PhD thesis: Bio-Hybrid Membrane Process for Food-based Wastewater Valorisation: a pathway to an efficient integrated membrane process design

Summary

The aim of this PhD is to develop **integrated membrane process** through individual investigation of various membrane operations for agro-waste valorization. Special emphasis is given to addressing **problems of membrane fouling** and **periodic discharge of huge volume**. A bold step has been taken to address problems of membrane fouling from a real industrial effluent i.e. an oily emulsion with a complex organic matrix using **biocatalytic membrane reactors**. A new method of immobilizing enzyme on the membrane surface using the concept of **superparamagnetism** have been developed, evaluated and optimized. The newly developed stimulus-responsive biocatalytic membrane reactor proved successful in **in-situ membrane foulants degradatio**n and **hydrolysis of lignoceluloisic biomass**. So it opens new horizon for industrial production, processing, environmental remediation or bio-energy generation. A suitable chemical cleaning condition for an emerging membrane type i.e. magnetic responsive membrane is established. FO process was evaluated to reduce total processable volume of vegetation wastewater.

State of the art

The **food industry** is by far the largest potable water consuming industry that releases about 500 million m^3 of wastewater per annum with very high organic loading [1]. Simple treatment of this stream using conventional technologies often fails due to cost factors overriding their pollution abating capacity. Hence, recently the focus has been largely centered on **valorization** through combinatorial recovery of valuable components and reclaiming good quality water using integrated membrane process. Membrane processes practically cover all existing and needed unit operations that are used in wastewater treatment facilities. They often come with advantages like simplicity, modularity, process or product novelty, improved competitiveness, and environmental friendliness.

Thus, the main focus of this PhD thesis is development of integrated membrane process comprising microfiltration (MF), forward osmosis (FO), ultrafiltration (UF) and nanofiltration (NF) for valorization of food based wastewater within the logic of zero liquid discharge. As a case study, the huge volume of foul smelling acidic dark liquid generated during the extraction of olive oil is taken. **Olive mill wastewater (OMWW)** is one among the numerous end-of-pipe treatment needing wastewaters very well known for its significant negative impact on the environment [2]. It is rich in biophenolic compounds that exhibit both **phytotoxicity and pharmacology** interesting bioactivities [3, 4]. Based on an extensive investigation of research articles and patents over the last 20 years, a paradigm shift from simple detoxification to valorization based on used treatment strategies was observed. Clear time line in the main strategies followed to solve OMWW related environmental pollution is plotted (Figure 1). Progressive development and significant rise in the use of integrated membrane process showed a huge potential for combined wastewater treatment and co-product valorization. The **successive integration** is observed to benefits from step-by-step pollutant removal, fractionation and concentration of the recovered biophenols. The literature study also showed that the most significant issues of integrated membrane process

that limited its **industrial scale** applications are: membrane fouling, pretreatments, consideration of membrane material, modules, process design and process economics, which all together forms the pillar for future developments within this field. Over all detailed analysis of the research articles and **patents**, revealed the main reasons why research efforts are not matching industrial practice and what could be done to alleviate these problems, so as to convert a recalcitrant wastewater to a vital alternative resource.

Problem statement

The challenges associated with the treatment of OMWW are: **absence of unique hydraulic** or organic loadings, **presence of biophenolic** compounds, **sever membrane fouling** specially at the start of the integrated scheme and periodic release of **large volume of wastewater**. Especially, presence of biophenolic compounds makes the wastewater detrimental to the environment. However, recovering these phytotoxic compounds can also add economic benefit to the simple treatment since they have interesting bioactivities that can be exploited in the food, pharmaceutical and cosmetic industries.

