Investigation of Hybrid Membrane System for Purification of Wastewaters from the Olive Industry with Simultaneous Recovery of Valuable Components

Report to the
Water Research Commission

by

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EXECUTIVE SUMMARY

This report describes laboratory work performed on the development of a hybrid membrane system for the treatment and simultaneous recovery of value-added products from wastewaters generated by the olive industry. These wastewaters are produced during table olive and olive oil processing in large quantities, especially so in the case of table olive brining and fermentation (this can consume up to 20 m^3 of water per ton of olives processed). The wastewaters present an environmental disposal problem due to their high organic load and high phenolic content.

The hybrid membrane system was comprised firstly of an ultrafiltration (UF) membrane to separate a valuable low molecular weight phenolic compound, called hydroxytyrosol (HT), from higher molecular weight phenolic compounds (tannins, etc.). Secondly, a nanofiltration (NF) system was investigated in order to concentrate the HT, and to separate it from salts, organic acids and water, which could then be recycled. Hydroxytyrosol is a powerful natural antioxidant with many potential uses in the pharmaceutical, food and beverage, and cosmetics industries.

The aims of the UF experiments were three-fold: firstly, to investigate the fouling potential of the wastewaters on membranes of different molecular weight cut-offs; secondly, to investigate the separation capabilities of these membranes upon the various chemical species that occur in the wastewaters; thirdly, critical flux and backflushing experiments were performed in order to investigate long-term operating potential of the system.

Although, as would be expected, fouling occurred in all the UF experiments, membrane flux was restored completely after chemical cleaning. The UF system was found to be able to successfully separate HT from the higher molecular weight compounds. Critical flux was not encountered up to the maximum operating pressure of the membrane. A backflushing regime was devised that allowed for extended operation in between chemical cleaning. Estimates of volumetric and product yields were made from results of the extended backflushing experiments.

The primary objective of the NF experiments was to investigate the separation capabilities of components of the permeate that would be obtained from the UF system. Fouling in the NF system is not an issue as the majority of fouling components in the wastewater (polymeric phenolics) are removed by the UF step. The critical issue in the NF step is retaining and concentrating the HT on the upstream side of the membrane, while passing salts through the membrane with the recovered (purified) water stream, such that this can be recycled back into the olive production process.

The NF experiments were only partially successful. In the best case, only around 50% of the HT was retained, while salt passage through the membrane was around 80%. It is therefore necessary to consider re-arranging the overall process: after UF, HT in the permeate should be extracted before the NF is applied to produce recyclable process water.

Some preliminary chromatographic purification experiments were performed on the UF permeate. Hydroxytyrosol yield and purity of these experiments was high (84 and 95% respectively), giving a basis for estimates from scaled up systems. Nanofiltration of the wastewater obtained after chromatographic purification has not yet been investigated: it needs to be determined whether the purified wastewater produced by this step will be suitable for recycling into the olive fermentation process.

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1. INTRODUCTION

The olive processing industry generates large quantities of noxious wastewater during the production of table olives and olive oil. South Africa, although still a minor player in the global olive industry, currently produces around 1 million litres of olive oil and 1000 tons of table olives annually. This accounts for less than 1% of global production, however the industry is burgeoning. There is a concomitant production of millions of litres of wastewater; accurate estimations of the total quantity produced are difficult because it is not closely monitored.

These wastewaters are characterised by a dark black colour, high organic load (chemical oxygen demand, or COD), high phenolic content, acidity, and in the case of table olives, high salinity; and are thus not easily biodegradable or recyclable. Due to these properties and the scale of production, such wastewaters are considered to be the most problematic of agro-industrial effluents, particularly in the Mediterranean regions, and have been the subject of extensive research over the past few decades (Brenes *et al*., 1995; Hafidi *et al*., 2005; Hamdi 1993; Lesage-Meessen *et al*., 2001).

Olives are generally grown and processed in arid Mediterranean-type climates, where clean potable process water is in short supply. Thus it is desirable to recycle wastewater or re-use it for other purposes; this would help to minimise local water demand while at the same time alleviate the troublesome wastewater issue. The problem is, however, not trivial due to the complexity of the wastewater. The table olive wastewaters, for example, contain 10-18% salt and are acidic, therefore they cannot be used for irrigation even if all the toxic organic components are removed. Currently these wastewaters are generally disposed of in evaporation ponds.

The phenolic component of the olive wastewater is predominantly responsible for the high organic load and resistance to biological degradation, but is of particular interest. The phenolics can be broadly separated into two fractions: a high molecular weight polyphenolic fraction (> 1 kDa) comprised of condensed tannins, lignans and related humic-like compounds, which are responsible for the dark colour of the wastewaters; and a lower molecular weight fraction comprised of mainly monomeric compounds.

The principal low molecular weight monomeric phenolic compound in the wastewater is hydroxytyrosol (3,4-dihydroxyphenylethanol, or HT). This compound is one of the most powerful known natural antioxidants, and has been the subject of much biochemical and pharmaceutical research due to its diverse biological properties (Bazoti *et al*., 2005; Bitler *et al*., 2007; Covas *et al*., 2006; Mateos *et al*., 2005; Obied *et al*., 2007; Rietjens *et al*., 2008, Romero *et al*., 2007; Schaffer *et al*., 2007; Visioli *et al*., 1999; Visioli *et al*., 2001; Walter *et al*., 1973), see Table 1.1. Hydroxytyrosol and related phenolic compounds have been shown to be the main biologically active components responsible for the beneficial properties of olives and olive oil (and their long shelf-life), and have been implicated in the low incidence of cardiovascular disease and cancer in Mediterranean countries where these are habitually consumed in the diet (Fitó *et al*., 2005; Tuck and Hayball, 2002).

Hydroxytyrosol is of high market value as a pure chemical compound (~US$1000/g, see http://www.caymanchem.com and http://www.extrasynthese.com), and thus offers the possibility of being a highly valuable by-product obtainable from the olive wastes. Recovery of this compound from wastewaters that arise from the production of olive oil has been extensively investigated; however, recovery of this compound from table olive wastewaters has received little attention, because most olives in the world are pressed for oil (~90%). Previous research has shown that HT occurs in fermentation brines at levels of 1-2 $g.L^{-1}$; one of the larger local table olive producers discards at least 500 000 L of this primary wastewater per annum, which illustrates the opportunity for beneficiation. A

HT extract obtainable from these wastewaters has significant potential for use in the food, beverage, cosmetic, or pharmaceutical industries as a natural, biologically active component and antioxidant.

Table 1.1: Pharmacological and biochemical research on HT

Description	**Reference**
Radical scavenging/antioxidant activity	O'Dowd *et al*., 2003; Moreno, 2003; Tuck and Hayball, 2002; Mc Donald *et al*., 2001; Espin *et al*., 2001; Visioli *et al*., 1999, 1998; Baldioli *et al*., 1996.
Antiviral activity	Micol *et al*., 2005; Lee-Huang *et al*., 2003.
Antibacterial (mycoplasmal) activity	Furneri *et al*., 2004, 2002; Capasso *et al*., 1999.
Anti-inflammatory effects	Miles *et al*., 2005.
Melanoma/ultraviolet irradiative protection	D'Angelo *et al*., 2005.
Erythrocyte (red blood cell) oxidative protection	Manna *et al*., 1999.
Lymphocyte (white blood cell) auto-immune response	Palmerini *et al*., 2005.
DNA oxidative protection	Quiles *et al*., 2002; Armstrong *et al*., 1997.
Prostate/urinary anti-mutagenicity	Malaveille *et al*., 2004; Quiles *et al*., 2002.
Colon anti-mutagenicity	Della Ragione *et al*., 2000.
Inhibition of LDL oxidation (arteriosclerosis)	Covas *et al*., 2000; Visioli *et al*., 1999; Aruoma *et al*., 1998.
Metabolism regulation	Polzonetti *et al*., 2004.
Alleviation of heavy metal intoxication	Casalino *et al*., 2002.
Nerve cell oxidative cytoprotection	Hashimoto *et al*., 2003.

The application of membrane systems to treat or fractionate olive wastewaters has been investigated by several researchers (Borsani and Ferrando, 1996; Dhouib *et al*., 2006; Paraskeva *et al*., 2007; Rautenbach and Mellis, 1995; Russo, 2007; Turano *et al*., 2002). This research is generally related to the treatment through concentration and purification of the wastewaters. However Russo (2007) describes a process for recovery of HT from 3-phase olive mill wastewaters, comprised of microfiltration, followed by ultrafiltration (UF) and reverse osmosis. Results from this system are impressive, but the olive mill wastewaters are becoming less of an issue as there is a general trend towards using the new 2-phase centrifugation process for oil production; this generates very little wastewater. The application of membrane systems to table olive fermentation brines has received little attention, and therefore was the focus of this current work.

Previous research (Burton *et al*., 2006; Garcin and Burton, 2007) describes the use of a novel membrane-assisted solvent extraction (MASE) process for the recovery of HT from the wastewaters. The process for producing table olives and olive oil, and concomitant generation of wastewaters is described in these reports. The MASE process was successful, but had the disadvantages of using organic solvent and suffered from instability at extended operation periods. Therefore, the current project was proposed to investigate a more environmentally friendly and sustainable membrane-based method that removes the need to use organic solvent for the extraction process and, in addition, may result in purification of the wastewater to such an extent that a portion of it may be re-used in the production process.

The procedure was based on a hybrid membrane system consisting of UF followed by nanofiltration (NF), which would ideally result in the fractionation of the wastewater into three components: 1) a concentrated polyphenolic fraction that can be composted or discarded as normal, 2) the concentrated monomeric phenolic antioxidant extract, and 3) purified water that contains salts and organic acids (which are required for the original olive fermentation). The purified wastewater could thus be re-used as process water, thereby reducing fresh water requirements, and alleviating the generation of polluting wastewaters through concentration. This would be of benefit to the olive producers, as the resource water cost for consumption could be reduced, whilst the energy cost of wastewater purification for recycling purposes could be offset by the simultaneous recovery of a highly valuable by-product.

The aim of the work presented in this report was twofold: firstly, to evaluate UF membranes of different molecular weight cut-offs (MWCOs) for the initial stage of the hybrid membrane system, in order to reject the high M_w phenolic components while the rest of the wastewater components are passed through the membrane. Secondly, several NF membranes were investigated in order to concentrate the antioxidant product through rejection, while ideally passing and purifying the remaining water to a level such that it can be recycled into the production process.

There were several specific objectives:

- To investigate the fouling characteristics of the wastewater upon various UF membranes.
- To investigate rejection (selectivity) characteristics for various components of the wastewaters which are either retained or passed through the UF membrane.
- To investigate maximising the productivity of the chosen UF membrane by determination of critical flux parameters.
- To develop a long-term operating regime for the UF system, through the use of backflushing, to extend the period between chemical cleaning of the membranes.
- To obtain estimates of possible yields in terms of wastewater volumes and over time, thereby enabling estimates of required membrane surface area for a given volume of wastewater.
- To investigate the separation capabilities of various NF membranes on UF permeate, specifically salts and monomeric phenols.
- To determine possible water recovery amounts from the NF system with a view towards recycling.

2. FINE ULTRAFILTRATION OF OLIVE FERMENTATION BRINE WASTEWATERS

The micro- and ultrafiltration of complex polyphenolic solutions is known to be problematic, particularly so for olive wastewaters (Russo, 2007). Membranes are prone to rapid fouling which is often irreversible. The UF part of the hybrid process is therefore perceived to be critical, and comprises the bulk of this report.

