Pilot Scale Treatment of Table Olive Brines: Beneficiation, Purification and Water Recovery for Re-Use

Report to the

WATER RESEARCH COMMISSION

by

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EXECUTIVE SUMMARY

BACKGROUND

Olives are exceedingly bitter and need to be cured to make them palatable before consumption. The curing process involves placing the olives in a brine solution whereupon a spontaneous lactic acid and/or yeast fermentation takes place. The brining process takes from 3 to 12 months, depending on cultivar and type (green or black), and is associated with various washing and rinsing steps. This results in noxious darkly-coloured and acidic wastewaters with a high organic load (COD < 70 $g.L^{-1}$), high phenolic content (< 5 $g.L^{-1}$), and high salinity (~10% NaCl, three times more than sea water). It is a water-intensive process, up to 10 kL of water is consumed per ton of olives processed. The wastewaters generated present an environmental disposal problem, as they are not amenable to biological treatment, and cannot be disposed of in municipal sewage systems or the environment for toxicity reasons. They are generally disposed of in evaporation ponds.

The wastewaters do, however, contain valuable components, in particular low molecular weight (monomeric) phenolic compounds with powerful antioxidant activity and numerous other beneficial effects on human health. In particular, hydroxytyrosol (3,4-dihydroxyphenyl ethanol, or HT, see Figure 1) occurs in the wastewaters at levels of around 1 $g.L^{-1}$. This is one of the most powerful known naturally occurring antioxidants; its activity is 10 times higher than similar compounds occurring in green tea and red wines, and twice that of co-enzyme Q10. HT has been the subject of extensive research due to its diverse biological properties, which include anticancer activity and cardioprotective effects; it inhibits LDL oxidation and subsequent platelet aggregation (arteriosclerosis), it has antimicrobial and antiviral activity, and more.

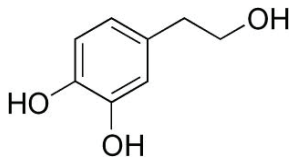

Figure 1: Hydroxytyrosol (3,4-dihydroxyphenyl ethanol)

Recovery of HT is therefore of significant interest, as it can be used in the pharmaceutical/nutracuetical, personal care products, and cosmetic sectors as an active ingredient, or in the food and beverage industries as a natural alternative to currently used synthetic antioxidants and preservatives. Purified HT (<98%), such as used for research purposes, is highly valuable, selling for ~ US$1000-2000 per gram.

RATIONALE

Based on previous laboratory research, a comprehensive treatment system was designed in order to recover the HT and simultaneously recover purified brine that can be re-used for table olive production, while minimising the amount of waste for disposal to the evaporation ponds. In this manner, the cost of wastewater treatment can be offset by the recovery of value added products that can be commercially exploited.

OBJECTIVES AND AIMS

The main objective of the project was to develop and construct a modular, containerised wastewater treatment system for on-site evaluation at an olive farm and processing factory, at pilot scale. Specific aims were:

- Design, construct, and install the system, and integrate it as an end-of-pipe treatment unit at the farm
- Recover purified brine for recycling back into olive production process
- Recover an antioxidant extract for purification and evaluation
- Evaluate overall process performance and the economic feasibility of the system.

METHODOLOGY

The process flow diagram for the system is shown in Figure 2. Wastewater batches from the factory were diverted into a holding tank for temporary storage. From there 1 kL batches of the discharge were processed through 2 sequential unit processes. Firstly, fine ultrafiltration (< 1 kDa) was performed to remove high M_w polyphenols (lignins and tannins) for disposal to waste, with the membrane permeate stream then containing low M_w phenolics (antioxidants), salt, organic acids and some other minor components. Secondly, this permeate stream was passed through a chromatography column containing a selectively adsorbent resin. The low M_w phenolics and some other minor organics bind to the resin, while a purified brine stream exits the column. When the column becomes fully loaded and can adsorb no more (resin saturated), it is rinsed and an ethanol solution is passed through. This causes the phenolics to desorb from the resin, the ethanol solution is then distilled to obtain a crude antioxidant extract and the ethanol is recovered for re-use. The ethanol also regenerates the column and, after rinsing, it is ready for the next loading cycle.

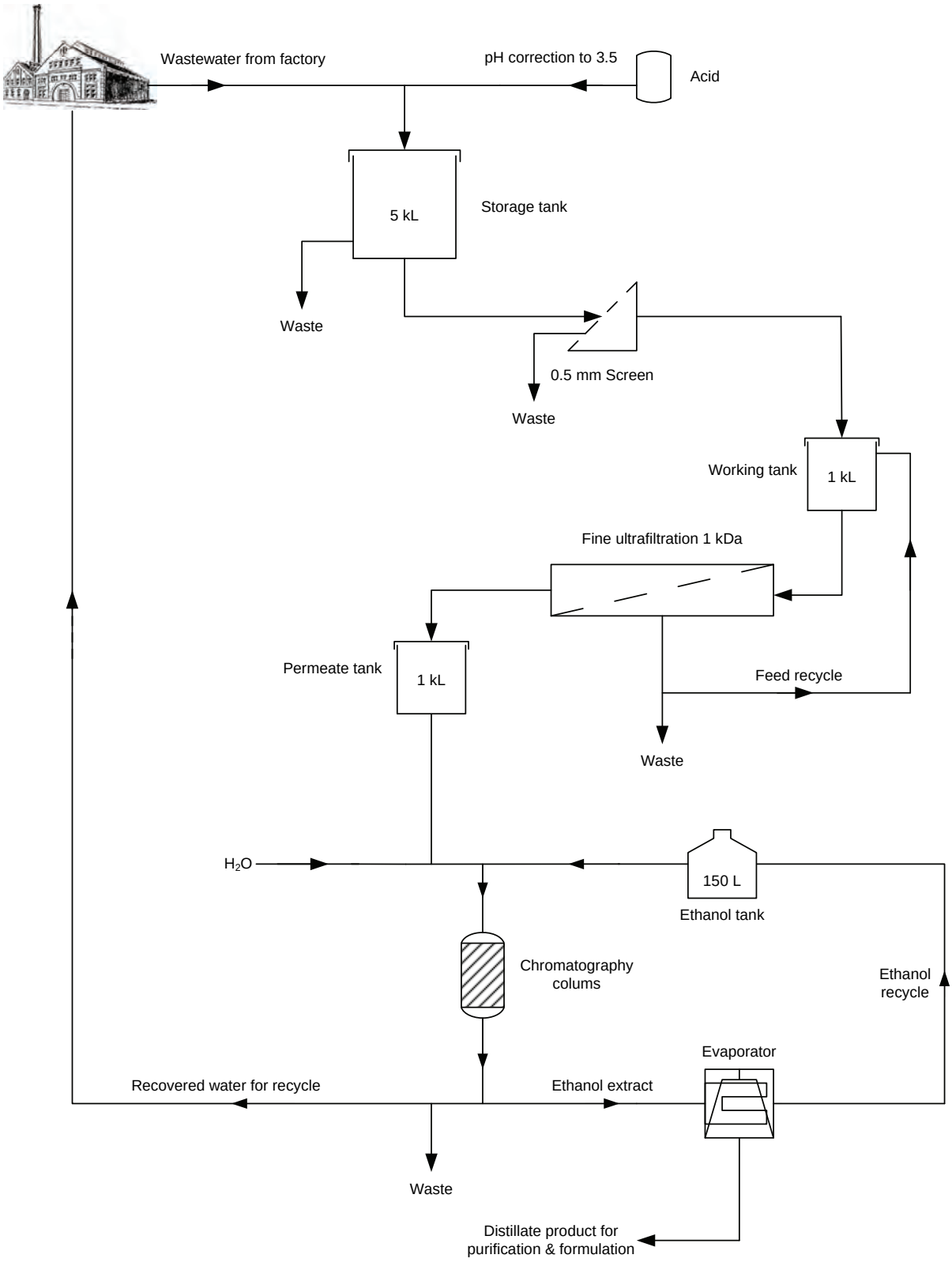

Figure 2: Process flow diagram for olive brine wastewater treatment plant

Pilot scale experimental batches were carried out over a six month period. The experimental objectives were to:

- Optimise and evaluate the membrane performance in terms of permeate flux and fouling, backwash frequency and duration, and chemical cleaning procedure and interval (CIP).
- Investigate the loading capacity, product recovery, purified water quality and cyclical performance of the column.
- Analyse the crude extract and investigate methods for the purification thereof.
- Evaluate the overall process in terms of volumetric productivity and yield based on plant size.