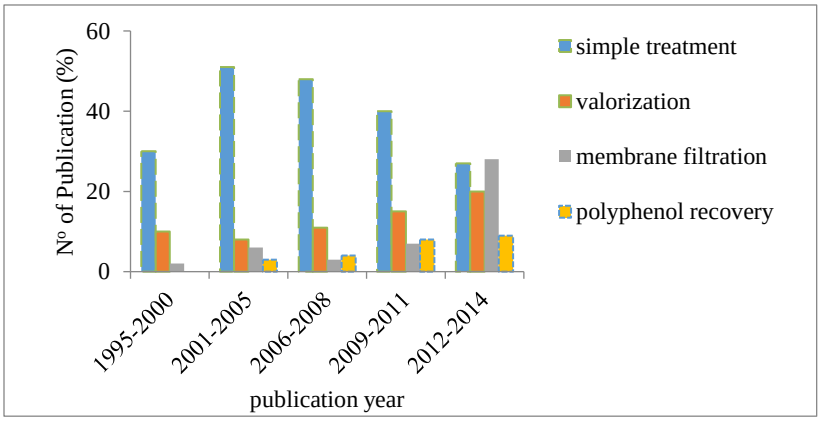

Figure 1: Time line in OMWW treatment obtained through analysis of published research articles (source: http://www.sciencedirect.com) from 1995 to August 2014 and patent publication from 1992 to 2013 (source: http://worldwide.espacenet.com.

Result and discussion

The first part of the experimental work gives special emphasis on the development of **biohybrid** membranes used to control membrane fouling during MF of OMWW.

Prior to filtration of OMWW, the hydrodynamic mixing and flow pattern of the lab-scale membrane module was characterized using the concept of **residence time distribution** analysis (RTD). The modules, which contains 7 polyethylene (PE) hollow fiber (HF) membranes with 0.4 µm pore size and internal/external diameter of 0.41/0.65 mm, were prepared by potting the HF membranes in a glass vessel with epoxy resin. The hydrodynamic study revealed that the module have well mixed conditions in the 95% of its volume.

Subsequently, OMWW that was pre-filtered using 35 µm mesh screen, were micro-filtered through the PE HF membranes under different operating conditions. Regardless of 99% TSS removal with rough filtration, continuous MF of OMWW over 24 h resulted in continuous **flux decline**. This is due to sever membrane fouling mainly caused by macromolecules like **pectins**.

To overcome the problem of membrane fouling, 500 mL of pre-filtered OMWW was kept in contact with the enzyme pectinases at three different enzyme concentrations to hydrolyze the pectin at 40°C and pH 4.2 (optimal condition for the enzyme) under mild stirring for 5 h. However, MF of the resulting mixture at 36 cm/s crossflow velocity and 0.1 bar transmembrane pressure (TMP), did not result in any improved flux. Kinetic studies of OMWW hydrolysis in a stirred tank reactor (STR) revealed that the enzyme pectinase is prone to enzyme product-inhibition, which explained why the flux did not improve while using the **free enzyme membrane bioreactor** (MBR). Such problem can mainly be resolved by using immobilized enzyme membrane reactor. Immobilization of enzymes on membranes to form biocatalytic membrane reactors (BMRs) is a typical example of **process intensification** which aims at hybridizing two or more traditional operations to make industrial production more efficient [5], since the coupling lowers chemicals and energy consumption, while increasing reaction yields and minimizing waste [6-8]. BMRs have been applied in different sectors e.g., in the pharmaceutical industry [9, 10], in the production of bio-renewables [11], and in waste valorisation [12].

As a proof-of-concept, a biocatalytic membrane reactor (BMR) with covalently immobilized pectinase on the HF membrane was used to develop ***self-cleaning*** **MF** membrane. Unlike the general trend observed in literature, all experiments took the bold step of realizing enzymatic membrane reactor on a real OMWW. The biocatalytic membrane with pectinase immobilized on the surface of the HF membranes gave a 50% higher flux compared to its counterpart inert membrane. This better performance was attributed to simultaneous *in-situ* foulant degradation of and removal of hydrolysis products from reaction site that overcome enzyme product inhibition. For all types of used membrane systems, analysis of cake resistance, membrane resistance and membrane fouling index indicated that the **biocatalytic membranes** exhibited the least fouling tendency.