2.1 Materials and Methods

2.1.1 Wastewater

Green olive brine (GOB) and black olive brine (BOB) were obtained from the Cape Olive Trust (Buffet Olives®) located in Dal Josafat, Western Cape. Typical properties of the wastewater are shown in Table 2.1. The wastewaters vary from season to season and by location and cultivar; however, the values shown in Table 2.1 can be considered to be representative of mass-produced local olive brines. The main difference between the two types of wastewater lies in the composition of the phenolic component. In GOB the phenolics are generally of low M_w (< 5 kDa) and are of yellow, green or brown colour. The BOB wastewaters are very dark in colour (black or purple), and incorporate a large M_w distribution of phenolic compounds (up to 2000 kDa).

Table 2.1: Physical properties of green and black table olive brines

Parameter	GOB	BOB
pH	3.8	3.6
Conductivity (mS.cm^{-1})	145	148
COD (g.L^{-1} of O_2)	56	28
Total solids (g.L^{-1})	122	144
Dissolved solids (g.L^{-1})	120	143
Total phenols (g.L^{-1})	589	3548

The low pH of the wastewaters is caused by the presence of short chain organic acids and some phenolic acids. Citric acid is added to fermentation brines as pH control, while lactic acid and traces of acetic acid are metabolic products. Elenolic acid is also present in the brines; this is a breakdown product of the secoiridoid glycoside oleuropein that occurs in high concentrations in olives, and is the parent compound of HT. Elenolic acid was not quantified because pure standards are not available, and it is difficult to quantitatively synthesize. The conductivity is high because of the salt (NaCl) added to fermentation brines – up to 20% w/v. Chemical oxygen demand is attributable primarily to phenolics, and then some other organics.

Figure 2.1 shows a size exclusion chromatography molecular weight distribution of phenolic compounds in BOB analysed previously (Garcin, 2005). The peak at 170 Da represents HT and lesser related compounds, while the larger compounds consist of tannins and lignin-like compounds that give the wastewater its dark colour.

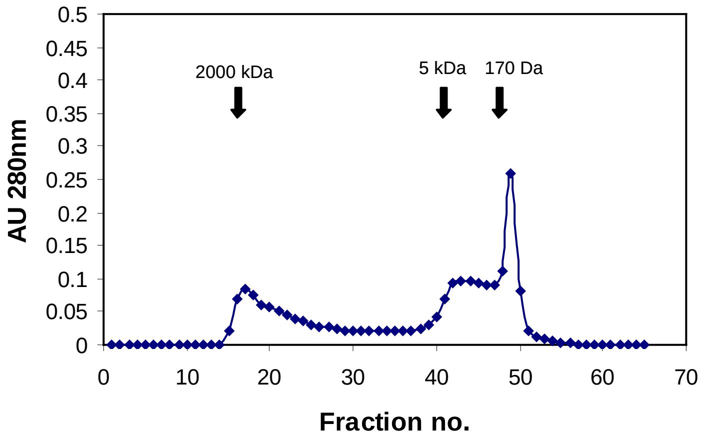


Figure 2.1: Size exclusion chromatogram showing the molecular weight distribution of phenolic compounds in black olive brine

Both wastewaters were subject to UF in order to evaluate the efficacy of the membranes. Wastewater samples (25 L) were stored at -20°C for long term, while 2 L aliquots were stored at 4°C during experiments. Necessary samples were pre-filtered with standard laboratory microfiltration paper (Whatman) before analysis or experiments.

2.1.2 Ultrafiltration extraction system and operation

Figure 2.2 shows a schematic diagram of the UF membrane system used for the experiments, and Figure 2.3 shows photographs of the actual system. The membranes used were from Tami Industries (Nyons, France) and comprised the "Valisette" test kit. This contained four tubular ceramic membranes of MWCOs of 50, 15, 5 and 1 kDa, as well as a stainless steel housing, manifolds and silicone rubber gaskets. The membranes were 250 mm long, 6 mm (ID) and 10 mm (OD), resulting in an active filtration area of 0.0047 m^2. Maximum operational transmembrane pressure (TMP) was 10 bar. Feed was recirculated by means of a Hydra-Cell positive displacement diaphragm pump (Wanner Engineering, Minneapolis, USA).

The particular pump was chosen because it is capable of high pressures at low flow rates (35 bar at 30 $L.min^{-1}$ and 1750 rpm). The pump motor was fitted with a variable speed (flow) controller, with an adjustable pulsation damper and an adjustable pressure relief valve on the delivery side. The pressure relief valve was set to 10 bar for UF experiments in order to protect the membranes in case of pressure spikes (due to a blockage, for example).

Feed and retentate pressures were measured using gauges, while permeate pressure was atmospheric. A needle valve was fitted downstream of the membrane unit in order to adjust TMP. Feed recycle flow rate was measured manually, while permeate volume was measured by weight using a digital mass balance (specific gravity of both wastewaters was approximately 1.1).

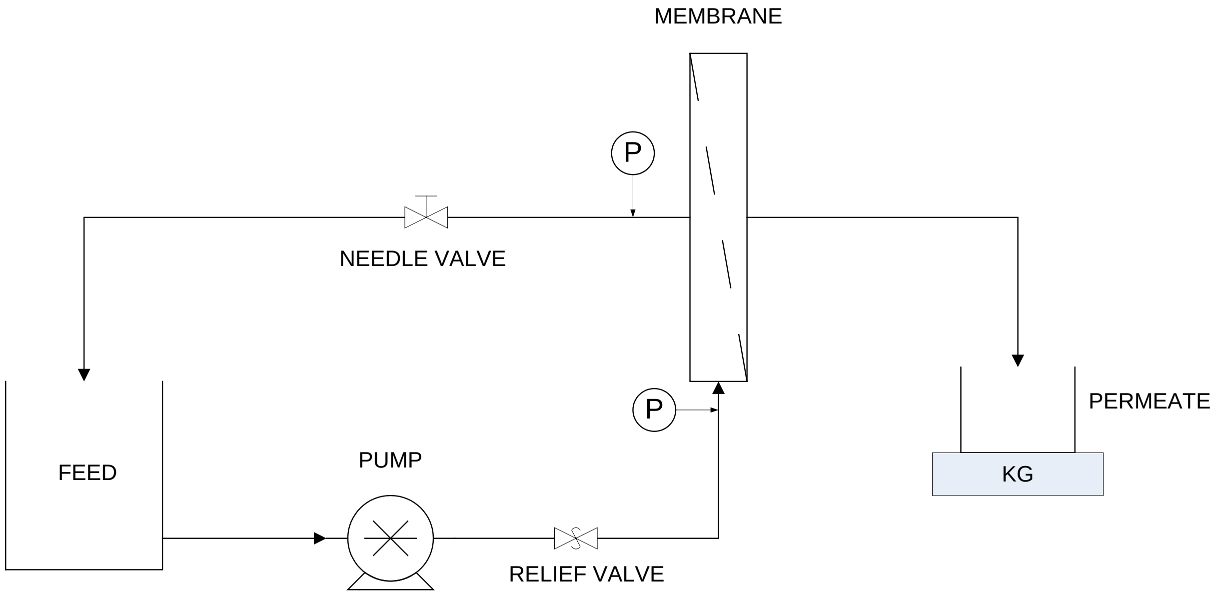


Figure 2.2: Schematic of UF membrane system

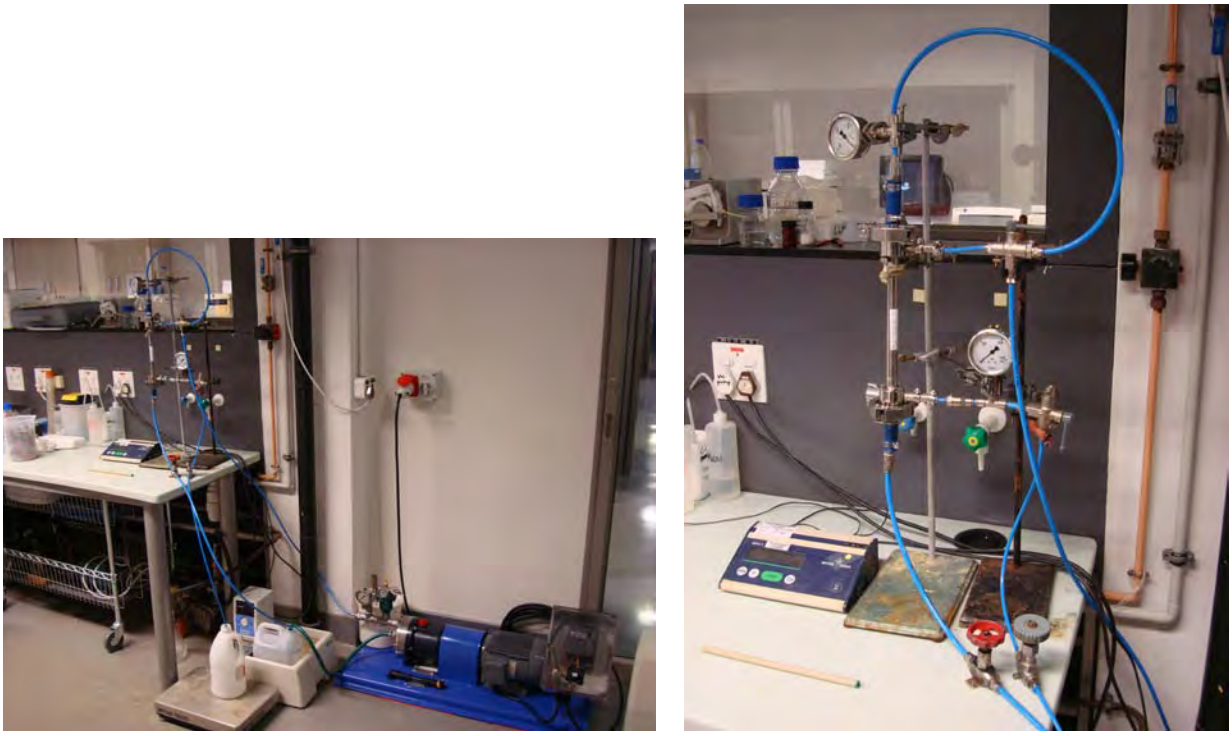


Figure 2.3: Photographs of the extraction system. A tubular ceramic membrane is shown in the forefront of the lower picture

2.1.3 Fouling and selectivity experiments

Fouling experiments were performed by recycling 2 L of wastewater feed through the membrane at a flow rate of 4 L.min^{-1}, which is equivalent to a tangential crossflow velocity of 2 m.s^{-1}. Transmembrane pressure was maintained at 5 bar (half the max. operational pressure) for all experiments. Permeate weight was recorded over time and 2 ml samples were taken periodically for analysis. Batch experiments with each membrane on both types of waste were run continuously for 60 minutes with no backflushing. Fouling was measured as a decrease in permeation rate per unit pressure (i.e. L.m^{-2}.hr^{-1}.kPa^{-1}). All experiments were performed at 25 ± 2°C; because of the relatively low flow and pressures used the recycling feed did not heat up appreciably; maximum temperatures were < 29°C, i.e. not high enough to significantly affect the fluid viscosity, and hence no cooling was used.

After an experimental run, membranes were chemically cleaned in order to remove fouling and restore flux. This was performed by reversing the permeate direction (outside-in as opposed to inside-out for filtration), firstly with 0.5 M NaOH at 85°C for 30 minutes, followed by rinsing with water to neutrality, then with 0.75 ml.L^{-1} H_3PO_4 at 50°C for 15 minutes, followed by rinsing with water to neutrality. In between use membranes were stored in 30% ethanol to avoid microbial contamination (these membranes have to remain wet).

Pure water flux (PWF) was measured initially, and after chemical cleaning, to determine if any irreversible fouling had occurred. Table 2.2 shows values of PWF for the new membranes.