RESULTS AND DISCUSSION

The pilot-scale system was designed, constructed and operated for 6 months during which data was collected and analysed, the yields and productivity of the process were established, and the economics of the treatment process were evaluated. Operation of the pilot plant has demonstrated that the technology is effective for treating such highly polluted brine wastewaters.

The membrane was able to satisfactorily separate the high M_w phenolic components from the waste stream resulting in clear brine stream that was then sent to the chromatography system; this was able to produce a purified brine stream for recycle, whilst retaining the antioxidants for recovery.

With the relatively small scale of the equipment used for the pilot plant, it was possible, on average, to process a 1 kL batch of wastewater per week, depending on the variable characteristics of the feed. Approximately 50-75% of this feed could be recovered as purified brine for recycling, while an additional ~300 L of fresh water was used for backwashing the membrane system and processing the chromatography column. Approximately half of this fresh water usage was also recovered for recycle. An average of 360 g of antioxidant product was produced per 1 kL batch of wastewater processed. The operational cost of production at the current level was around R20 per gram of antioxidant in the form of a crude extract, with a corresponding zero cost for the treatment and recovery of the wastewater.

There is scope for significant improvement of the throughput, yields and productivity of the plant by using continuous (overnight) and simultaneous operation of the membrane and chromatography systems, as well as various modifications that can be made to improve the overall process.

CONCLUSIONS

The majority of the objectives were achieved. Yields and productivity of the system were, however, lower than anticipated for 2 main reasons: firstly, throughput of the membrane system was lower than anticipated due to high suspended solids concentrations in the feed and subsequent fouling of the membrane. This can be improved by installing a pre-filter for the wastewater before membrane processing. Secondly, yields from the chromatography column could be improved by using pure ethanol for elution (denatured ethanol was used during this project), and the aspect ratio of the column needs to be changed (longer and thinner), to achieve a more uniform flow distribution through the column.

Nonetheless, the system may be financially viable even at pilot scale. The system would need to be scaled up by a factor of 10-20 to treat all the brine emanating from the farm where the pilot scale studies were performed. This does not imply scaling-up of the footprint of the treatment plant by the same amount, merely scaling-up of the unit operations accordingly. This could possibly be achieved by increasing the footprint of the current plant by a factor of 3-4.

A spin-out company is being formed to exploit the IP generated during the project and develop the process into a full scale treatment system. Thereafter it is intended to roll out the technology to other olive producers, and investigate other possible applications of the technology. The process is only feasible if there are value-added products to be obtained from a waste stream; if wastewater treatment alone is considered, it is expensive due to the high cost of the speciality membranes and the chromatography resin used.

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LIST OF ABBREVIATIONS

CeBER	Centre for Bioprocess Engineering Research (at UCT)
CIP	Cleaning-in-place
COD	Chemical oxygen demand
Da	Daltons
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
HT	Hydroxytyrosol
IP	Intellectual property
kDa	Kilo Daltons
kL	Kilo litres
LSM	Living standards measure
M_w	Molecular weight
MWCO	Molecular weight cut-off
N	Normality
NaCl	Sodium chloride
NaOH	Sodium hydroxide
P&ID	Piping and instrumentation diagram
PLC	Programmable logic controller
TBHQ	Tetra hydroxyl butyl quinine
UCT	University of Cape Town
UV	Ultra violet light
WRC	The Water Research Commission

1. INTRODUCTION

The purpose of WRC project K5/2010 was to investigate the development and operation of a combined membrane-chromatography pilot-scale system for the treatment of wastewater brines that result from the table olive fermentation process. In brief, table olives are produced as follows: after harvesting, the olives are washed and then placed in a brine solution (8-12% NaCl) and adjusted to pH 4-5 using citric or other acids. A spontaneous lactic acid bacteria and/or yeast fermentation then takes place; this reduces the bitterness of the olives and improves the texture. The fermentation can last anywhere from 2 to 12 months, depending on whether the olives are green or black, whether they are sliced or not, and various other factors such as cultivar and pre-treatment. Green olives often also undergo a pre-treatment with sodium hydroxide to remove excess bitterness prior to fermentation. After fermentation the olives are rinsed, packaged, pasteurised, and then sent to market.

The brines are environmentally toxic, due to acidity, salinity, and high concentrations of phenolic compounds contained therein, which can also give them a dark colour. The brines have a high chemical oxygen demand (COD) of up to 70 $g.L^{-1}$. They cannot be disposed of in municipal waste treatment systems or into the environment, and are mostly disposed of in evaporation ponds. The wastewaters do however contain low molecular weight phenolic compounds with powerful antioxidant activity and of high value. A pilot-scale treatment system was thus developed with four main objectives:

- Recovery of a purified brine wastewater stream for recycling into the table olive production process
- Extraction and purification of the antioxidants
- Minimisation of the final effluent volume for disposal
- Evaluation the economic feasibility of the process

The objective of the wastewater treatment aspects of the project were minimisation and recovery; reduction of chemical oxygen demand (COD) was not an objective as the minimised disposal volumes are discharged to the (aerated, lined) evaporation ponds. This is perceived to be a suitable solution for the wastes as they have little further value and are resistant to biodegradation.

The process devised for the treatment of the brine wastewaters was based on previous work conducted during WRC project K8/814. The overall treatment process developed in the laboratory during this project resulted in the reduction of the disposable waste to < 30% of the original volume, recovery of process water (>70%) and inorganics such as NaCl for re-use within the olive industry, and successful extraction of valuable antioxidant compounds from the waste stream. On the basis of

this research, a further pilot-scale project was proposed, in order to assess scale and field application, long-term operation and the economic viability of the process.

In brief, the process developed is as follows: after pre-treatment (pH adjustment, coarse pre-filtration), the wastewater is directed through a ceramic fine ultrafiltration membrane with a molecular weight cut-off (MWCO) of 1 kDa. The purpose of the membrane is to retain the high molecular weight phenolic compounds such as lignins and tannins in a progressively more concentrated feed stream, while the lower molecular weight components permeate through the membrane. The permeate stream thus consists of water, salts, organic acids, and low molecular weight phenolic compounds of interest (antioxidants), as well as some other minor components. A ceramic membrane was chosen because of the particularly aggressive nature of the wastewater and harsh chemical cleaning required. The concentrated feed stream is disposed as per normal to the evaporation ponds.

The permeate stream is then directed through a chromatography column containing a selectively adsorbent resin that retains the phenolic compounds, while the rest of the components pass through the column, resulting in a purified brine stream that is suitable for re-use in the olive production process. The purified brine can be re-used either as make-up water for olive fermentations, or for final product packaging. Once the column is saturated with phenolic compounds, it is rinsed, then the phenolics are eluted from the column using ethanol as a solvent. The ethanol is then distilled to recover a crude “olive extract”, containing high concentrations of phenolic antioxidants, predominantly hydroxytyrosol. Figure 2 shows a schematic process flow diagram of the overall process.

A feature of the process developed is that the financial and energy costs associated with wastewater treatment and recovery for re-use can be offset by the production of value-added products, which makes an appealing case for olive producers who are often reluctant to invest in wastewater treatment plants due to the expense. The main innovation of the technology is that antioxidant extraction and wastewater purification are a result of the same process with shared unit operations. The process can be considered green, as no environmentally harmful chemicals or solvents are used, water and salts are recovered and recycled, and the discharge amounts of potentially harmful wastewaters generated by the industry can be reduced.

Using such technology, it is also possible to undercut the cost of competitors’ antioxidant products, because of the different waste resources and technology being exploited. Competitors use wastes resulting from olive oil pressing, or leaves from tree pruning to produce their products. These are solid or semi-solid sludge-like matrices, and require complex processing, extraction and purification techniques. These extracts contain numerous other components including lipids, pectins, carbohydrates and general cellular debris that complicate purification processes.