Membranes with either physically or covalently attached enzymes have long been investigated but still face many problems [13]. Enzyme immobilization via physical adsorption on the membrane surface requires retentive surfaces in order to avoid enzyme leakage through membrane pores [14]. Such required enzyme retention lowers the degrees of freedom to optimize the membrane performance with respect to creating high fluxes and the passage of desired components. Immobilizing enzymes on larger retainable carrier particles, such as alginate beads, also poses serious problems of isolating and recovering them from other retained components with possibly similar particle size. **Covalent immobilization** on the membrane is not conceptually attractive, since in a prolonged run, membrane fouling and enzyme activity loss demand chemical cleaning of the membrane and enzyme replenishment, respectively. The ultimate fate of such membranes would then be disposal, since detaching the inactivated enzyme to regenerate the membrane would be difficult, if not impossible. Hence, a novel immobilization technique that fulfils the conditions of the BMRs, but still facilitates membrane chemical cleaning and enzyme renewal is urgently needed.

To surmount this problem, a **new class of superparamagnetic (BMR^{SP})** was developed, verified and optimized. This development is novel for its use of superparamagnetic nanoparticles (NP^{SP}) both as (1) support material for enzyme immobilization to form an enzyme immobilized superparamagnetic particles (Enz^{SP}) and (2) as fillers in an organic inorganic (O/I) hybrid membrane to form a superparamagnetic membrane (M^{SP}). Both the Enz^{SP} and the M^{SP} thus exhibit superparamagnetism which allows the Enz^{SP}, initially homogeneously dispersed in the

bulk reaction mixture, to be attracted onto the M^{SP} surface. The synergies of the magnetic responsive M^{SP} and the Enz^{SP} enhance the formation of a dynamic layer of Enz^{SP} on the M^{SP} surface. More specifically, by applying an external magnetic field parallel to the surface of the M^{SP}, the particles dispersed in the bulk stream will align parallel to the applied magnetic field [15-17]. The relatively **stronger magnetic force** at the surface of the membrane due to the presence of M^{SP}, creates a magnetic field gradient that helps in relocating the NP^{SP} in the liquid to the surface of the membrane (Figure 2). Since the north and south poles of the NP^{SP} attract each other, they align the NP^{SP} and form chains. On the contrary, particles coming close to each other with the magnetization direction parallel will repel each other, thus leaving spaces between the aligned nanobiocatalysts. **The dynamic layer** of Enz^{SP} can thus be considered as a specific reactor resulting from an array of **microreactors** formed by the voids between the nanoparticles connected by magnetic forces. The M^{SP} thus serves as a magnetic field actuator, i.e. create a localized magnetic field, while also helping in a magnetophoretic dispersion of the NP^{SP} on and near the membrane surface.

Their superparamagnetic properties can again be employed later on to **recover the Enz^{SP}** from the M^{SP} surface whenever the M^{SP} has for instance been severely fouled and needs chemical cleaning, or when the Enz^{SP} has been denatured, by simply switching off the external magnetic field and a subsequent mechanical stirring. This novel approach, BMR^{SP}, offers a **paradigm shift** in addressing two of the most critical bottlenecks of BMRs currently hindering their widespread use: easy recycling of the enzyme, and extending the membrane working life cycle beyond the enzyme's active period. Similar to direct onto membrane immobilization, the enzyme immobilized on NP^{SP} will indeed be denaturized in time. In this case, the membrane remains intact, as both denaturation and oversaturation effects are mostly restricted to the dynamic Enz^{SP}/NP^{SP} layer. The novelty of the current system also is the ease to remove the denatured Enz^{SP} and re-supply with fresh Enz^{SP}, while direct onto membrane immobilization forces one to dispose a full membrane module.