Table 2.2: Pure water flux of different M_w cut-off membranes

Membrane	Pure water flux (L.m^{-2}.hr^{-1}.kPa^{-1})
50 kDa	0.045 ± 0.001
15 kDa	0.031 ± 0.001
5 kDa	0.027 ± 0.002
1 kDa	0.026 ± 0.002

2.1.4 Critical flux experiments

After initial fouling and selectivity experiments had led to the choosing of a specific MWCO membrane for further investigation, critical flux experiments were performed. Critical flux can be defined as the point where the rate of gel layer deposition exceeds the rate of removal due to tangential crossflow velocity of the feed. Once this point is exceeded rapid fouling of the membrane occurs and there is a decline in permeate flux or an increase in TMP, depending on how the system is operated. In this work, a step-wise increase in TMP was used to investigate critical flux, while fouling was monitored by measurement of permeate flux.

2.1.5 Backflushing experiments for flux recovery during extended operation

Backflushing experiments were then performed in order to develop a protocol for long-term operation in between chemical cleaning. This involved varying the intensity, length, and frequency of backflushing. Effectiveness of the different backflushing operations was judged by the restoration of permeate flux after backflushing.

Extended operation with backflushing allowed for the estimation of productivity and yield, and thus an estimation of required membrane surface area for the treatment of a given volume of wastewater in a given amount of time. It also allowed for the maximum product and water recovery ratios to be determined – it is only possible to concentrate the feed a certain amount before osmotic pressure exceeds the applied TMP and permeation ceases. Backflushing was performed with tap water (rather than permeate as is usually the case in industrial operations) because permeate quantities were small due to the small membrane size used (in fact they were less than the liquid hold-up of the system), and in addition, the permeate was saved for further purification and NF experiments.

2.1.6 Analytical methods

Standard laboratory analyses (as shown in the parameters of Table 2.1) have been described previously by Burton *et al*. (2006) and Burton and Garcin (2007). All reagents were of analytical or high performance liquid chromatography (HPLC) grade as required, and were supplied by either Sigma-Aldrich or Merck. Purified de-ionised water was used for all analyses, and was obtained from a Millipore purification system.

Total phenols were measured by spectrophotometric assay using the Folin-Ciocalteau reagent by means of a modified method according to Garcia *et al*. (2001). Gallic acid in the range 0-100 mg.L^{-1} was used as standard, and samples were appropriately diluted to fall within this range.

Hydroxytyrosol was measured by HPLC on a Beckman System Gold (and is associated rubbish software). The column was a reversed phase Phenomenex Luna C18(2) of 5 μm particle size and dimensions 150 × 4.6 mm. The mobile phase was $H_2O/CH_3OH/CH_3COOH$ in the ratio 80:20:2.5 at a flow rate of 1 ml.min^{-1}. Ultraviolet (UV) detection was at 280 nm. Pure HT standard was obtained from Extrasynthese (Gamay, France).

Organic acids were also measured by HPLC on the same system. The column was a Phenomenex Rezex ROA of dimensions 300 × 7.8 mm. The mobile phase was 0.005 N H_2SO_4 at a flow rate of 0.5 ml.min^{-1} with UV detection at 210 nm; relevant sodium salt standards were used for peak area quantification and retention times.

2.2 Results and Discussion

2.2.1 Fouling and selectivity experiments

Figure 2.4 shows permeate flux decline over time for the GOB and BOB during filtration with the different MWCO membranes as a consequence of membrane fouling. The increase in membrane resistance due to fouling is shown as a decrease in membrane permeability (the two are inversely related). There are two mechanisms of fouling: 1) pore blocking, in which species are deposited on or within the pores of the membrane, and 2) cake (or gel) layer formation, which is more gradual and is a cumulative result of a fouling layer being deposited over the entire membrane surface. The purpose of tangential crossflow filtration is to minimise cake layer formation, by removing deposited species through high fluid crossflow velocity.

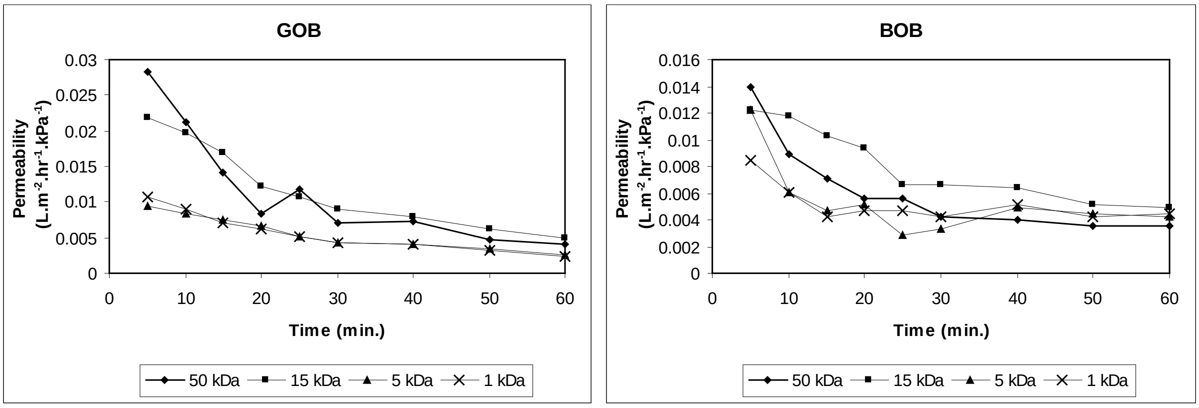


Figure 2.4: Permeate flux decline over time due to membrane fouling

Initial flux of GOB was approximately double that of BOB for all membranes, due to the high concentration of high M_w phenolics in the BOB that do not occur GOB. Initially for GOB, fouling using the 50 kDa membrane (seen as a decrease in flux) occurred more rapidly than for the 15 kDa membrane, indicating that pore blocking was occurring to a larger extent in the larger MWCO membrane, although the nature of the species causing this is not certain. After 20-30 minutes the rate of fouling declines, which indicates that cake layer formation was the more likely mechanism of fouling. Cake layer formation is thus shown to ameliorate the negative effects of pore blocking. There was very little difference in permeate flux between the 5 and 1 kDa membranes, which both had a gradual decline in flux, indicating that cake layer formation was the predominant mechanism of fouling and pore blocking had a lesser effect.

In the BOB filtration experiments, there was a rapid initial decline in flux for all the membranes, except (inexplicably) the 15 kDa membrane. It is likely that phenolic compounds of intermediate M_w were responsible for pore blocking effects, after which cake layer formation resulted in more gradual fouling. Irrespective of the wastewater or membrane used, flux appeared to stabilise at a level of around 0.005 $L.m^{-2}.hr^{-1}.kPa^{-1}$. This indicates that the rate of cake layer removal (by tangential flow) was approaching the rate of cake layer deposition. It must be noted that no backflushing was performed during these experiments; this would improve long-term operation in terms of restoration of flux – the aim here was to investigate fouling during continuous operation. In this respect, the 15 kDa membrane performed best with both types of wastewater. Pure water flux was essentially 100% restored after chemical cleaning, which illustrates that no permanent (irreversible) fouling occurred.

In terms of separation, the major components of the wastewater are phenolics, salt and organic acids. The main objective here was to investigate the ability of the different membranes to separate HT (M_w 154 Da) from the rest of the higher M_w phenolics, which are ideally retained by the membrane. This was performed by measuring the yield (concentration times permeate volume) and purity (HT % of total phenolics) in permeate from the different membranes. A further objective was to investigate the effect of the UF membranes upon salt and organic acid permeation, both initially and with increased fouling.

Table 2.3 shows concentrations of total phenols and HT, purity, and yield in the *total* permeate from the different experiments. The yield is directly proportional to extraction rate, as all experiments had the same membrane surface area and were performed for the same amount of time. Feed total phenol concentrations were 589 ± 41 and 3548 ± 195 $mg.L^{-1}$ for GOB and BOB, while HT concentrations were 144 ± 21 and 1205 ± 174 $mg.L^{-1}$ respectively.

Table 2.3: Total phenols (TP) and HT in permeate from different MWCO membranes

	TP conc. ($mg.L^{-1}$)	**HT conc.** ($mg.L^{-1}$)	**HT purity** (%)	**HT yield** (mg)
GOB				
50 kDa	188	56	30	15
15 kDa	204	86	42	23
5 kDa	225	92	41	10
1 kDa	253	99	39	13
BOB				
50 kDa	3233	961	30	129
15 kDa	1987	961	48	184
5 kDa	1663	862	52	105
1 kDa	1487	815	55	104

TP = total phenols, HT = hydroxytyrosol, GOB = green olive brine, BOB = black olive brine

For GOB, both total phenols and HT concentrations *increase* with decreasing membrane pore size, which is unexpected and counterintuitive. This is possibly a result of fouling: with cake layer formation an additional resistance to permeation is observed, which may decrease the observed MWCO, as fouling is more severe with the larger MWCO membranes. In any case it is not a bad effect.

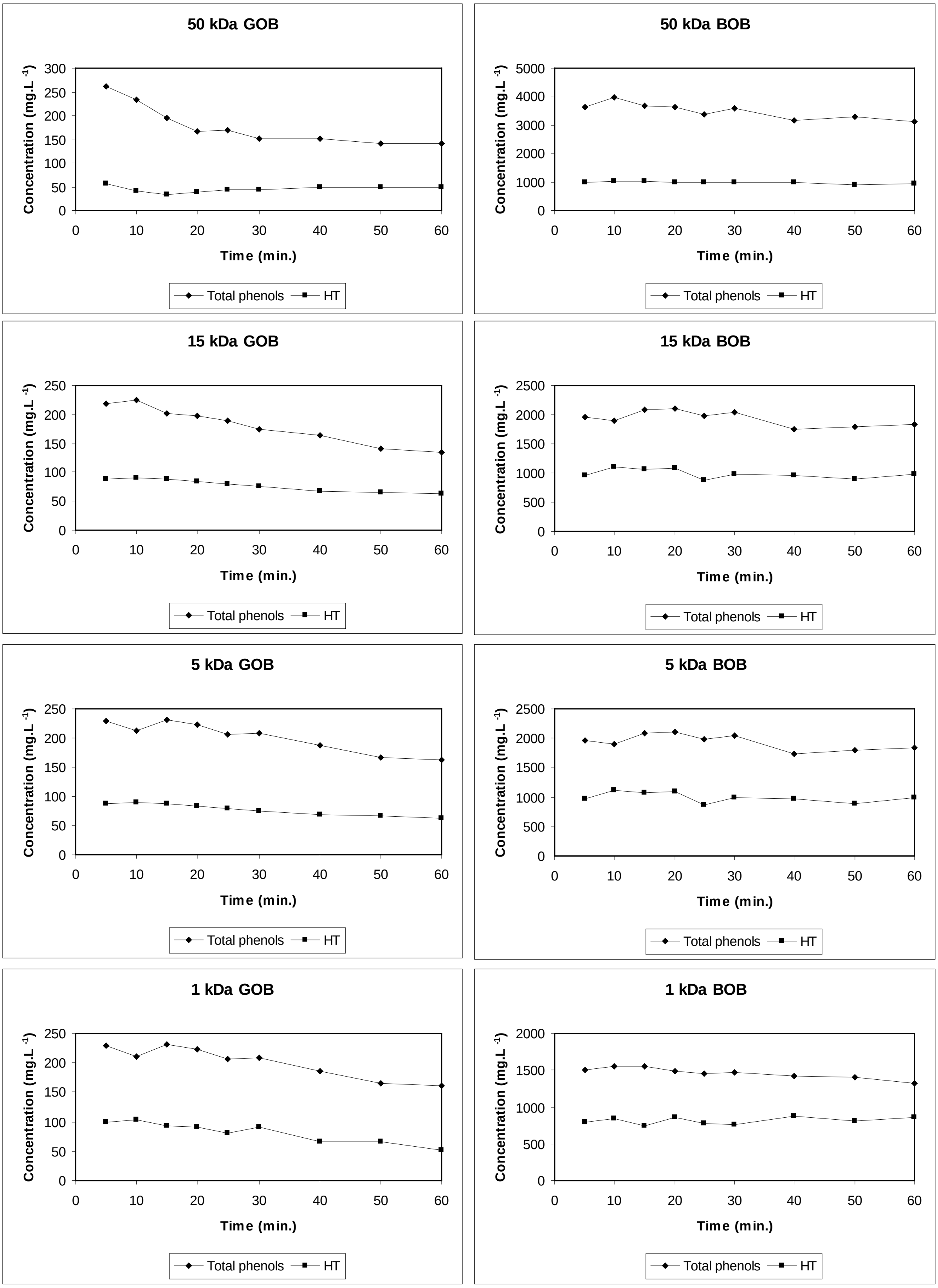
For BOB the situation is reversed, as would be expected. Total phenol and HT concentrations decrease with decreasing pore size. The purity of HT, i.e. the percentage of total phenols permeating the membrane, is seen to increase. The percentage purity of HT is more or less the same for the 15, 5 and 1 kDa membranes. Percentage purity can be understood as the selectivity of the membrane towards HT permeation, with higher M_w phenols being retained. At this point it should be noted that membrane MWCO is a nominal value; in reality there is a pore size *distribution*, which allows for passage of phenols of greater size than the nominal MWCO. It is not an absolute cut-off.