The technology that was developed in this work is for the liquid waste brines generated by the production of table olives, and therefore allows for relatively easy processing. Furthermore, the extracts produced in this manner have fewer other components that complicate further purification, and correspondingly the crude extracts produced can have higher concentrations of hydroxytyrosol than those reported above (> 20%). Considering that the brines contain ~ 1g/L of hydroxytyrosol, and that Buffet Olives (the biggest local producer) discards 500 000-1 000 000 L of this brine annually, there is a considerable resource to be exploited at this one farm alone.

From a market perspective, naturally derived antioxidants are becoming increasingly popular in many sectors as alternatives to conventional synthetically derived preservatives (e.g. THBQ), with the added benefit that they have health-enhancing properties. This is driven by growing consumer awareness and market demand. Hydroxytyrosol (See Figure 1), which occurs predominantly in olives, is one of the most powerful known natural antioxidants and free radical scavengers; it is more than 10 times as effective as compounds occurring in green tea and red wines, and twice as effective as coenzyme Q10. Pure hydroxytyrosol (> 90%) costs from R15,000 to well over R50,000 *per gram* because of the purification processes employed; this essentially limits the use of such to medicinal and scientific research. Extracts containing hydroxytyrosol (at concentrations from 2-12%) cost from R20-R70 per gram. These extracts are not generally well characterised in terms of the other components, and can thus be of dubious quality.

The intention is to develop the process further and scale up for commercial application, through the creation of a spin-out business that will exploit the intellectual property generated during the project. The proprietors of the Buffet Olives farm have agreed to continued operation of the pilot-plant after the end of the research project (May 2012), and are interested in seeing plans for further development, as they are intrigued by the possibility of re-using their wastewater and minimising effluent discharge, at little or no cost to them except for some infrastructural expenses. They are willing to allow continued operations for the indefinite future while the process is further developed.

2. SYSTEM DESIGN, CONSTRUCTION AND COMMISSIONING

This section describes the design, construction and installation of the integrated system, which is a containerised on-site unit at the Buffet Olives farm near Paarl. The objectives of this phase of the project were:

- Process and system design for membrane and chromatography units
- Specification, sourcing and acquisition of required equipment and consumables
- Construction of the membrane and chromatography systems
- Commissioning of the systems, including computerised monitoring and control

2.1 Process description

The schematic piping and instrumentation diagram (P&ID) for the system, including the membrane and chromatography processes, is shown in Figure 3 (the distillation of extract is not included, it is a separate stand-alone unit operation). Operation of the membrane and chromatography systems were both comprised of several sub-processes which are described in detail below. These sub-processes were designed into the P&ID, such that they could be controlled by a programmable logic controller (PLC). The membrane and chromatography systems were designed to operate independently of each other, in a sequential manner. This allowed for monitoring of process parameters, and independent optimisation thereof.

For the membrane system, there are 3 basic modes of membrane operation: normal filtration with periodic backwashing; chemical cleaning, comprising base (NaOH) and acid (H_3PO_4) washes with rinsing to neutrality after each; and soaking in hyperchlorite, also for cleaning purposes. Filtration was designed as a batch-wise tangential flow process with feed recycle.

Pretreatment
Wastewater is re-directed from normal discharge (to digester) into a 5 kL holding tank (T1) directly from the factory. From here it is transferred by Pump 1 through a 0.5 mm screen into the working tank (T2). The working tank is fitted with a dosing system for pH adjustment (to pH 3) using HCl. The wastewater is acidified in order to: reduce oxidation reactions during processing, prevent microbial activity in the feed, and to hydrolyse conjugated phenolics into simpler compounds. Once the working tank is full a filtration batch can commence. The holding tank is fitted with manual valve (MV14) for settled sludge discharge to waste when required. Waste discharge is to the factory sump, which acts as an aerated digestor. Pump 1 is mobile and connects through tubing with detachable fittings such that it can be used to pump this and other waste back into the digestor.

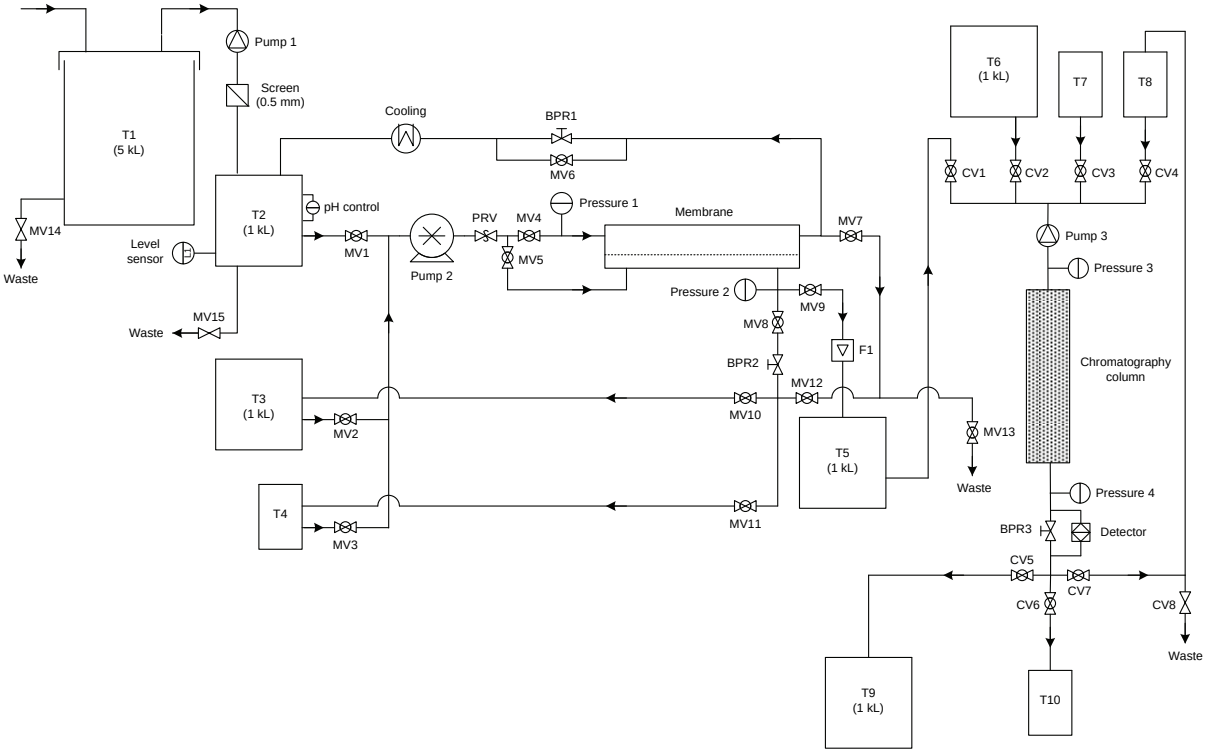

Figure 3: Piping and instrumentation diagram for olive brine wastewater treatment plant

Filtration

The purpose of filtration is to separate and retain high molecular weight phenolic compounds in the feed from the low molecular weight phenolics (antioxidants), which pass through the membrane. Wastewater is pumped from the working tank to the membrane module by a high pressure pump (P2). There is an adjustable pressure relief valve (PRV) fitted between the membrane and the pump, set to 10 bar as a safety feature. This prevents damage to the membrane due to high pressure in the event of a blockage or valve failure. The mode of normal membrane operation is recycled tangential flow, with a back pressure regulator (BPR1) fitted to the retentate path to create transmembrane pressure. Permeate from the module is directed to a secondary holding tank (T5), while the permeate flow rate is measured by a flow meter (F1). When the permeate flow rate declines below a certain value, chemical membrane cleaning is required. Mechanical and electronic pressure gauges were fitted to the membrane inlet and permeate outlet for operational data, the difference between the two being the approximate applied transmembrane pressure. The electronic pressure gauge was programmed *via* the PLC to act as a fail-safe by cutting out the high pressure pump in the event of system overpressure. A cooling system was fitted to the retentate stream to avoid excessive temperature build-up in the working volume, however this was later bypassed as it was found to be unnecessary and caused excessive back pressure. The cooling system consisted of a stainless steel coil immersed in a chilled water bath. The working tank was also

fitted with a level sensor that shuts down the pump in the case of the working volume dropping too low.