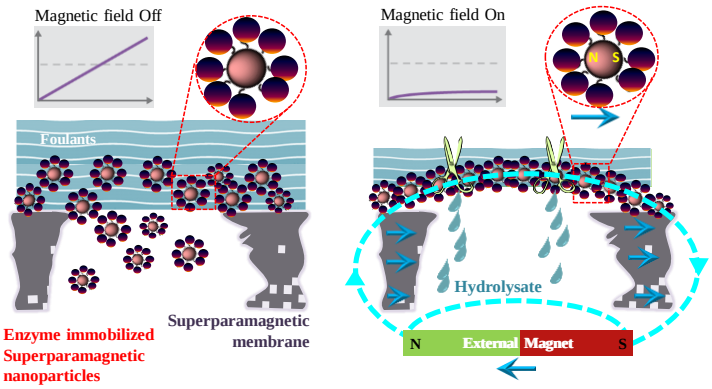

Figure 2: Permeation of an 8 nm NP^{SP} through 0.2 µm diameter pores of an M^{SP} (a) in the absence of an external magnet and (b) in the presence of an external magnet.

To the best of our knowledge, **this is the first work** that employed this novel concept in a BMR. As a proof-of-concept, the BMR^{SP} is applied in an *in-situ* enzymatic membrane cleaning for a pectin/pectinase system, as encountered in a typical vegetation wastewater. It was also tested in an arabinoxylan/xylanase system, as typically used in bio-ethanol production or present in

brewery and bakery wastewaters[18]. Ultimately, the effect of using a superparamagnetic enzyme mixture is also evaluated.

Firs the membrane preparation (M^{SP}) and enzyme immobilization on the NP^{SP} were optimized. To prepare the M^{SP}, dope solutions containing 12% PVDF in DMF were mixed with variable polyethylene glycol (PEG) coated 8 nm diameter NP^{SP} concentrations (0.08 to 0.5 w/w %). For enzyme immobilization instead of PEG, (3-aminopropyl) trimethoxysilane was used to introduce reactive amine groups on the surface of the NP^{SP}. Both particles exhibit an average particles size of 8 nm. A detailed description of the preparation and characterization of both the PEG coated and the aminated NP^{SP} is given in [19].

All **enzyme immobilizations** were performed using glutaraldehyde as a crosslinker. A 2.4 mg of aminated NP^{SP} was mixed with 0.8 mL of a 25% aqueous glutaraldehyde solution and stirring for 2 h at room temperature to introduce a terminal aldehyde functional group to the NP^{SP}. The aldehyde derivatized NP^{SP} were then mixed with different concentrations of pectinase or endo-β-1-4-D-xylanase dissolved in a sodium acetate buffer for 10 h to form enzyme immobilized NP^{SP} (Enz^{SP}).

The specific **enzyme loading capacity** of the NP^{SP} ranged from 215 to 218 mg per gram of NP^{SP}. The covalently linked pectinase retained 70±7% of the specific activity of similar amount of free enzyme, as measured using a 5 mL of 4 mg/mL pectin solution at 37°C in an STR. Due to their superparamagnetic properties and the enhanced stability, it was possible to use the immobilized enzyme over 10 repeated cycles in an STR with a slight performance loss over time. The slightly decreased activity after 10 cycles can be due to particle aggregation, particle loss during the multiple step wash and may be enzyme deactivation upon extensive shaking [20].

To evaluate the effectiveness of the BMR^{SP} system, the M^{SP} prepared from 0.33 w/w % NP^{SP} (highest flux and lowest fouling propensity) were selected. The Enz^{SP} were confined on the surface of M^{SP} using three sets of Neodymium magnets each with 24.4 kg pull capacity, which were located at 5 cm below the bottom support of the dead-end membrane module (outside the filtration cell). As a control, inert NP^{SP}, i.e. without immobilized enzyme, were dispersed on a similar membrane in a parallel experiment. The system was operated in a continuous mode at a constant reactor volume of 500 mL and feed concentration of 0.3 mg/mL on 0.0079 m^2 membrane area. As a proof-of-concept, the system was operated under **accelerated fouling** conditions, presuming a worst case scenario, by fixing the flux at 17 $L/m^2.h$, which is well above the critical flux of 10 $L/m^2.h$. Permeate recirculation was avoided deliberately to prevent **product inhibition** of the enzyme [21]. Fouling resistance, manifested as a rise in TMP, was used to evaluate the system efficiency. An interesting phenomena here is, the hydrolysis product in the permeate are high added value component, which can be valorized for instance to **bioethanol**. Permeates were thus sampled, and later analyzed to assay the BMR^{SP} productivity.