The best membrane is the one that achieves both the highest HT purity and the highest yield, i.e. the amount of HT that permeates through the membrane, which is a function of permeate flux, which decreases with increased fouling. In this respect, the 15 kDa membrane is seen to be the best for both GOB and BOB, overall. However, the observed selectivity changes over time for any particular membrane as fouling occurs. Figure 2.5 shows total phenol and HT concentrations over time during experiments with the different membranes and wastewaters.

In experiments on GOB, total phenols concentrations are seen to decrease fairly significantly, while the effect on HT is less marked. This occurs in conjunction with the decrease in permeate flux, which demonstrates that the development of a fouling layer affects the selectivity of the membrane (the values shown in Table 2.1 are averages of the concentrations over time). For the BOB there is also a decrease in total phenol concentrations – note that if the scale of the y-axis on the BOB graphs was the same as the GOB graphs the situation would be seen to be much more pronounced. Hydroxytyrosol concentrations, however, remain relatively consistent in the BOB permeate, which shows that permeation thereof is not affected by the development of a fouling layer. This is beneficial for the separation process, although the overall rate of extraction does decline due to the decline in flux (permeate flow rate).

The effect of fouling layer development on total phenol concentration is nicely illustrated in Figure 2.6. The top panel shows GOB samples over time, with the rows from top to bottom representing the different membranes, decreasing from 50 kDa MWCO. Similarly the bottom panel shows BOB samples. Time scale is the same as represented for the samples shown in Figure 2.2. It is evident how the decrease in total phenols over time causes a decrease in observable colour in the permeate, especially for the BOB. The effect is also observable for a decrease in membrane pore size, although there was very little difference between the 5 and 1 kDa membranes.

It is also interesting to note that since there is little or no colour in the later samples from the 5 and 1 kDa membranes, it can be assumed that all phenolic compounds present are < 500 Da. Pigmented phenolic compounds, starting with the dimeric anthocyanidins (responsible for the red and purple colours in fruit and vegetables) and then increasing in M_w, are not present. This bodes well for future downstream processing for the purification of HT; this will most likely be a column chromatography process, and pigmented phenolics are known to adversely affect this process as they often bind irreversibly to the column media.


50 kDa GOB

50 kDa BOB

15 kDa GOB

15 kDa BOB

5 kDa GOB

5 kDa BOB

1 kDa GOB

1 kDa BOB

Figure 2.5: Total phenols and HT concentrations in permeate over time

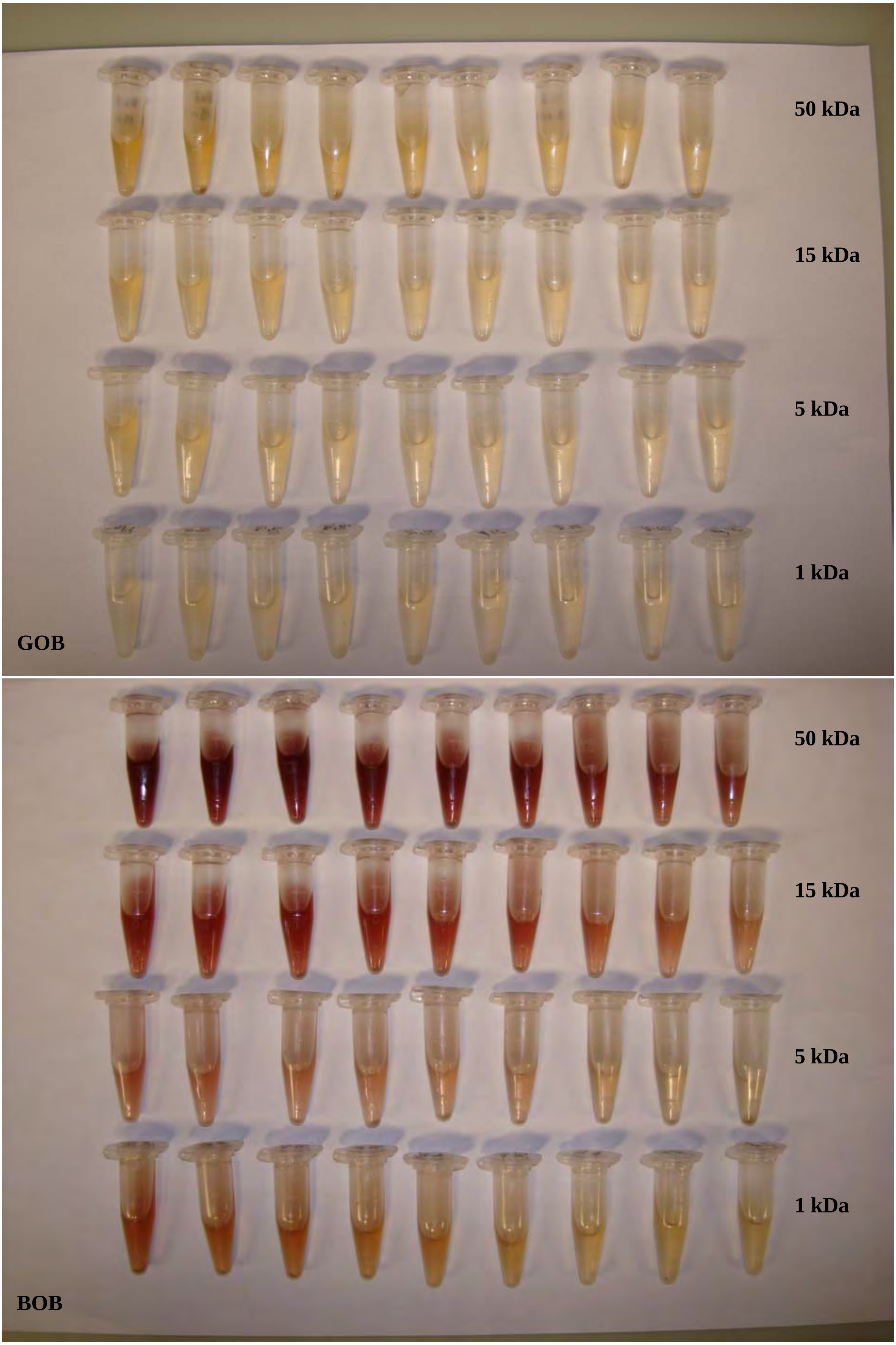


Figure 2.6: Photographs of permeate flux samples over time using different MWCO membranes

Colour was not measured, as it is not a reliable quantitative measure of any singular specific component in these wastewaters. This is because different phenolic species have different absorptivities in both the UV and visible light wavelengths, while organic acids also absorb in the low UV range. Hydroxytyrosol itself is colourless in the visible wavelengths, but absorbs strongly at 280 nm. It may thus be possible to quantify HT approximately by measurement at this wavelength, on condition that it makes up a sufficiently large percentage of total phenols in the permeate. Most phenolic compounds absorb strongly at 280 nm.

If one considers the HPLC chromatogram shown in Figure 2.7, which is of the last BOB permeate sample shown in Figure 2.6, it is evident that HT (at 7.07 min.) accounts for approximately 60% of the total peak area, and thus for approximately 60% of the phenolics measured in the sample. This correlates well with the HT percentage purity shown in Table 2.1.

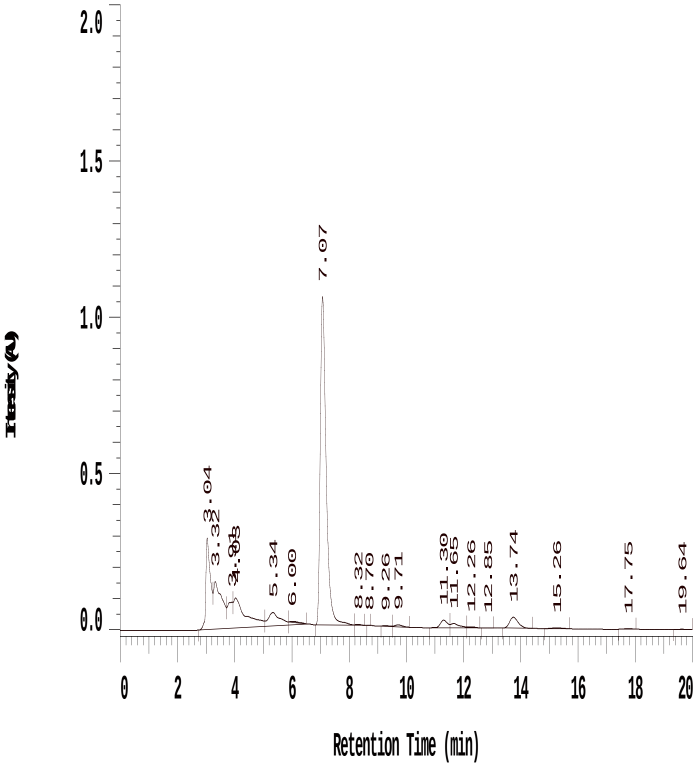


Figure 2.7: HPLC chromatogram of BOB permeate from the 1kDa membrane at 60 minutes

Conductivity, pH, and organic acids of the total feed, permeate and retentate are shown in Table 2.4. In nearly all cases there is a slight increase in pH from the initial feed to the permeate and final retentate. This corresponds to a general decrease in concentrations of citric and lactic acids. Mass balances on these showed between 5-25% loss; thus it is suspected that these compounds formed part of the fouling layer, which was removed and discarded during chemical cleaning.

Similarly, the conductivity decreased slightly in all cases, indicating that salts were also incorporated in the fouling layer. There is not, however, too much concern about the fate of organic acids and salts, as separation and removal of these compounds is the objective of the NF part of the overall hybrid membrane system, and is to be investigated in the future. The more critical part of the current UF process was to separate HT from other, larger phenolic compounds, as discussed above.