Flush
Flushing is required before backwashing in order to remove concentrated feed from the high pressure pump (2) and piping. This is performed for a short period only, and ensures that the permeate side of the membrane is not contaminated with feed wastewater. Flushing is performed with clean water from tank T3, while the flushed volume is directed back to the working tank (T2).

Backwash
Backwash is performed periodically to remove the fouling layer that builds up during normal filtration. Clean water from tank T3 is directed through the permeate side of the membrane, and a second backpressure regulator (BPR2) forces clean water through to the feed side of the membrane and this is returned to the feed tank, bypassing BPR1 so as to achieve the maximum transmembrane pressure during backwash.

Chemical cleaning
When backwashing is no longer sufficient to restore membrane flux, chemical cleaning is performed. First there is a short flush to remove wastewater feed from the system. Washing with base is performed first (20 g/L NaOH), followed by a short flush and then rinsing to neutrality with clean water. Rinsing is performed with clean water in the same manner as backwashing except permeate coming out the feed side of the membrane is discarded. After this acid washing is performed (0.75% H_3PO_4), followed by flushing and rinsing to neutrality again. The acid and base washes are combined for neutralization and discarded to waste. After extended operation when the base and acid washing fails to restore permeate flux to a suitable level, the membrane is soaked overnight in a hyperchlorite solution, with flushing and rinsing thereafter, as above.

The chromatography process that occurs after filtration of a wastewater batch also comprises several steps:

Chromatography load
When a 1 kL batch of wastewater has been processed through the membrane system, it is pumped from tank T5 through the chromatography column using a controllable variable speed peristaltic pump (Pump 3). The purpose of the chromatography is to adsorb low molecular weight phenolics of interest, while allowing salts, organic acids, and purified water to pass through. Pressure is measured before and after the column, while the outlet is monitored using a purpose built UV detector at 280nm for measuring phenolic compound concentration (WRC project K8/865). The detector has a 0-10 V output that is inversely proportional to phenolic concentration. When this voltage starts to drop then it can be assumed that

the column is saturated with phenolics and the next step can begin. Wastewater exiting the column during the loading stage is ideally colourless, odourless, and preferably sterile, and thus suitable for process recycle or packaging. This wastewater is collected in tank T9.

Chromatography rinse 1
When the column is fully loaded with a batch of permeate from the membrane system it is necessary to rinse out residual salts and acids before recovering the phenolic compounds by elution with an ethanol solution. Clean water from tank T6 is directed through the column and collected in tank T9. This adds to the water available for process recycle or packaging. The output from the column can be manually monitored using pH and conductivity meters, although a set volume of liquid is usually sufficient to clean the column.

Chromatography elute
An ethanol solution is used for elution of the phenolic compounds (tank T7). This is collected in the product tank (T10). The UV detector can be used to monitor phenolic compounds exiting the column using adsorption measurements at 280nm. The UV detector was custom built for this purpose using LED's and photodiodes, as a low cost alternative to commercially available spectrophotometers. This was the subject of WRC project K8/865.

Chromatography rinse 2
After elution, residual ethanol in the column needs to be removed. Clean water from tank T6 is once again directed through the column, but is discarded in this case because of residual ethanol. A set volume of water is used for this rinse, after which loading can re-commence and the cycle be repeated.

Chromatography clean
After a certain amount of chromatography cycles, the resin needs to be cleaned and regenerated, using hydrogen peroxide (2% H_2O_2 at pH 11.5 with NaOH). After this, rinse 2 is performed as above.

2.2 Physical design and construction

When the components shown in Figure 3 had been specified and sourced, the physical 3-D design was done on a CAD program. The membrane and chromatography systems were incorporated into a skid-mounted system, together with the pumps, valves, and associated equipment. The skid-mounted system design is shown in Figure 4. The design was done in collaboration with Atl-Hydro as consulting engineers.

With reference to Figure 4: The grey box on the left of the skid houses the PLC control unit. This controls all the (solenoid) valves and pumps for the various sub-processes described in Section 2. Below to the right is the high pressure pump, which feeds the membrane unit. A peristaltic pump feeds the chromatography column. There is also a chiller unit underneath the peristaltic pump, which is used for cooling of the working liquid. The piping used was food-grade polypropylene with heat-welded joints. This was a low-cost alternative to stainless steel; the piping is capable of handling the required system pressures and has good chemical resistance.

The skid unit and other equipment was designed to be housed in a modified shipping container that is installed on-site on the olive farm, as shown in the cutaway of Figure 5. The construction process and equipment installed are shown in Figures 6-12.

