were chemical and enzymatic cleaning (backpulsing or other mechanical methods were not included) and comparison between two different spacer sizes (small and large) for enzyme recovery. Experiments were made in laboratory and pilot scale. In addition, methods for utilization and disposal of waste streams from membrane-based enzyme recovery process are considered.

Screening for enzymes and chemicals for membrane cleaning was made in laboratory. Results showed that for the tested fouled membranes commercial cleaning solutions were more effective than enzymes from Genencors own production. Enzyme doze was high, but results were still lower than those obtained with relatively dilute chemical solutions.

In laboratory and pilot cleaning experiments with old membranes, cleaning solutions C (alkali) and D (oxidizing) showed some promise. Products are not designed for membranes and could cause problems if used in long run. Both could be utilized as emergency options or similar products better suited for membranes could be interesting.

Based on laboratory experiments solution B (enzymatic) was included to cleaning protocol instead of enzyme mix used before in pilot cleanings. B solution seemed to help bit, but cleaning success still varies quite lot for different runs and feeds. Sufficient rinsing and stress to the foulant layer seem to be important factors for cleaning as well as cleaning agents used. With biological and varying feeds variation in cleaning results is something that must be accepted, and managed, also regular replacement of membranes is to be expected. Cleaning of S spacer membrane does not cause additional problems compared to L spacers.

S spacer membrane doesn’t seem beneficial. Even if unit capacity increases with additional membrane area enzyme passage and flux decline. This could be due changes in flow hydrodynamics caused by thinner feed spacers and channel height.

Still filtrations with Products A and B show some potential. With fully conditioned membrane and well optimized filtration parameters for high passage and enzyme flux it could be possible to get more enzyme recovered with S membrane. More testing is needed to reach full potential of process (also for L membranes). Also, testing for differences in spacer geometry and feed channel free volume could be done to find explanation in differences for enzyme separation.

Membrane process in enzyme recovery produces lot of waste waters and cellular waste mass. To improve process economy new ways to treat and utilize wastes should be found. Literature part of this work presents options for waste dewatering and utilization of biomass. For example, different types of filter presses combined with heat could be useful. Biomass could be utilized for example in energy production. From waste waters phosphorus recovering could be interesting option. Phosphorus is nonrenewable material used for example in fertilizer production.

Microfiltration is suitable option for enzyme recovery. Problems with low passage for some enzymes and membrane fouling limit applicability of process. Membrane process also produces large amounts of waste slurries and water which increase costs of operation as dewatering and waste water treatment are needed.

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