Table 2.4: Bulk properties of feed, permeate and retentate of wastewaters

		pH	Conductivity ($mS.cm^{-1}$)	Citric ($mg.L^{-1}$)	Lactic ($mg.L^{-1}$)
GOB					
50 kDa	Feed	3.84	146	123	115
	Permeate	3.9	131	124	121
	Retentate	3.9	135	107	98
15 kDa	Feed	3.74	147	130	140
	Permeate	3.92	128	102	101
	Retentate	3.77	131	113	105
5 kDa	Feed	3.7	151	103	106
	Permeate	4.14	117.9	70	57
	Retentate	3.76	142	87	89
1 kDa	Feed	3.69	157.4	101	102
	Permeate	3.81	145	92	83
	Retentate	3.75	149	97	98
BOB					
50 kDa	Feed	3.36	48.6	27	201
	Permeate	3.45	40.7	22	168
	Retentate	3.43	42.4	22	176
15 kDa	Feed	3.49	57	27	197
	Permeate	3.54	51	23	157
	Retentate	3.55	53	24	182
5 kDa	Feed	3.49	48	22	163
	Permeate	3.66	42	15	89
	Retentate	3.59	45	20	134
1 kDa	Feed	3.6	56	23	206
	Permeate	3.82	49	16	120
	Retentate	3.6	51	21	176

2.2.2 Critical flux experiments

Having determined the separation characteristics of the membranes, it was then necessary to attempt to maximise the productivity, i.e. operate at the highest possible permeate flux. To this end a step-wise increase in TMP was applied to the chosen membrane and fouling effects were observed. The 1 kDa MWCO membrane was chosen for all further experiments, as this membrane showed the best selectivity in terms of HT purity, and in addition, there was little difference in membrane flux between the 1 and 5 kDa membranes. All further experiments were conducted on BOB only, this wastewater being of more interest due to its high HT content and more complex phenolic nature than GOB. Figure 2.8 shows results of the critical flux experiments.

The step-wise pressure increase interval was 2 bar, starting at 2 bar and ending at 8 bar. As expected, there is an increase in flux with increased TMP, and a decline in flux over time in all cases due to gradual membrane fouling (pore blocking). However, there is no sudden and dramatic decrease in flux, which indicates that critical flux was not reached at these conditions for this wastewater. This implies that it is possible to operate near the maximum operational pressure of the membrane without the rapid formation of a gel layer.

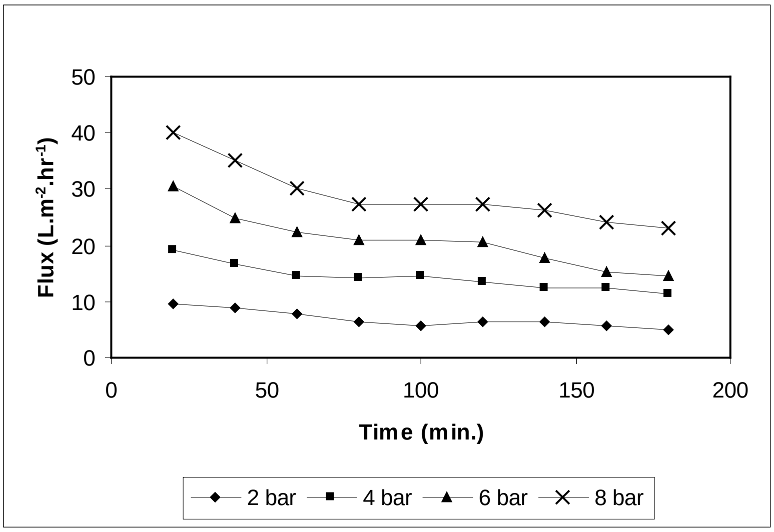


Figure 2.8: Flux decrease over time during operation at different TMPs

Figure 2.9 shows membrane permeability over time for the same experiments. Permeability, and thus membrane fouling, is seen to be more or less the same for the different TMPs. This further illustrates that critical flux was not reached, and gel layer formation is independent of TMP at these operating conditions for this wastewater. What is interesting to note is that after approximately 120 minutes of operation the permeability starts to decline again after having stabilised somewhat during 60-120 minutes of operation. This is indicative of gradual gel layer formation and a corresponding loss of permeability. This suggested that for future backflushing experiments, periodic backflushing should commence no later than 120 minutes after the start of operation in order to avoid the build-up of a gel layer.

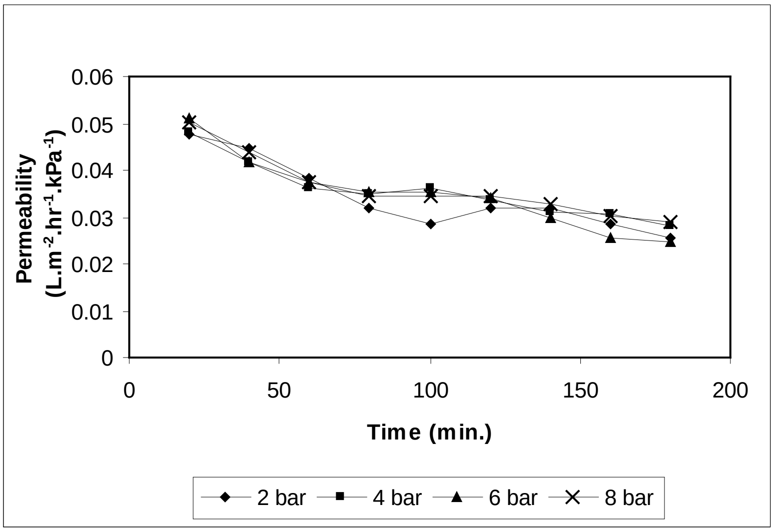


Figure 2.9: Membrane permeability decrease over time at different TMPs

2.2.3 Backflushing experiments during extended operation

Based on the results above, and in order to maximise productivity, it was decided to start backflushing experiments at an operational TMP of 8 bar, with a two minute backwash at 8 bar every hour. As can be seen from Figure 2.10 this approach was largely unsuccessful, with permeability *decreasing* after the backwash instead of being restored. The reason for this is unclear, but it is possible that the backwash removed what gel layer had been formed, making the membrane susceptible to further pore blocking, without the pore blocking components having been dislodged during the backwash.

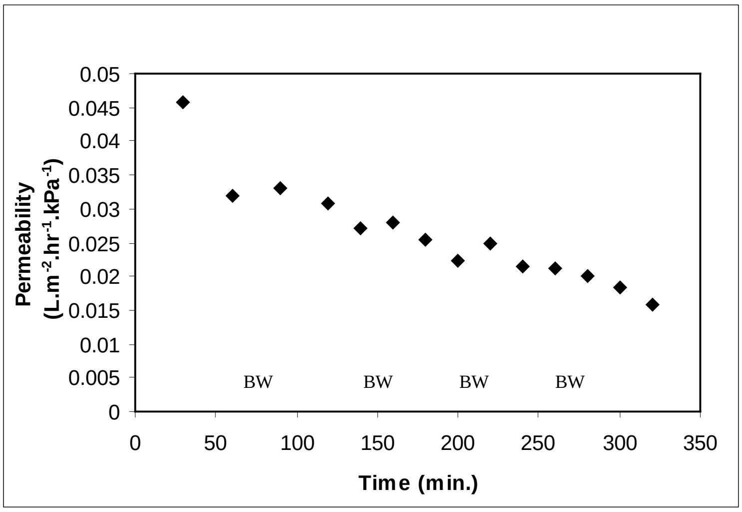


Figure 2.10: Backflushing experiment during continuous operation at 8 bar TMP. Backwashing (BW) was performed at 120, 180, 240 and 300 minutes.

An experiment at 6 bar TMP operation with a 6 bar backwash yielded similar continuous fouling results, although not as severe as with operation at 8 bar. These results indicated that backflushing needed to be more intense, i.e. at higher pressure, or had to be performed for longer periods of time. Thus it was decided to operate the system at 6 bar TMP, with backflushing at 8 bar.

Initially backflushing was performed for two minutes every hour, and while the situation was improved compared to the results shown above, flux recovery was only marginal, and thus backflushing was performed for five minutes at 8 bar. Figure 2.11 shows results of this experiment during extended operation. There was between 5-15% improvement of flux after backwashing. Permeability declined to a level of around 0.015 L.m^{-2}.hr^{-1}.kPa^{-1} (equivalent to flux of ~10 L.m^{-2}.hr^{-1} at 6 bar pressure), where it appeared to stabilise.

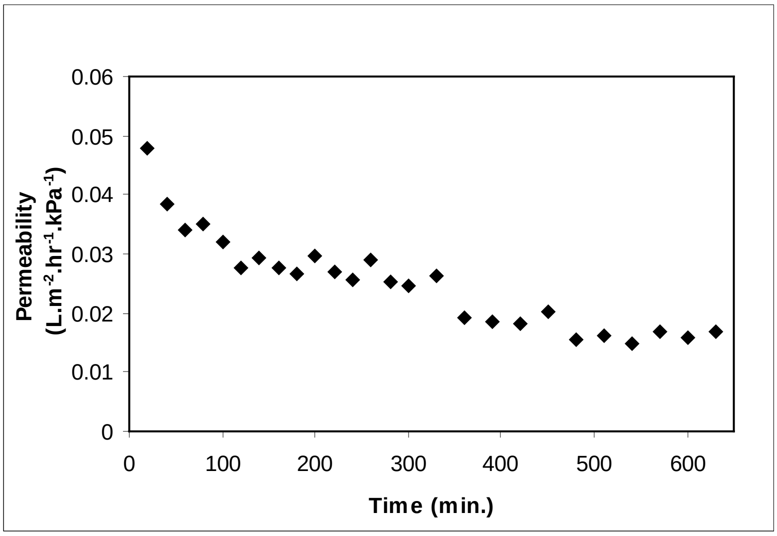


Figure 2.11: Decline of flux due to fouling in a backflushing experiment during continuous operation at 6 bar TMP. Backwashing was performed at first at 120 minutes and then every 60 minutes thereafter.

By integrating the area under the curve in Figure 2.11 it is verifiable that 1.37 L of permeate was obtained from 2 L of wastewater feed. A proper mass balance across feed and permeate streams was complicated by the use of tap water for backflushing, which served to dilute the feed stream by an unquantifiable amount. Normally permeate is used for backflushing in industrial membrane systems, however because of the small scale of these experiments and the need to save permeate for further experiments and analysis, tap water was used instead.

Approximately 70% of the initial feed ended up as permeate. The concentration of HT in the combined permeate was 952 mg.L^{-1}, meaning that 54% of the initial HT in the feed was recovered; this was at a purity of (in terms of total phenols) 62% – slightly better than data shown in Table 2.1. This is not bad for a single step concentration, given that HT accounts for only around 30% of the total phenols in the feed. The total yield for the experiment was 1.3 g of HT from 2 L of wastewater. The overall extraction rate of HT was calculated to be 0.02 g.L^{-1}.m^{-2}.hr^{-1}.kPa^{-1}. These values can be used as estimates for scaled up production, although it must be noted that there is variation in the wastewaters coming from the olive factory, and therefore these figures should be used with caution as the extraction rate will depend on the concentration in the initial feed.

The above extended backflushing experiment was repeated with a different batch of BOB wastewater in order evaluate reproducibility of the backwashing protocol and evaluate yields, and to investigate relative concentrations of HT and total phenols in the permeate over time.