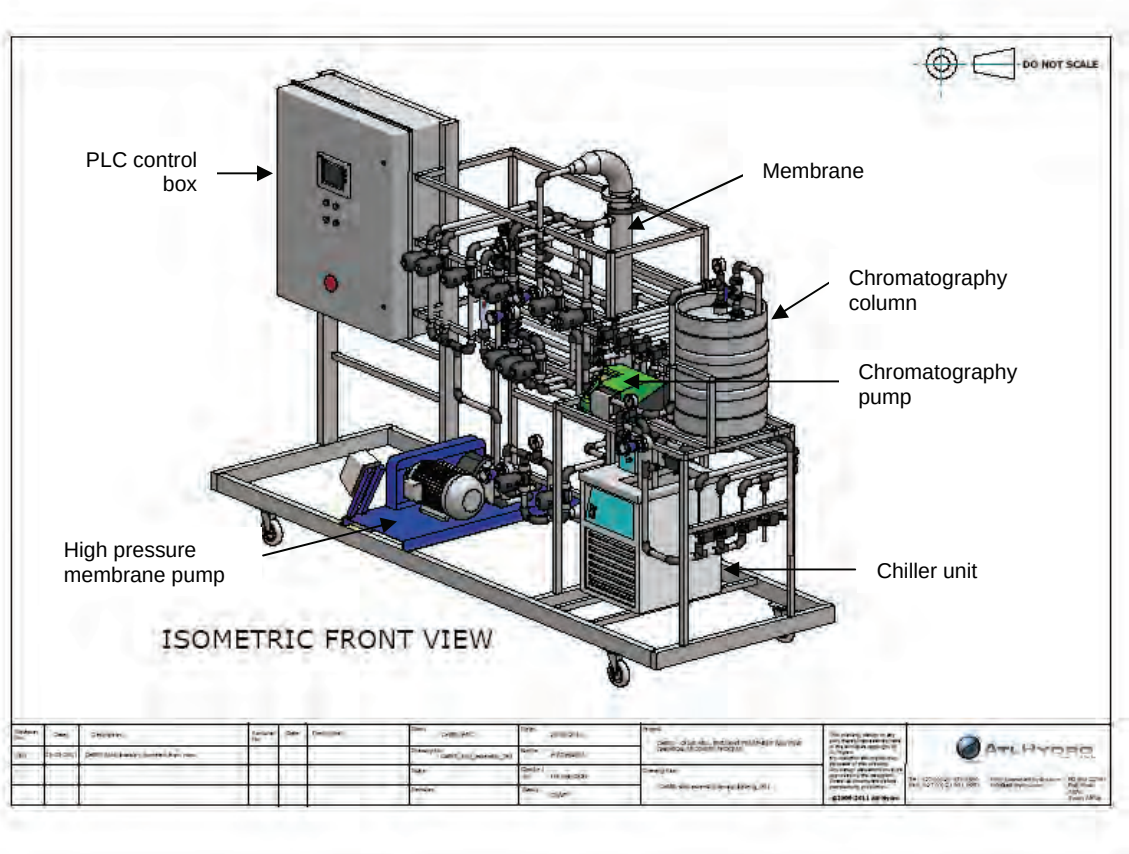

Figure 4: Skid mounted membrane and chromatography systems

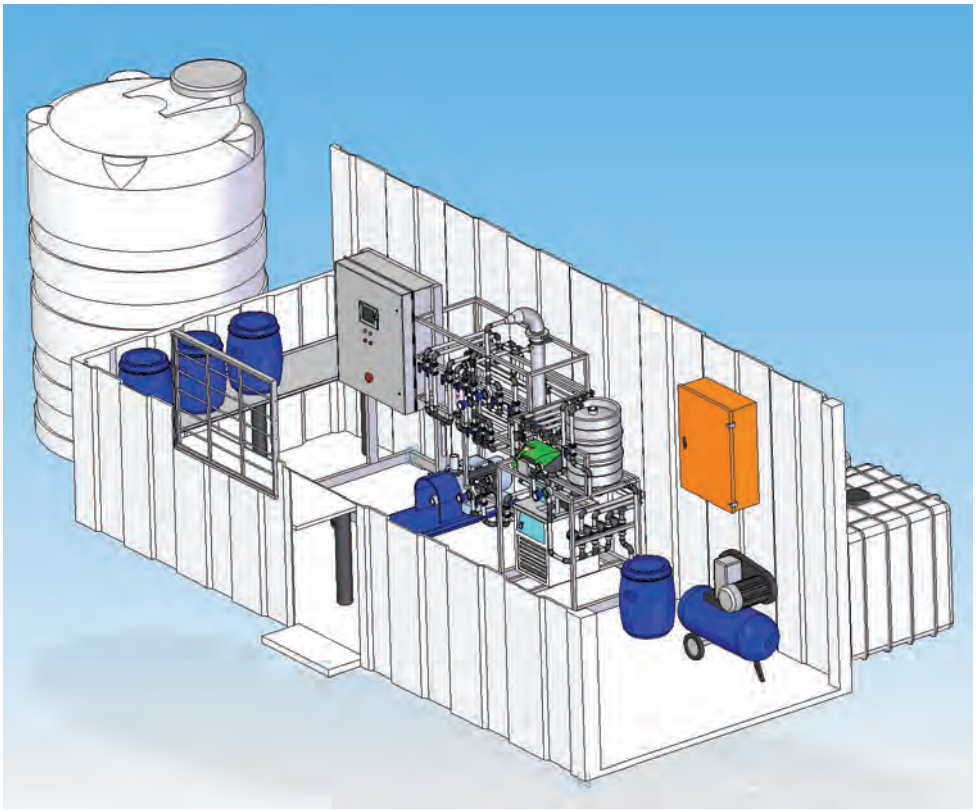

Figure 5: Layout of the containerised system

Figure 6: Site preparation for the container and external tanks. The concrete building in the background stands on top of the factory wastewater sump

Figure 7: Delivery of the container unit

Figure 8: Shelving and wiring installation.

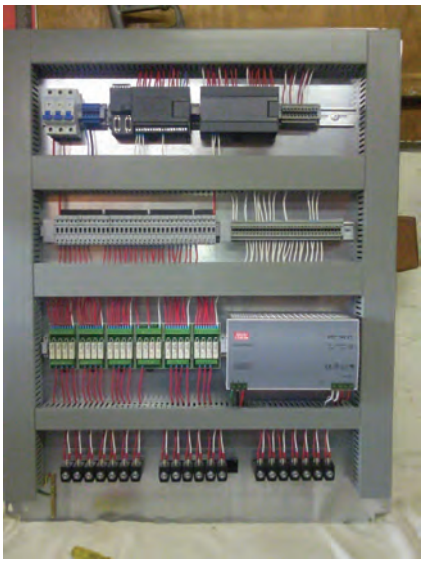

Figure 9: PLC control box and wiring

Figure 10: Tank installation and earthworks for piping

Figure 11: Installation of the skid-mounted system

Figure 12: Completed system ready to operate

The construction of the skid mounted system was outsourced because the Chemical Engineering workshop at UCT was under renovation and therefore unavailable. Atl-Hydro were the lead consultants in this process, and sub-contracted various aspects of the project as necessary. During the skid construction process, plumbing and electricity supply to the container, and related services, were secured locally. Chemicals, consumables and analytical requirements for the pilot scale studies were also acquired and prepared.

2.3 Commissioning

When construction was complete, the plant was thoroughly checked to ensure that all components were working properly. The commissioning process became iterative with faults being corrected as others were discovered. The main items checked include correctly operated PLC switches and voltages for all electrical components, pumps and solenoid valves. Monitoring devices were calibrated as necessary and then checked for correct communication back to PLC or computer. Fail-safe devices such as level- and pressure sensors were checked for correct operation and that they effected the appropriate control signals.

Once there was confidence that all components were operating correctly, dry testing was performed. This involved checking that all components worked correctly for programmed sequential operations, again with troubleshooting and fixing as required.

Wet testing was then performed to establish that all liquid flows followed the correct paths with no problems during sequential operations, at low pressures (i.e. from tanks to unit operations to other tanks, without any pressure regulation), and that fail-safes actuated if they do not. When everything was seen to be in order pressure and flow rate tests were performed. This was done to check that there were no leaks on the system, and that the equipment was performing according to design specifications.

The final phase of commissioning of the completed plant involved doing dummy production runs (using water first, then wastewater) through the entire operational process until everything was seen to be satisfactory. The plant construction was then signed off for the contractors, and proper operations commenced.

3. SYSTEM OPERATION

3.1 Operational protocol

The standard operating protocol for the membrane system was as follows:

1. Discharge from the factory collected in the 5 kL holding tank such that several 1 kL batches could be performed on the same wastewater in order to evaluate reproducibility of results.
2. 900 L of wastewater decanted from holding tank into 1 kL working tank. Take sample.
3. Wastewater circulated through pump back to holding tank while pH adjustment is done by the dosing system. Take initial feed sample.
4. Filtration commences with desired backwash regime, adjust filtration pressure manually using BPR1 and backwash pressure using BPR2.
5. Note permeate flow rate value initially, and then before and after every backwash
6. At end of filtration batch take feed sample (retentate) and volume measurement, and bulk permeate sample and volume measurement.
7. Rinse membrane system with water, measure pure water flux.
8. Discard retentate to wastewater sump, clean and rinse working tank.
9. Set membrane system to clean cycle.
10. Measure pure water flux of cleaned membrane.

The standard operating procedure for the chromatography system was as follows:

1. Run water through column at desired flow rate to determine column pressure.
2. Load membrane permeate onto column, take column permeate samples every 100 L and bulk sample at end.
3. Rinse column with water, measure permeate conductivity and pH every 50 L and take samples until pH neutral and conductivity ~ 0.
4. Elute column with 150 L of ethanol at desired concentration, take bulk eluate sample.
5. Rinse column with 100 L water, discard to waste.
6. Chemically clean column if necessary and rinse.

Phenol concentrations exiting the column are monitored throughout the chromatography process using a UV detector. The ethanol eluate is distilled to obtain a crude phenolic extract; the recovered ethanol is kept for re-use. The samples and crude extract are taken to the laboratory for analysis.

Operation of both systems is performed through the touch screen on the PLC. This controls parameters such as backwash frequency and duration, chromatography flow rates etc. Manual control of the system is also possible using the PLC.

3.2 Materials and methods

All reagents were of analytical or 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, using 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 high performance liquid chromatography (HPLC) on a Thermo-Fischer system using Chromquest software. The column was a reversed phase Phenomenex Luna C18(2) of 5 µm particle size and dimensions 250 x 4.6 mm. The mobile phase was water/methanol/acetic acid in the ratio 80:20:2.5 at a flow rate of 1 $ml.min^{-1}$. UV detection was at 280 nm. Pure hydroxytyrosol 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 x 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.

Free acidity was measured by volumetric titration to pH 8.3 using 0.01 M NaOH, according to APHA standard methods.

Total solids were measured by evaporating 50 ml samples to dryness and then weighing. Suspended solids were measured by filtration (0.45 μm) of a 50 ml sample and then weighing of the filter.

Merck reagents A and B were used to measure COD in conjunction with a digestion block and a spectrophotometer. Potassium hydrogen phthalate was used as a standard, at concentrations of 425 and 850 $mg.L^{-1}$ in distilled water, corresponding to COD values of 500 and 1000 $mg.L^{-1}$ respectively.

3.3 Operational data

Post-commissioning operations started in November 2011. This was several months later than anticipated for several reasons: construction of the system was delayed due to the slow arrival of equipment from overseas; the olive producers had a period of minimal discharge due to poor a harvest in the preceding season; a period of particularly foul weather made operations impossible. An extension of the project

was thus requested such that sufficient operational data could be collected. The system was run for six and a half months up to the writing of this report.