The novel BMR^{SP} system described here resulted in up to **75% reduction in membrane filtration resistance** through the membrane surface cleaning action of the Enz^{SP} (Figure 3).

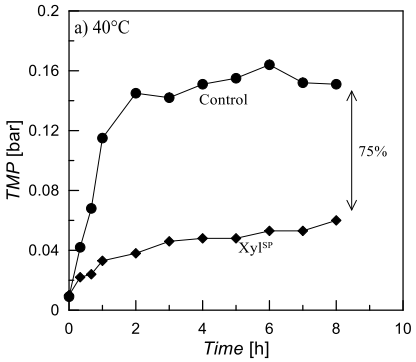

Figure 3: Performance difference between neutral MF and BMR^{SP} at 17 L/m^2 h flux, 0.3 g/L arabinoxylan and at 40^{0}C.

The **repeated use stability** of both immobilized enzymes in a BMR^{SP} during continuous filtration of 0.3 mg/mL arabinoxylan also gave an interesting performance. After 8 h of continuous filtration and foulant deposition, the TMP was limited to 0.1 bar during the 1st and 2nd cycle filtration with a slight performance loss over 4 cycles. This performance loss over cycle number could be correlated to the occurrence of local structural changes in the immobilized enzyme, resulting in a regional collapse and compaction of the Enz^{SP} layer [22]. Moreover, the system is operated in the supercritical flux regime, where mass transfer rate might prevail over reaction rate. Consequently, the enzymes might get oversaturated with foulants and might become isolated from newly arriving foulants. Yet, the observed TMP was low enough to enable an 8 h continuous filtration over 4 consecutive cycles without a major hurdle.

The possibility to mix different enzymes either coated as an enzyme mixture per nanoparticle or combined as a **mixture of single enzyme** nanoparticle for consolidated bioprocessing, also offers ample opportunities. Hence, a blend of xylanase and pectinase immobilized Enz^{SP} was used to form a mixed dynamic layer of Enz^{SP} on the membrane. Effecting multiple enzymatic reactions in a single stage BMR by using a blend of different Enz^{SP} could actually facilitate **process intensification** by avoiding the need for multistage BMRs e.g. to treat fouling of mixed wastewaters, to enzymatically hydrolyse complex sugars [23, 24], to enhance the efficiency of an enzyme by the *in-situ* generation of its substrate, to remove undesired by-products of an enzymatic reaction etc [7]. Here again, the filtration resistance of the BMR^{SP} fed with 50:50 mixture of pectin and arabinoxylan were significantly lower than a parallel control system. The proposed BMR^{SP} concept thus also proved successful in tackling complex foulant mixtures.

In membrane operation, in general, the necessity to fix flux well below the critical flux in order to limit fouling might make processes economically not viable [25]. In the present system however, due to the **effective *in-situ* foulant degrading** capacity of the Enz^{SP}, and the simultaneous permeation of hydrolysis to avoid enzyme-product inhibition [21, 26], it was possible to operate the system well above the critical flux without facing severe filtration resistance.

Moreover, the stability of the BMR^{SP} was investigated over a broad range of operational conditions: 0.01-3 g/L foulant concentration, 1-9 g per m^2 of membrane area Enz^{SP}, 5-45 L/m^2.h flux and different filtration temperatures. A much higher biocatalytic efficiency was obtained in the BMR^{SP} than in a batch reactor, ascribed to an absence of enzyme-product inhibition and a high mass transfer rate induced by the convective flow through the biocatalytic bed. When the feed concentration increased by a factor of 300 (0.01 to 3 g/L), the rate of fouling only increased by a factor of 8.

When the system was operated under mass transfer rate balanced by reaction rate, it exhibited **no significantly increase TMP, activity decay or enzyme leakage** during a 200 h lasting continuous filtration (Figure 4).