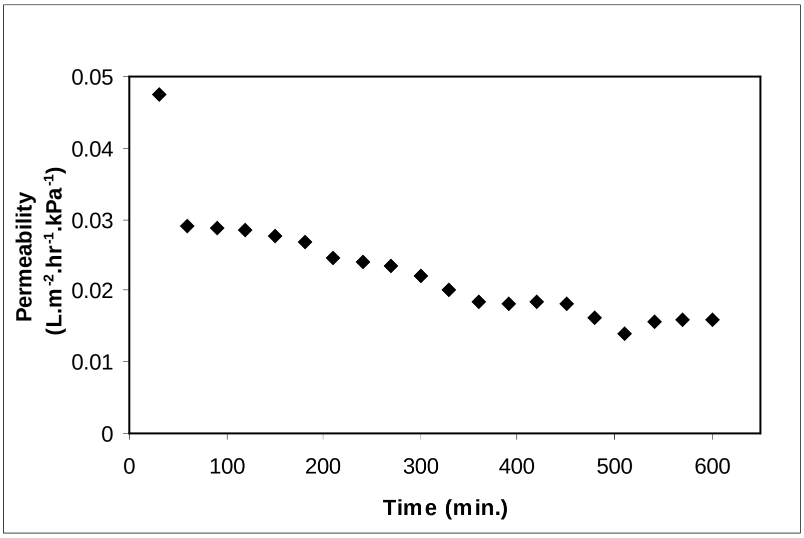


Figure 2.12: Repeat backflushing experiment using the same conditions as in Figure 2.8 but with different wastewater

As in Figure 2.11 a rapid initial drop in permeability was observed, with stabilisation of permeability at a value of around 0.015 L.m^{-2}.hr^{-1}.kPa^{-1}, despite there being lower permeate flux recovery after backwashing which was possibly due to slightly different compositions of the wastewater. Nevertheless, the final permeability value can plausibly be used as a worst-case scenario for determining the required membrane surface area for scaled up wastewater volumes and extraction rates.

Figure 2.13 shows total phenol and HT concentrations in the feed and permeate streams over time. After the first backwash at 120 minutes there is a marked decrease in total phenols concentration in the feed, and a corresponding increase in the total phenols concentration in the permeate. This illustrates that although the backwashing had little effect on flux recovery, it did have an effect on fouling in terms of membrane selectivity. Subsequent backwashing when the fouling was more established had a less marked effect. The ratio of HT to total phenols is seen to increase over time from around 50% to >70%. This again illustrates the effect of fouling layer formation upon membrane selectivity. As the membrane fouls, more of the higher M_w polyphenols are retained, while passage of HT through the membrane is largely unaffected, although the rate declines due to decreasing flux.

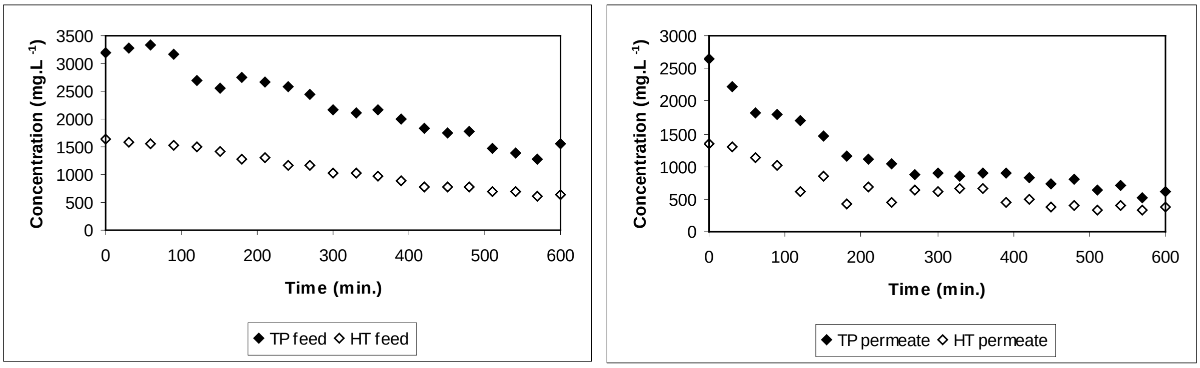


Figure 2.13: Relative concentrations of hydroxytyrosol (HT) and total phenols (TP) in the feed and permeate streams during backflushing experiment

The increase in selectivity towards HT over time as the membrane fouls is further illustrated in Figure 2.13. Initially the permeate is light pink in colour, but becomes progressively clearer until it is essentially colourless. Performing wavelength scans on the permeate over time illustrates this to be the case.

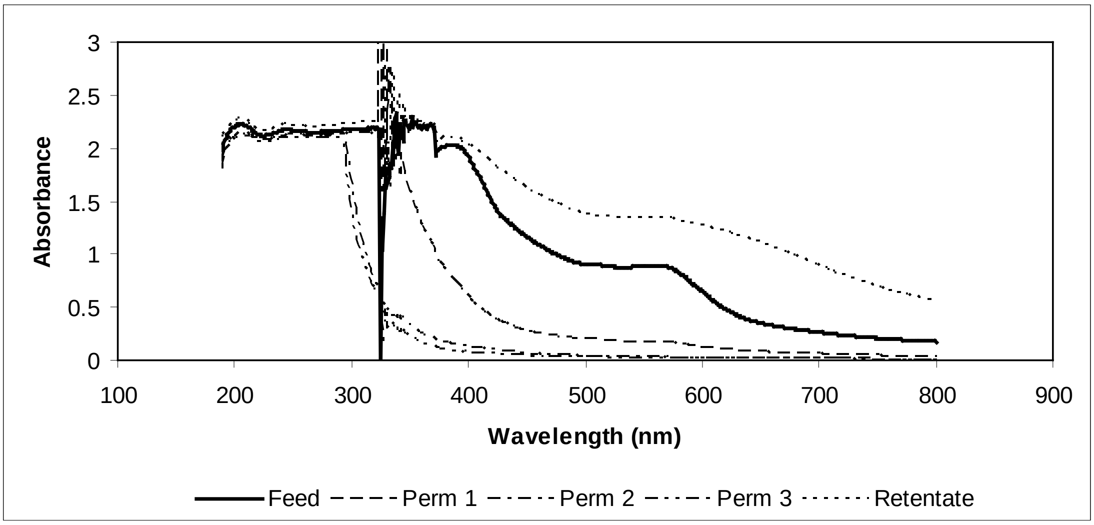


Figure 2.14: Wavelength scans of wastewater feed, permeate and retentate during UF. Perm 1: permeate during 0-60 minutes, Perm 2: 60-300 minutes, Perm 3: 300-600 minutes. (The erratic lines at 325 nm are due to a change from the UV to the visible lamp in the spectrophotometer).

The feed is seen to have high absorbance in the UV range (200-325 nm), with gradually declining absorbance in the visible wavelengths (400-700 nm). Absorbance in the UV range is due to both monomeric and polymeric phenols, while absorbance in the visible range is due to polymeric (coloured) phenols alone. Monomeric phenols (such as HT) are generally colourless. It can be seen that the majority of polymeric phenols are retained in the feed and hence retentate streams, while absorbance in the visible regions is markedly lower in the permeate streams.

This phenomenon offers an opportunity for monitoring of permeate quality: HT purity (HT % of TP) of the permeate could be qualitatively measured by monitoring at two fixed wavelengths, e.g. 280 nm in the UV range and 400 nm in the visible range. The ratio of absorbance at these two wavelengths in the permeate stream would give an indication of membrane selectivity towards HT; similarly such measurements in the permeate stream would give an indication of polymeric phenols being retained. This concept is being further developed in WRC research consultancy K8/865, using low-cost narrow bandwidth light-emitting diodes (LEDs) and photodiode detectors coupled to flow cells for on-line monitoring.

3. NANOFILTRATION EXPERIMENTS

The rationale for investigating NF membranes for post-treatment of the UF permeate from the olive fermentation brines was several-fold. Firstly, the brines contain > 10% salt (NaCl) – approximately three times the concentration found in sea water. Osmotic pressure gradients are therefore excessive and so RO membranes are not an option for water purification and reclamation in this case, as applied pressures would be excessive and permeate yields low. Nanofiltration membranes, on the other hand, have moderate to high monovalent salt passage while exhibiting good retention of organics. Nanofiltration membranes thus have much lower osmotic pressure gradients due to this salt passage. Secondly, if little besides water and salt passes through the membranes there is the possibility that this NF permeate could be re-used for the brining process, thereby saving on both salt and water costs for the producers. The purpose of the work presented here was thus to investigate the ability of the NF membranes to retain and concentrate the HT and others organics (acids) from the UF permeate, while at the same time producing re-useable process water.

3.1 Materials and Methods

3.1.1 Wastewater

Because of the significant area size difference between the UF and NF membranes, it was first necessary to investigate the separation characteristics of the different NF membranes using synthetic wastewater. Permeate quantities from the UF experiments were not sufficient for execution of all the NF experiments, due to the much larger volumetric hold-up of the latter system. Synthetic wastewater was made up from tap water and contained 0.5 $g.L^{-1}$ gallic acid and 100 $g.L^{-1}$ NaCl. Gallic acid is a monomeric phenol similar to HT (molecular weights are 154 and 170 respectively). Batches of 5 L were made up for each experiment.

Real UF permeate was used in a later experiment. This was made by combining all the remaining permeate from the experiments in Section 2, diluting this up to 20 L, and adding salt to reach a conductivity of 80 $mS.cm^{-1}$, similar to that of the original wastewater.

3.1.2 NF membrane system and operation

The NF system was essentially the same as that used for UF experiments, as shown in Figure 2.2. Figure 3.1 shows a photograph of the system. Three membranes of different MWCO were obtained from Dow (Midland, MI, USA). These were of the Filmtec range, and were spiral-wound polypiperazine amide membranes, with an active surface area of 2.6 m^2. The NF2450 membrane has a nominal MWCO of 200, and is designed to reject organics and pass monovalent salts; NF90-2450 has a nominal MWCO of 90 and is designed to have low salt passage; the NF270-2450 has a nominal MWCO of 270, and is designed to have medium to high salt passage. The elements are designed for a maximum operational pressure of 40 bar at 45^oC and have a pH tolerance range of 2-11.

No backwashing is performed on these membranes as they are designed to operate in one direction only. Cleaning is achieved by releasing back pressure on the module resulting in a crossflow "flush". Chemical cleaning can be periodically performed but was not necessary during these experiments.

As before, TMP was applied through the use of a throttling needle valve, feed flow rate was measured manually, and permeate was measured using a mass balance.

Figure 3.1: Photograph of the NF membrane system

3.1.3 Selectivity and recovery experiments

Pure water flux was performed before and after all experiments to check for membrane fouling. Firstly the experiments were performed using the synthetic wastewater. The membrane module was slowly filled to remove all air, and then the flow rate was adjusted to 10 $L.min^{-1}$ to achieve the necessary crossflow velocity recommended by the manufacturer. The system was then brought up to pressure to get a suitable permeate flow. Pressures of 5-20 bar (half the maximum operational pressure) were used, depending on the membrane. Samples were collected periodically from the feed/retentate and permeate streams. Gallic acid concentrations in the samples were measured by HPLC in order to evaluate the monomeric phenol rejection, while conductivity was measured to monitor salt passage through the membranes. The same procedure and analyses were performed on experiments using the real wastewater. Permeate flux was measured over the course of the experiments as for the UF experiments. Analytical methods were as described in Section 2.1.6.

3.2 Results and Discussion

3.2.1 Synthetic wastewater experiments

Pure water flux and operational flux (measured as membrane permeability) for the three membranes are shown in Figure 3.2. Values shown for the different membranes were consistent throughout experiments; they did not decrease due to fouling as in the UF experiments.