Many batches were attempted during this period, several of which were terminated prematurely while different operational problems were encountered and resolved. Some instances of equipment failure and repair also interrupted progress. Results for eight batches are presented here. The batches were labelled by the date of commencement. Batch A20111114 and A20111121 were performed using the same wastewater; likewise batches B21111129, B20111216 and B20120124 using a different factory discharge; C20120222 and C20120329 another discharge; and D20120411 a final discharge. The different discharges varied substantially in their composition, the details of which are shown in Table 1, as the analysis of the membrane feed volumes for each batch.

All of the wastewaters used were black olive fermentation brines. These contain many polyphenolic lignins and tannins that tend to have an adverse effect on membrane performance, and as such represent a worst case scenario for operation. The performance of the membrane system is presented first. This was analysed in terms of flux (permeate flow) and how this is affected by wastewater composition, then in terms of the separation characteristics. This is followed by analysis of the chromatography data, then a summary is presented of the total volumes of water treated in each batch, the fresh water used, and purified brine recovered.

Membrane flux over time for the various batches is presented in Figure 13. Flux is reported as $L.hr^{-1}.kPa^{-1}$ such that membrane performance over time could be compared for different operating pressures. The variation of wastewater feed composition had a significant effect on the membrane performance. The first two (A) batches were performed on “low-strength” wastewater, compared to the usual black olive brine composition at Buffet Olives (the olives were imported for packaging from other farms). Batch A20111114 shows the effect of transmembrane pressure on membrane flux with different backwashing pressures. It was established that it was possible to operate near the maximum system pressure (800 kPa) without encountering critical flux i.e. rapid drop in flux with increasing pressure due to excessive fouling. It was also established that the backwash pressure was optimal at around 100 kPa above the operating pressure: any less than this and flux recovery was not sufficient after backwash, while if the difference was too large (i.e. 200 kPa) then backwash water volumes caused the feed volume to increase significantly, since backwashing was performed with clean water and directed back to the working tank. The optimal was therefore determined to be 700 kPa for normal operation and 800 kPa for backwash, which is the maximum operating pressure of the system. Run A20111121 was performed without backwashing to establish a baseline scenario; it can be seen that flux drops rapidly to a level below 0.04 $L.hr^{-1}.kPa^{-1}$. This value was designed to be the minimal acceptable flux for feed flow rate to the downstream chromatography system.

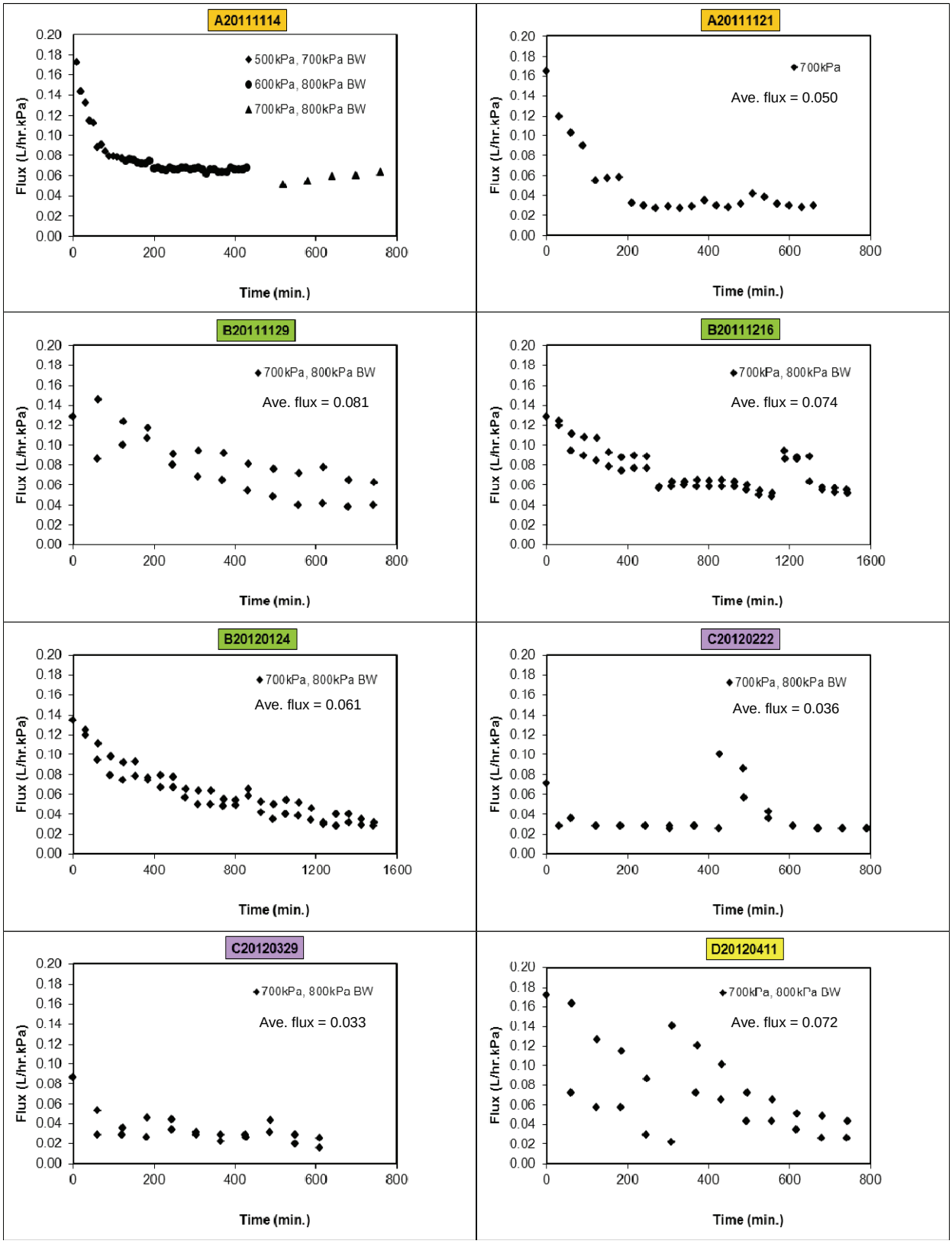

Figure 13: Membrane flux over time for different wastewater batches

The “B” batches were performed on typical black olive brine, having high total solids content of around 120 $g.L^{-1}$ and moderate suspended solids of around 1 $g.L^{-1}$. Data points are shown for flux immediately before and after backwashing, so that the effect on flux recovery could be ascertained. In all 3 batches a general decline in flux was observed, as is to be expected: there is accumulated fouling of the membrane that is not removed by backwashing, and also an increase in the concentration of retained solids in the feed stream, both of which lead to declining flux.

Batches B20111216 and B20120124 show poorer flux recovery after backwashing than the previous batch (B20111129). This is indicative of irreversible fouling as a consequence of pore blocking, as well as the usual fouling due to surface deposition. This was later remedied by overnight soaking in hypochlorite solution. CIP was performed during batch B20111216 just before 1200 min. of run time; it is difficult to say if this was beneficial or not because there were severe temperature fluctuations of the feed due to erratic weather at the time (18^oC followed by 45^oC the next day).

The “C” batches were performed on wastewater that had very high suspended solids content and the effect of this was immediately evident. Flux almost immediately dropped to a level of around 0.03 $L.hr^{-1}.kPa^{-1}$ and backwashing had a minimal effect. CIP was performed in batch C20120222 after 420 of run time, but the flux declined rapidly back to the previous value. The run was repeated in batch C20120329 after a delay to have the high pressure pump serviced; similar results were obtained. These runs resulted in the poorest membrane performance, and illustrate the need to remove suspended solids prior to membrane filtration. This was a contrary result to that obtained in project K8/814 where no prefiltration of wastewater was performed. In that project, wastewater was decanted after solids had settled and were not problematic, whereas in this project the wastewaters were well mixed due to the decanting process from the holding tank into the 1 kL working tank. There is a need for a proper pre-filter to remove these suspended solids at the farm as the 0.5 μm is clearly not sufficient; a separate microfiltration unit or a sand filter need to investigated in this regard.

The last batch (D20120411) was performed using wastewater with a high total solids content, and this too led to a rapid decline of flux, despite the suspended solids content being similar to the “B” batches. Chemical cleaning was necessary after only 5 hours of operation. This batch however led to the best hydroxytyrosol yield from the membrane because of the high concentrations thereof in the feed.