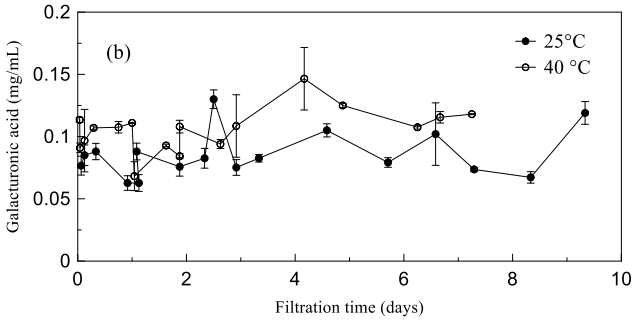

Figure 4: Constant productivity of BMR^{SP} continuously operated over 10 days at constant flux, feed concentration and feed volume.

In conventional BMRs e.g., through **covalent enzyme immobilization**, the enzyme loading capacity of the membrane is limited by the available surface area. Formation of multi-layers of enzyme results in cross-linking of the enzyme, causing molecular crowding[27]. In the current system however, in order to increase the amount of enzyme on the surface of the membrane, the amount of Enz^{SP} was increased. By increasing the amount of Enz^{SP}, it was possible to pack a multilayer of enzyme with a **better distribution** i.e. increase the surface area for enzyme immobilization over a given membrane area. As a result, an increase in the **reactor productivity** was achieved by increasing the enzyme loading. This further allowed the BMR^{SP} to hydrolyze higher loads of foulants while keeping a low if not zero increase in TMP over time at constant flux.

Overall, physical localization of a nanoscale Enz^{SP} on the M^{SP} surface, despite the unfavorable difference in pore and particle size, in a reversible superparamagnetic way can **out-compete** currently employed approaches in BMRs. This offers an important additional flexibility to the proposed membrane-cleaning strategy, since it does not require retentive surfaces and can then even be operated with highly porous membrane surfaces allowing sustainable operation at higher fluxes.

In addition to reducing fouling propensity, both Enz^{SP} and NP^{SP} dynamic layers prevent direct membrane-foulants interaction, hence serving as an additional **anti-fouling coating** on the membrane which re-disperses a possibly formed cake layer at the moment the Enz^{SP} or NP^{SP} are being re-dispersed. The observed immobilized enzyme and membrane stability, together with the high reusability suggests an estimated wide use of this BMR^{SP} in the production of bulk chemicals, pharmaceuticals**, bio-based commodities** and biodiesel in near feature [7, 9]. The BMR^{SP} concept as a whole can be employed to “re-engineer” not only membranes, but also any fouling prone surface.

Comparison between chemical and enzymatic cleaning shows that, cleaning once fouling of the membrane has become severe is less effective than continuous maintenance cleaning through *in-situ* degradation which prevents incipient fouling. Nevertheless, in many cases no single cleaning reagent will recover the flux entirely, and it is often advantageous to use a combination of cleaning reagents in sequence.

While using enzymatic *in-situ* degradation of foulants to keep sustainable membrane productivity over long-term, there may be a need for **periodic chemical flushing**. This may be important

especially to remove partially permeable hydrolysis products that are trapped inside the membrane pore structure. However, as an emerging field of research, the knowledge regarding the hypochlorite cleaning effect on M^{SP} is unavailable. Hence, a comprehensively quantification of the effects of **hypochlorite exposure** on changes in the physico-chemical characteristics, hydraulic permeability and fouling propensity of the M^{SP} was investigated. The ageing caused change in the physico-chemical characteristics and enhanced fouling propensity of the membrane due to step-by-step degradation of the polymeric coating layer of used NP^{SP}. Yet 400 ppm NaOCl solution at pH 12 was less detrimental to the overall membrane integrity. Hence, hypochlorite cleaning under these conditions can be used to give short back-flush, after magnetically removing the Enz^{SP} from the surface of the membrane.