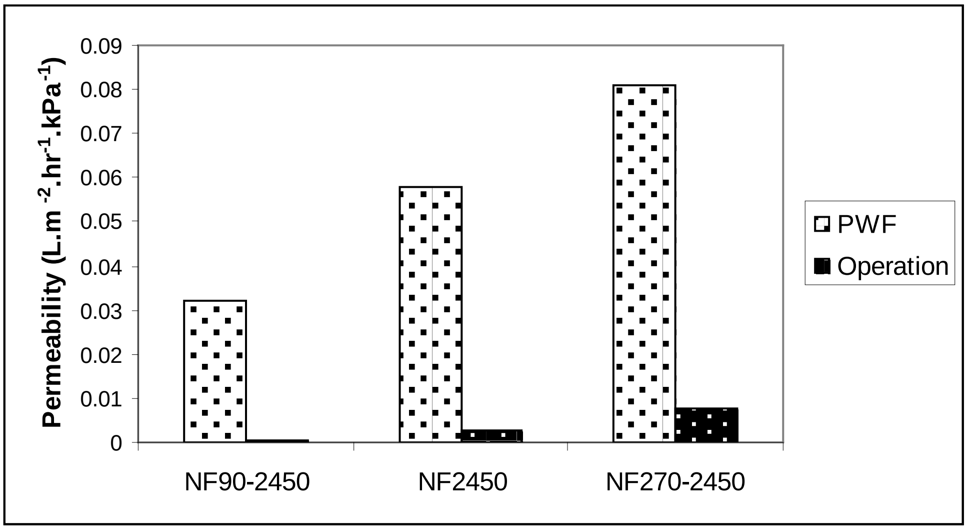


Figure 3.2: Pure water flux and operational flux in different NF membranes

As would be expected, membranes with increasing pore size had increasing PWF. What was unexpected was the drastic reduction in flux using the synthetic wastewater. The operational flux as a percentage of PWF was 1.2, 4.5 and 9.8% respectively. The dissolved substances in the synthetic wastewater have a massive effect on membrane permeation despite their relative passage, as discussed below. Nonetheless, operation was well within the bounds of acceptability given that the maximum applied TMP in these experiments was only half the maximum allowable operational TMP indicated by the manufacturer. Although these figures appear low, actual permeate flow rates varied from between 2-10 L.hr^{-1} (due to the large surface area of the membranes), compared to the average 50 ml.hr^{-1} for the UF system described previously, and thus the experiments were relatively short.

Figure 3.3 shows feed and permeate stream concentrations for gallic acid and salt over time in the different membranes, and Table 3.1 shows a summary of these results. The NF270-2450 and NF2450 membranes both had very high salt passage, but showed very little retention of gallic acid (4 and 10% respectively), and thus are not suitable for concentration of the UF permeate. This is not surprising given that the nominal MWCO of these membranes is 270 and 200 Da respectively, and the molecular weight of gallic acid is 170 Da. The NF90-2450 fared better than the other membranes, but still only retained a little over 50% of the gallic acid, while at the same time retaining about 20% of the salt. This is explained by the fact that the spiral-wound membranes are depth, not surface, filters and are thus not absolute barriers. Also, there is a statistical pore size distribution around the nominal MWCO.

Table 3.1: Average phenol (gallic acid) retention and salt passage for NF membranes

Membrane	Phenol retention (%)	Salt passage (%)
NF270-2450	4.1	95.3
NF2450	10.7	93.0
NF90-2450	52.4	81.2

Because of the factors discussed above, none of the membranes appear particularly suitable for the process of concentrating the permeate obtained from the UF system, in order to recover the HT and reclaim process water and salt. Salt retention by the membrane is not particularly problematic, but even in the best case it would be unacceptable to lose 50% of the HT to the permeate stream. In addition, the HT concentration in the reclaimed water could preclude its re-use as process fermentation water through inhibition.

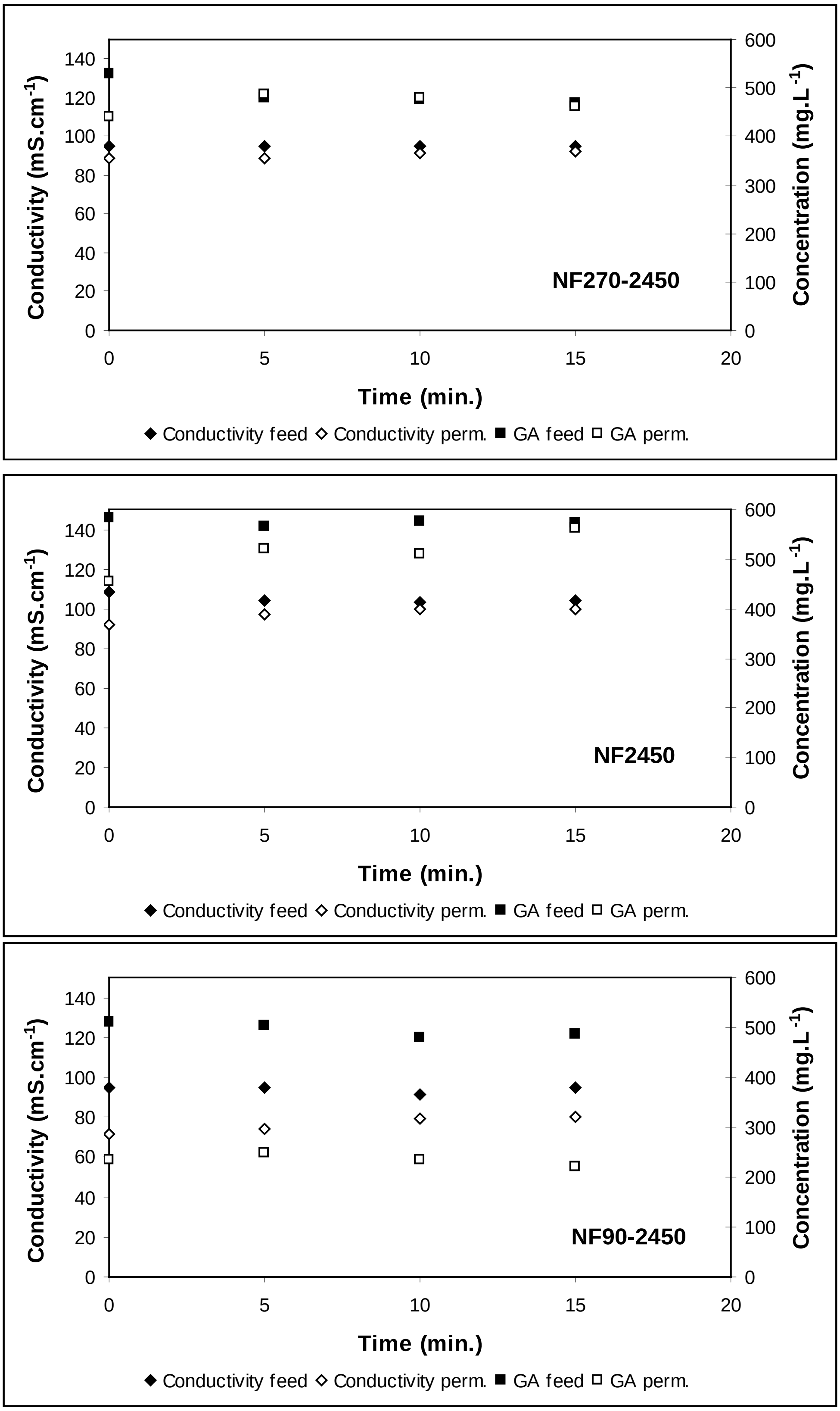


Figure 3.3: Variation in salt (conductivity) and gallic acid concentrations in the feed and permeate over time in different NF membranes

The NF90-2450 membrane was used in an experiment with real UF permeate wastewater to verify the above results, and also to observe the effect of the membrane upon the organic acid components of the real wastewater. This experiment was run for a longer time than the previous experiments in order to establish how much permeate could be obtained from a given feed volume (concentration factor), before osmotic effects due to increasing feed concentration (or other factors e.g. Donnan exclusion) lead to decline in flux.

Results from this experiment are shown in Figure 3.4. Hydroxytyrosol retention started at 71% at the beginning of the experiment, but this dropped to 43% by the end, while salt passage increased from 52 to 72%. Passage of total organic acids (as measured by HPLC) also increased from 51 to 78%. Thus previous results with the synthetic wastewater were verified: the NF membranes are not capable of concentrating the HT in the UF permeate sufficiently.

Flux measured as membrane permeability during this experiment was consistent (at a level of 0.00031 $L.m^{-2}.hr^{-1}.kPa^{-1}$, similar to the synthetic wastewater NF90-270 experiment); this despite the increasing concentrations of salt and HT in the feed recycle stream due to partial retention of the solutes. 10 L of the real UF permeate wastewater was used for this experiment, 7.2 L of which was recovered as permeate.

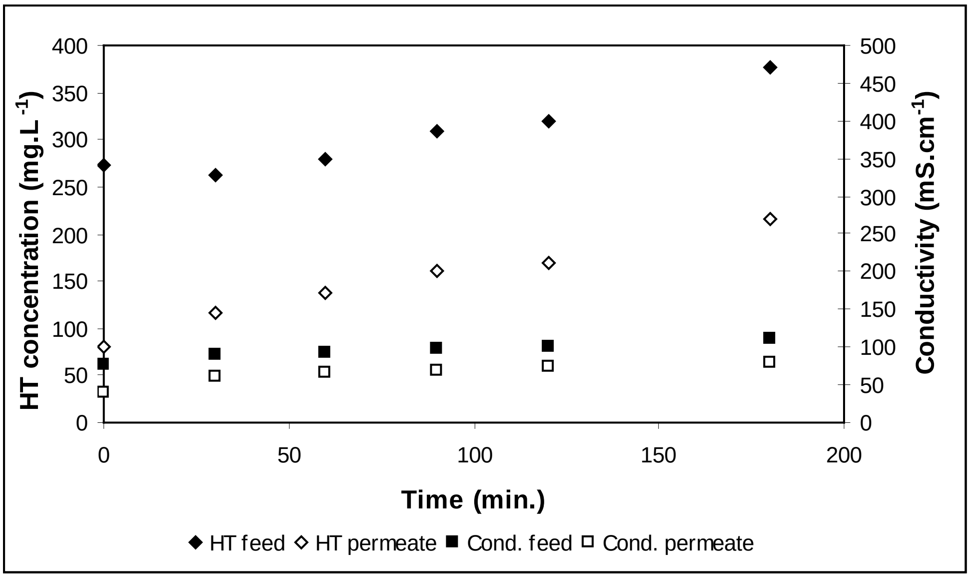


Figure 3.4: HT and salt concentrations in feed and permeate streams over time during NF experiment on real wastewater UF permeate

The problem of unsuccessful retention of HT by the NF membrane suggests that a different approach be taken in the overall process. Instead of concentrating the UF permeate before further chromatographic purification of the extract, it would make more sense to extract HT before the UF permeate is sent for NF purification. This would most likely be a chromatographic adsorption process.

Alternatively, other brands of NF membranes could be investigated, but these would probably suffer from the same problems. Decreasing the pore size further than 90 Da is not a possibility, as one then heads into the realm of RO membranes; salt retention becomes significant and osmotic pressure would rapidly become insurmountable.

4. PURIFICATION OF ULTRAFILTRATION PERMEATE

The purpose of this section was to investigate the recovery of HT from the UF permeate stream. As mentioned above, it is necessary to recover the HT before a NF step, this because of incomplete HT retention by the NF membrane and hence product loss. Purification was investigated with and without performing a “clean-up” pre-treatment step using either activated carbon or diatomaceous earth (DE). C-18 reversed phase chromatography was then used to recover and purify the HT. The objective of the purification process was to determine estimates for yields and purity of HT recovered from the UF permeate.

4.1 Materials and Methods

UF permeate used for purification was obtained from the final backwashing experiment in Section 2.2. Celite® 545 DE was obtained from Merck, and applied to samples at a concentration of 10 g.L^{-1}. Powdered activated carbon (AC) was also used at 10 g.L^{-1}. Samples were magnetically stirred in beakers for 30 minutes for clean-up, and were then centrifuged and filtered (0.22 μm) to remove the AC or DE.

Strata-X solid phase extraction tubes (Phenomenex) containing 500 mg of C-18 reversed phase media were used for purification. The columns were activated with methanol (12 ml), and then equilibrated with water (12 ml). 10 ml of the relevant sample was then loaded onto the column, which was then washed with water (12 ml) and eluted with 12 ml of 15% methanol.