The selective separation properties of the membrane for the different batches are shown in Table 1. The membrane had very little effect on the pH and conductivity, essentially salts and acids were in equilibrium between the feed and permeate streams (retentate refers to the reduced feed stream at the end of the batch). This was verified by the free acidity measurements; equilibrium mass balances closed to

within ± 5% between feed and retentate + permeate. The pH reported in the table refers to the original pH of the raw wastewater, this was adjusted to pH 3 in all cases as described in Section 3.1.

In all cases there were no detectable suspended solids in the permeate stream, which is to be expected because of the very fine MWCO of the membrane, in the nanometer range.

Table 1: Analysis of membrane process data

Batch	Volume	pH	Cond.[a]	TS[b]	SS[c]	Acidity	TP[d]	HT[e]	COD[f]
	(L)		($mS.cm^{-1}$)	($g.L^{-1}$)	($g.L^{-1}$)	($g.L^{-1}$)	($g.L^{-1}$)	($g.L^{-1}$)	($g.L^{-1}$)
A20111114									
Feed	900	5.84	41.5	60	0.89	1.72	0.31	0.087	18.2
Permeate	450	-	-	39	-	1.57	0.06	0.047	–
Retentate	750	-	-	49	2.71	1.16	0.35	0.076	12.9
A20111121									
Feed	900	5.78	39	60	0.93	1.69	0.31	0.082	16.5
Permeate	390	-	-	35	-	1.65	0.06	0.039	–
Retentate	510	-	-	48	2.84	1.70	0.47	0.115	11.9
B20111129									
Feed	900	4.82	78.4	122	1.02	3.95	4.35	1.027	42.6
Permeate	670	-	-	60	-	3.51	0.82	0.636	–
Retentate	380	-	-	175	2.82	3.16	8.83	1.311	35.7
B20111216									
Feed	900	5.24	74.3	120	1.15	3.89	4.3	1.041	43.7
Permeate	840	-	-	56	-	3.47	0.82	0.604	–
Retentate	240	-	-	182	3.67	2.40	12.58	1.790	33.8
B20120124									
Feed	900	5.18	70.8	143	1.1	3.82	4.39	0.891	42.2
Permeate	850	-	-	54	-	3.36	0.72	0.592	–
Retentate	320	-	-	195	3.26	1.85	10.03	0.933	34.5
C20120222									
Feed	900	4.06	69.5	109	3.57	2.75	1.87	0.383	37.8
Permeate	370	-	-	48	-	2.28	0.10	0.081	–
Retentate	610	-	-	128	4.85	2.69	2.72	0.516	30.3
C20120329									
Feed	900	4.21	70.1	111	3.72	2.73	1.78	0.341	34.4
Permeate	260	-	-	42	-	2.25	0.12	0.085	–
Retentate	660	-	-	119	5.01	2.81	2.40	0.432	28.8
D20120411									
Feed	900	4.18	67.8	110	1.06	5.32	5.12	1.543	51.2
Permeate	500	-	-	53	-	4.52	1.26	0.872	–
Retentate	550	-	-	127	2.28	4.60	7.28	1.732	45.7
Average									
Feed	**900** ± 0	**4.91** ±0.67	**63.9** ±14.0	**104** ±28	**1.68** ±1.14	**3.23** ±1.16	**2.80** ±1.83	**0.674** ±0.49	**35.8**
Permeate	**541** ± 207	**-**	**-**	**48** ±8	**–**	**2.83** ±0.98	**0.50** ±0.44	**0.370** ±0.317	**–**
Retentate	**503** ± 165	**-**	**-**	**128** ±53	**3.43** ±0.95	**2.55** ±0.99	**5.58** ±4.39	**0.863** ±0.643	**29.2**

a) Conductivity b) Total solids c) Suspended solids d) Total phenols e) Hydroxytyrosol f) Chemical oxygen demand. Note that Permeate + Retentate volumes are > Feed volume because of additional backwash water added during operation.

The most important function of the membrane is the molecular separation of the lower molecular weight monomeric phenolic compounds from the higher molecular weight compounds. The monomeric compounds are generally < 500 Da. There is a large range of the polyphenolic compounds, as shown in the molecular weight distribution in Figure 14. The higher molecular weight compounds (lignins, tannins) are responsible for the dark colour of the black olive brines, and are a result of polymerisation reactions of the smaller phenolics. The green olive brines do not initially contain many of the higher molecular weight polyphenols and thus are of a lighter yellow or green colour, however they tend to go dark over time due oxidative polymerisation, especially if exposed to sunlight and if the pH is not reduced.

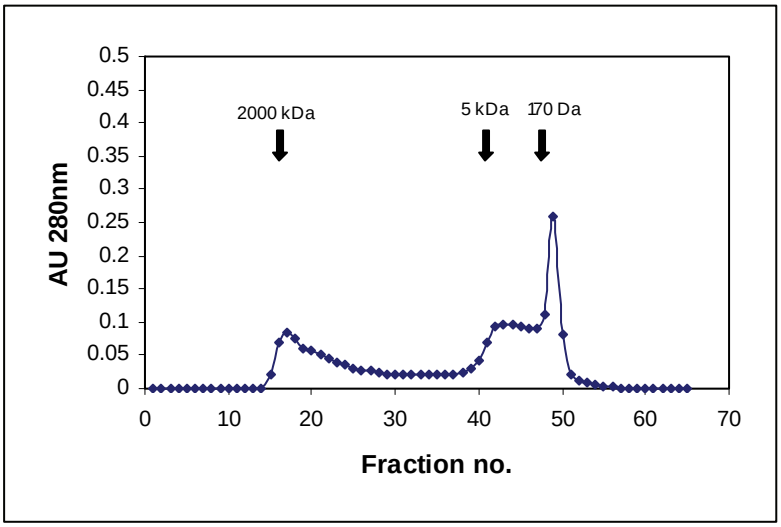

Figure 14: Molecular weight distribution of phenolic compounds in black olive brines. The peak near 170 Da is hydroxytyrosol.

With regard to Table 1, hydroxytyrosol made up between 20-30% of the total phenols in the feed. In the permeate, however, hydroxytyrosol was responsible between 65-80% of the total phenols, as most of the higher molecular weight polyphenols had been retained by the membrane. The permeate was much lighter in colour than the feed (see Figure 15), although not entirely colourless. After hydroxytyrosol (which is colourless), the other minor components of the feed included tyrosol, 3,4-dihydroxyphenylacetic acid, and other unidentified compounds, possibly biphenols such as anthocyanins which give the permeate its colour.

Recovery of hydroxytyrosol from the feed stream into the permeate stream was less than expected, compared to results obtained in the laboratory during project K8/814. Concentrations in the permeate stream were 50-60% of that in the feed (with exception of the 2 “C” batches that had high suspended solids content). This indicates that although hydroxytyrosol has a significantly lower molecular weight (154 Da) than the cut-off of the membrane (1000 Da), there is not free passage of

this compound through the membrane. This is possibly a consequence of the fouling layer on the membrane surface, and molecular interactions with the components therein.

During the pilot scale studies analysed here, a total of 1.96 kg of hydroxytyrosol was recovered from 7.2 kL of wastewater using the membrane system. The average yield was thus 0.27 $g.L^{-1}$ of wastewater, at a recovery of 40% of the total put through the system. Of this, the best three "B" repeated batches produced 1.44 kg from 2.7 kL of wastewater, i.e. a yield of 0.53 $g.L^{-1}$ at a recovery of 54%. A value between the average and the best should realistically be used for prediction of future yields and scaled-up design, although this could possibly be improved by the removal of suspended solids before filtration. There was a strong negative correlation between the average flux observed and suspended solids content of the feed during the processing of the different batches.

Although not a particular objective of the project, the COD of the waste stream was reduced by an average of 20% through the removal of low molecular weight phenolics and acids into the permeate stream.

Permeate from the membrane system was then passed through the chromatography column in order to capture the hydroxytyrosol and other minor phenolics, and to produce the purified brine for recycling. Results are shown in Table 2. The UV detector was used to monitor the phenolic concentration of the water or ethanol exiting the column. Phenolics were not detected exiting the column during loading of the permeate in any of the batches (i.e. no breakthrough), indicating that the absorbance capacity of the resin is greater than 15 g (phenolics).L^{-1} (resin), based on the data from batch B20111216. From this it can be concluded that the column was never saturated with phenolics, and it should be possible to load the column with larger quantities of permeate before this occurs. In addition, the column also absorbed organic acids, as was determined by decreased acidity and increased pH of recovered brine after loading.