Second major limitation identified during the treatment of OMWW is presence of **large volume** of wastewater that comes in short period following the harvest of olive fruit. To alleviate this problem, FO was investigated to concentrate the wastewater. This process is believed to be less energy demanding, suppose that draw solution does need to be regenerated, and with low foul propensity. By operating at 3.7 molal $MgCl_2$ draw solution and 6 cm/s crossflow velocity, single-step FO resulted in an average flux of 5.2 kg/m^2.h. and **71% volume concentration** factor with almost **complete retention** of all the pollutants. Moreover, the system gave a stable performance over ten days when operated continuously. After FO, both NF and UF were used to fractionate the **recovered biophenols** from the concentrate streams of FO. Compared to polymeric UF membrane, ceramic NF gave better flux of 27 kg/m^2.h at 200 L/h feed flow rate and 7 bar TMP. Finally, when FO was used as a final polishing step to recover highly concentrated biophenols from permeate of the UF; it gave an average flux of 5 kg/m^2.h and VCF of 64%.

In conclusion, a great success has been made in tackling the two most important challenges of vegetation wastewater valorisation using the concept of **biohybridization and FO**. The bio-inspired NP^{SP} provides strong evidence that magnetically controlled enzyme immobilization have an immense potential in membrane fouling prevention and paves a potential breakthrough for continuous wastewater filtration. By setting bio-inspired NP^{SP} biocatalytic membrane reactor at the heart, it is possible to successfully use **integrated membrane process** for continuous valorisation of food based wastewater. In addition to fouling prevention, they open a new horizon for applications in localized biocatalysis to intensify performance in industrial production, processing, environmental remediation or bio-energy generation.

Based on investigation of individual units throughout this PhD thesis, the following integrated membrane schemes are suggested (Figure 5):

1. When the wastewater has high suspended solids as well as high organic content (fouling propensity), rough filtration, biocatalytic MF, NF can be the main integration with FO being used as a final polishing step to obtain highly concentrated biophenols, e.g, from permeate of UF (option 1), or eventually it can also be used subsequent to MF (option 2) of Figure 5.
2. When the wastewaters' macro-pollutant load is limited, FO can precede MF/UF/NF and can also be used as final polishing step to get more concentrated biophenols (Figure 5: option 3).
3. When the feed wastewater has no enough biophenols to encourage recovery, one can envisage on the use of FO to concentrate the stream. This will eventually reduce the total processable volume thereby reducing the need for large onsite and offsite storage facilities and transportation to offsite treatment facilities. Most olive oil production centers are located along the sea side. Since FO process is able to retain more than 98% of all the

pollutants, it is possible to use the FO membrane as barrier to retain pollutants while letting the purified water diffuse into the seawater. The seawater will serve as the source of osmotic driving force as shown in option no 4 of Figure 5.

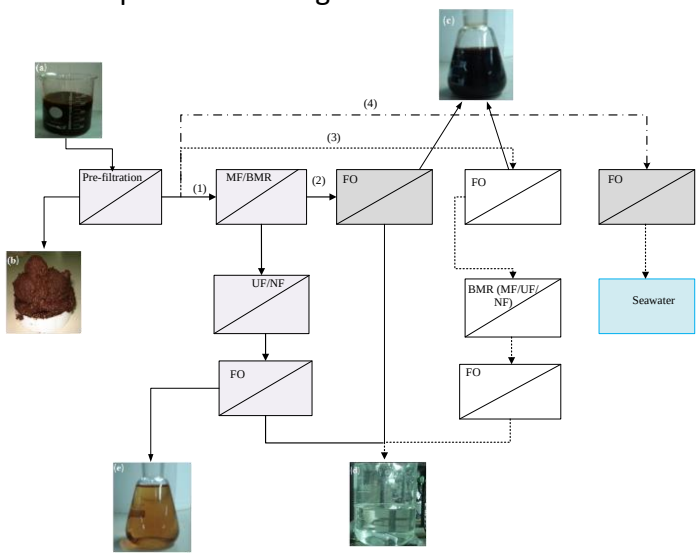

Figure 5: Suggested possible integrated membrane process based on results of individually investigated membrane operations, where a) feed water, b) sludge after screening with 35µm stainless steel wire mesh, c) FO concentrated biophenol and d) permeate of FO.

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