Hydroxytyrosol and organic acids were measured using HPLC as described in Section 2.1. Percentage purity of HT was measured by % total mass (not as a percentage of total phenols as before) as follows: 25 ml (in round bottom flask) or 1 ml (in Eppendorf) of sample was dried in a oven (80^{o}C), then lyophilised in a freeze drier to constant weight. These samples were then brought to room temperature in a desiccator before weighing.

4.2 Results and Discussion

Activated carbon treatment of the UF permeate resulted in a totally clear sample, while samples treated by DE retained a slight brown colour present in the original UF permeate. Organic acid concentrations (as determined by HPLC) were largely unaffected by both AC and DE treatments. Results of the SPE extraction experiments are summarised in Table 4.1.

Table 4.1: Hydroxytyrosol purification using solid phase extraction with activated carbon and diatomaceous earth (Celite) pre-treatment

	UF permeate	Activated carbon	Celite
Total solids (mg.L^{-1})	30272	29539	30198
HT conc. (mg.L^{-1})	1044	333	1029
HT loaded (mg)	10.4	3.3	10.3
Eluate HT conc. (mg.L^{-1})	729	163	701
Eluate HT mass (mg)	8.8	2.0	8.4
% Recovery	84	59	82
% Purity	95	98	96

Activated carbon adsorbed 733 mg.L^{-1} of dissolved substances in the UF permeate while the Celite adsorbed only 74 mg.L^{-1}. The mass adsorbed onto the AC is almost completely accounted for by HT, which is undesirable as it is a product loss. Little HT was lost by the Celite. The HT mass loaded onto the columns is calculated from the HT concentration after clean-up multiplied by the volume loaded (10 ml). Similarly eluate concentration was measured by HPLC and then multiplied by eluate volume to obtain the mass eluted; from these the percentage recovery was calculated.

From these results it would appear that the pre-treatment steps are largely unnecessary, however it should be pointed out that the column loading rates were very low in these experiments – around 2% in terms of HT. It is possible (and even likely) that pre-treatment will enhance the loading capacity and purity of the eluate when the system is operated at maximum column loading (break through) levels. Diatomaceous earth initially appears more suitable for this task than AC (that absorbs HT product), but further studies will be needed to verify this. In particular, lower AC application rates should be investigated.

The purity of the HT recovered after one chromatographic step is very high at > 95%; at this value the HT is suitable for most applications in the food, beverage, cosmetic or pharmaceutical industries. The quantity of HT recovered from any particular wastewater batch will vary depending on the initial concentration in the waste; however, this work provides some initial estimates for percentage recovery possible. The quantity recovered from a particular wastewater batch will also depend on the concentration factor obtained from the UF process. In this particular case, 800 $mg.L^{-1}$ was obtained, based on initial wastewater volume.

5. CONCLUSIONS

The following conclusions can be drawn from the experiments in this work:

- The UF membrane system has been shown to be capable of successfully separating low M_w phenolics, particularly HT, from higher M_w pigmented compounds. This is particularly evident with the BOB, which in general contains more HT than the GOB.
- Membranes with larger pore sizes foul more rapidly than those with smaller pores, although initial or PWF is proportional to MWCO.
- The fouling layer affects the passage of total phenols, which declines as the fouling layer develops and higher M_w phenols are retained. However, the passage of HT is affected to a lesser extent, which means that purity improves as flux declines due to fouling.
- The 15 kDa membrane gave the highest yield of HT (and, by implication, extraction rate), but the separation of low M_w phenols from higher M_w compounds was better with the 5 and 1 kDa membranes.
- The passage of salts and organic acids through all the UF membranes was largely unaffected, although a small amount of these compounds appeared to be entrained in the fouling layer.
- Critical flux experiments showed that it is possible to operate near the maximum operating capacity without significant increases in membrane fouling, however…
- Extended continuous operation with backflushing showed that it was advisable to operate below the maximum, as this allowed for a better flux recovery after backwashing.
- Volumetric concentration values for the UF feed/retentate stream will depend on the varying composition of the wastewater. However, using a typical wastewater sample the feed was concentrated by 70% without the complete loss of flux, and thus further concentration and extraction is possible.
- Estimates for UF extraction yields and rates were obtained; this allows for estimates of required membrane surface area for scaled up systems for the treatment of larger wastewater volumes.
- Pilot-scale UF experiments should be performed to refine estimates for extraction rates and yields through the generation of more extensive long-term data.
- The best NF experimental results were unsatisfactory in terms of separating salts and HT in the UF permeate stream: 20% of the salt was retained while 50% of the concentrated HT product was lost.
- In light of the above point, chromatographic recovery of HT from the UF permeate (before NF) was investigated. High product yield and purity was obtained (84 and 95% respectively) without any pre-treatment clean-up process. Pre-treatment may, however be necessary at higher column loading rates.
- Maximum chromatographic column loading rates need to be determined in future work, as chromatographic purification is expensive and will be responsible for a large percentage of the overall process cost.
- Further NF experiments need to be performed on the residuals from the chromatographic process. It needs to be determined what the purity of this water is, and whether it will ultimately be suitable for recycling back into the olive fermentation process.

REFERENCES

BAZOTI FN, GIKAS E, PUEL C, COXAM V and TSARBOPOULOS A (2005) Development of a sensitive and specific solid phase extraction-gas chromatography-tandem mass spectrometry method for the determination of elenolic acid, hydroxytyrosol, and tyrosol in rat urine. Journal of Agricultural and Food Chemistry **53** (16) 6213-6221.

BITLER C, MATT K, IRVING M, HOOK G, YUSEN J, EAGAR F, KIRSCHNER K, WALKER B and CREA R (2007) Olive extract supplement decreases pain and improves daily activities in adults with osteoarthritis and decreases plasma homocysteine in those with rheumatoid arthritis. Nutrition Research **27** (8) 470-477.

BORSANI, R and FERRANDO, B (1996) Ultrafiltration plant for olive vegetation waters by polymeric membrane batteries. Desalination **108** 281-286.

BRENES M, REJANO L, GARCIA P, SANCHEZ A and GARRIDO A (1995) Biochemical changes in phenolic compounds during spanish-style green olive processing. Journal of Agricultural and Food Chemistry **43** (10) 2702-2706.

BURTON SG,COWAN DA, GARCIN C, VAN SCHALKWYK A and WERNER C (2006) A customised bioreactor for beneficiation and bioremediation of effluents containing high value organic chemicals. Research Report No. 1361/1/06, Water Research Commission, Pretoria, South Africa.

COVAS M, DELATORRE K, FARRÉ-ALBALADEJO M, KAIKKONEN J, FITÓ M, LÓPEZ-SABATER C, PUJADAS-BASTARDES M, JOGLAR J, WEINBRENNER T, LAMUELA-RAVENTÓS R and DELA TORRE R (2006) Postprandial LDL phenolic content and LDL oxidation are modulated by olive oil phenolic compounds in humans. Free Radical Biology and Medicine **40** (4) 608-616.

DHOUIB A, ALOUI F, HAMA, N and SAYADI S (2006) Pilot-plant treatment of olive mill wastewaters by *Phanerochaete chrysosporium* coupled to anaerobic digestion and ultrafiltration. Process Biochemistry (**41**) 159-167.

FITÓ M, CLADELLAS M, DELA TORRE R, MARTÍ J, ALCÁNTARA M, PUJADAS-BASTARDES M, MARRUGAT J, BRUGUERA J, LÓPEZ-SABATER MC, VILA J and COVAS MI (2005) Antioxidant effect of virgin olive oil in patients with stable coronary heart disease: a randomized, crossover, controlled, clinical trial. Atherosclerosis **181** (1) 149-158.

GARCIN CJ (2005) *Investigation into the bioremediation and beneficiation of olive-derived wastewaters from the Western Cape*. PhD thesis, Chemical Engineering Department, University of Cape Town, Cape Town, South Africa.

GARCIN CL and BURTON SG (2007) Implementation of a generic membrane-based system for beneficiation and treatment of Agro-Industrial wastewater. Research Report No. KV 186/07, Water Research Commission, Pretoria, South Africa.

HAFIDI M, AMIR S and REVEL J (2005) Structural characterization of olive mill waster-water after aerobic digestion using elemental analysis, FTIR and 13C NMR. Process Biochemistry **40** (8) 2615-2622.

HAMDI M (1993) Future prospects and constraints of olive mill wastewaters use and treatment: A review. Bioprocess and Biosystems Engineering **8** (5) 209-214.

LESAGE-MEESSEN L, NAVARRO D, MAUNIER S, SIGOILLOT J, LORQUIN J, DELATTRE M, SIMON J, ASTHER M and LABAT M (2001) Simple phenolic content in olive oil residues as a function of extraction systems. Food Chemistry **75** (4) 501-507.

MATEOS R, GOYA L and BRAVO L (2005) Metabolism of the olive oil phenols hydroxytyrosol, tyrosol, and hydroxytyrosyl acetate by human hepatoma HepG2 cells. Journal of Agricultural and Food Chemistry **53** (26) 9897-9905.

OBIED HK, BEDGOOD JDR, PRENZLER PD and ROBARDS K (2007) Bioscreening of Australian olive mill waste extracts: Biophenol content, antioxidant, antimicrobial and molluscicidal activities. Food and Chemical Toxicology **45** (7) 1238-1248.

PARASKEVA CA, PAPADAKIS VG, TSAROUCHI E, KANELLOPOULOU DG and KOUTSOUKOS PG (2007) Membrane processing for olive mill wastewater fractionation. Desalination **213** (7) 218-229.

RIETJENS S, BAST A and HAENEN G (2008) The olive oil antioxidant hydroxytyrosol protects the aorta against oxidative stress. Chemico-Biological Interactions In Press, Corrected Proof.

ROMERO C, MEDINA E, VARGAS J, BRENES M and DECASTRO A (2007) *In vitro* activity of olive oil polyphenols against *Helicobacter pylori*. Journal of Agricultural and Food Chemistry **55** (3) 680-686.

RUSSO C (2007) A new membrane process for the selective fractionation and total recovery of polyphenols, water and organic substances from vegetation waters. Journal of Membrane Science **228** 239-246.

SCHAFFER S, PODSTAWA M, VISIOLI F, BOGANI P, MULLER WE and ECKERT GP (2007) Hydroxytyrosol-rich olive mill wastewater extract protects brain cells *in vitro* and *ex vivo*. Journal of Agricultural and Food Chemistry **55** (13) 5043-5049.

TUCK KL and HAYBALL PJ (2002) Major phenolic compounds in olive oil: metabolism and health effects. Journal of Nutritional Biochemistry **13** 636-644.

TURANO E, CURCIO S, DE PAOLA MG, CALABRO V and IORIO G (2002) An integrated centrifugation-ultrafiltration system in the treatment of olive mill wastewater. Journal of Membrane Science **209** 519-531.

VISIOLI F, CARUSO D, PLASMATI E, PATELLI R, MULINACCI N, ROMANI A, GALLI G and GALLI C (2001) Hydroxytyrosol, as a component of olive mill wastewater, is dose-dependently absorbed and increases the antioxidant capacity of rat plasma. Free Radical Research **34** 301-305.

VISIOLI, F, ROMANI, A, MULINACCI, N, ZARINI, S, CONTE, D, VINCIERI, FF and GALLI, C 1999, Antioxidant and other biological activities of olive mill waste waters. Journal of Agricultural and Food Chemistr*y* **47** (8) 3397-3401.

WALTER WM JR, FLEMING HP and ETCHELLS JL (1973) Preparation of antimicrobial compounds by hydrolysis of oleuropein from green olives. Applied and Environmental Microbiology **26** (5) 773-776.