After loading, the column was rinsed to neutrality and conductivity of ~ 0 to ensure that unbound acids and salts were removed before recovery of the extract. The quantities of rinsing water used for the different batches is shown in Table 3. Elution was then performed using ethanol/water mixtures. 150 L of ethanol solution was used for elution of each batch (except D20120411), which was the volume of the feed tank, corresponding to 3.75 bed volumes of resin. This was found to be sufficient to elute the majority of the phenolics, excepting those that had irreversibly bound to the column.

Table 2: Analysis of chromatography column data

Batch	Volume	HT	TP	HT purity	HT yield	HT recovery
	(L)	($g.L^{-1}$)	($g.L^{-1}$)	(% TP)	(g)	(%)
A20111114						
Load	450	0.047	0.061	77	—	—
Elute (50% EtOH)	150	0.114	0.145	79	17.1	81
A20111121						
Load	390	0.039	0.060	65	—	—
Elute (50% EtOH)	150	0.084	0.119	71	12.6	83
B20111129						
Load	670	0.636	0.827	77	—	—
Elute (50% EtOH)	150	2.358	2.911	81	353.7	83
B20111216						
Load	840	0.604	0.821	74	—	—
Elute (33% EtOH)	150	2.638	2.998	88	395.7	78
B20120124						
Load	850	0.592	0.729	81	—	—
Elute (25% EtOH)	150	2.415	2.625	92	362.3	72
C20120222						
Load	370	0.081	0.108	75	—	—
Elute (33% EtOH)	150	0.150	0.176	85	22.5	75
C20120329						
Load	260	0.085	0.121	70	—	—
Elute (50% EtOH)	150	0.119	0.151	79	17.9	81
D20120411						
Load	500	0.872	1.263	69	—	—
Elute (25% EtOH)	225	1.337	1.486	90	300.8	69

Different concentrations of ethanol were used for elution, and this affected the composition of the eluate in terms hydroxytyrosol purity, and the recovery thereof. General observations were that as the ethanol concentration increased, the yield from the column increased but the purity of the extract decreased (hydroxytyrosol % of total phenols in the extract). This was observable as darker coloured extracts with increasing ethanol concentrations. Visual inspection of the column after processed batches showed that there were coloured compounds attached to the upper resin layers. It was not possible to elute these compounds even using 100% ethanol. This, however, did not appear to affect the hydroxytyrosol absorbance capacity of the column at the loading rates observed. When the colour became severe, the column was cleaned and regenerated using the H_2O_2 solution as described in the operational protocols. This was performed after every 5^{th} or so run.

The recovery (i.e. loaded vs. eluted) was in most cases around 80%. The unrecovered portion presumably remained bound to the column, as there were only very low concentrations detected in the rinse water (< 0.005 g), and concentrations in the ethanol solutions towards the end of elution were also minimal as determined by UV detection.

A total of 1.483 kg of hydroxytyrosol was recovered after chromatography from the original 7.2 kL of wastewater, or 0.210 $g.L^{-1}$. Considering the "B" batches alone, the recovery was 0.412 $g.L^{-1}$. This compares favourably to a value of 0.48 $g.L^{-1}$ reported by Agalias *et al.* (2007), who used a similar chromatography system to recover hydroxytyrosol from olive mill wastewaters (oil production), although these researchers employed complex procedures before chromatography and were working at smaller (laboratory) scale. There was no noticeable deterioration of the adsorbance capacity of the chromatography resin over the course of the project with repeated cyclic loading. The column needs to be run for longer to determine the lifespan of the resin.

An average value of above 0.04 $L.hr^{-1}.kPa^{-1}$ for membrane flux means that a 900 L batch of wastewater can be processed in 32 hours, i.e. 4 working days (or less than 2 days if operated continuously). The chromatography system takes ~ 24 hours to process a 650 L batch of permeate, including the rinsing and elution steps. The chromatography process can be started while the membrane process is still in operation, thus it is possible to process a batch in the space of a working week. If an average hydroxytyrosol yield of 0.4 $g.L^{-1}$ is assumed, the productivity of the system works out to 360 g of product per week for the current pilot scale system.

Table 3 shows a summarised version of wastewater treatment process. The initial feed volume that would ordinarily be disposed was reduced to the retentate volume. The water used is made up of rinse water from the chromatography system and water used for backwashing the membrane system (retentate + permeate – feed). The recovered water is made up from permeate that has passed through the chromatography system and the chromatography rinse water. A lot more water was used during the course of the project than that shown in Table 3; tanks were washed and rinsed, and there were many abandoned batches where problems were encountered, however, the data presented shows what is possible using the system. The figures are quite acceptable if only "B" batches are considered; this would be the level to strive for in future operations.

If it is assumed that 900 L can be processed per week, and that Buffet Olives produces a maximum of 1 000 kL of wastewater per annum, then the system would need to be scaled up by a factor of 20 to treat all the wastewater produced on the farm. This could account for up to 360 kg of product per year at the current yields, possibly substantially more if the yield from the membrane system is improved by incorporation of a pre-filtration system to remove suspended solids.

Table 3: Volumetric analysis of water and wastewater

Batch	Feed	Retentate	Permeate	Chromatography rinse water	Water used	Water recovered
	(L)	(L)	(L)	(L)	(L)	(L)
A20111114	900	750	450	120	420	570
A20111121	900	510	390	110	110	500
B20111129	900	380	670	160	310	830
B20111216	900	240	840	180	360	1020
B20120124	900	320	850	170	440	1020
C20120222	900	610	370	100	180	470
C20120329	900	660	260	80	100	340
D20120411	900	550	500	650	800	1150
Total	**7200**	**4020**	**4330**	**1570**	**2720**	**5900**
Average ± SD	**900**	**503 ± 176**	**541 ± 221**	**196 ± 187**	**340 ± 228**	**738 ± 305**

Lastly, Figure 15 shows samples of the processed wastewater taken during a production batch, and Figure 16 shows the corresponding phenolic HPLC chromatograms for the samples. The recovered water, comprised of purified brine and rinsing water is suitable for re-use as either make-up water for fermentations, or for packaging of the market-ready olives, as this is sterilised and therefore there are no concerns about microbial contamination. The recovered water from the different batches had pH of ~5, conductivity of ~ 30 $mS.cm^{-1}$, and acidity of ~ 2 $g.L^{-1}$, comprised of predominantly lactic acid (~ 60%) and acetic acid (~ 20%), as determined by HPLC. Recovered wastewater samples have been stored in a refrigerator for several months without any perceivable change or contamination.

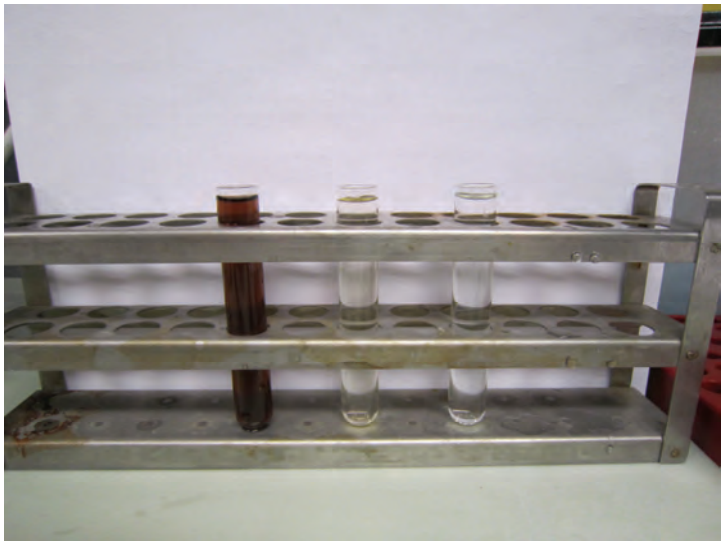

Figure 15: Wastewater feed sample (left), permeate from the membrane system (middle), and recovered process water after chromatography (right).

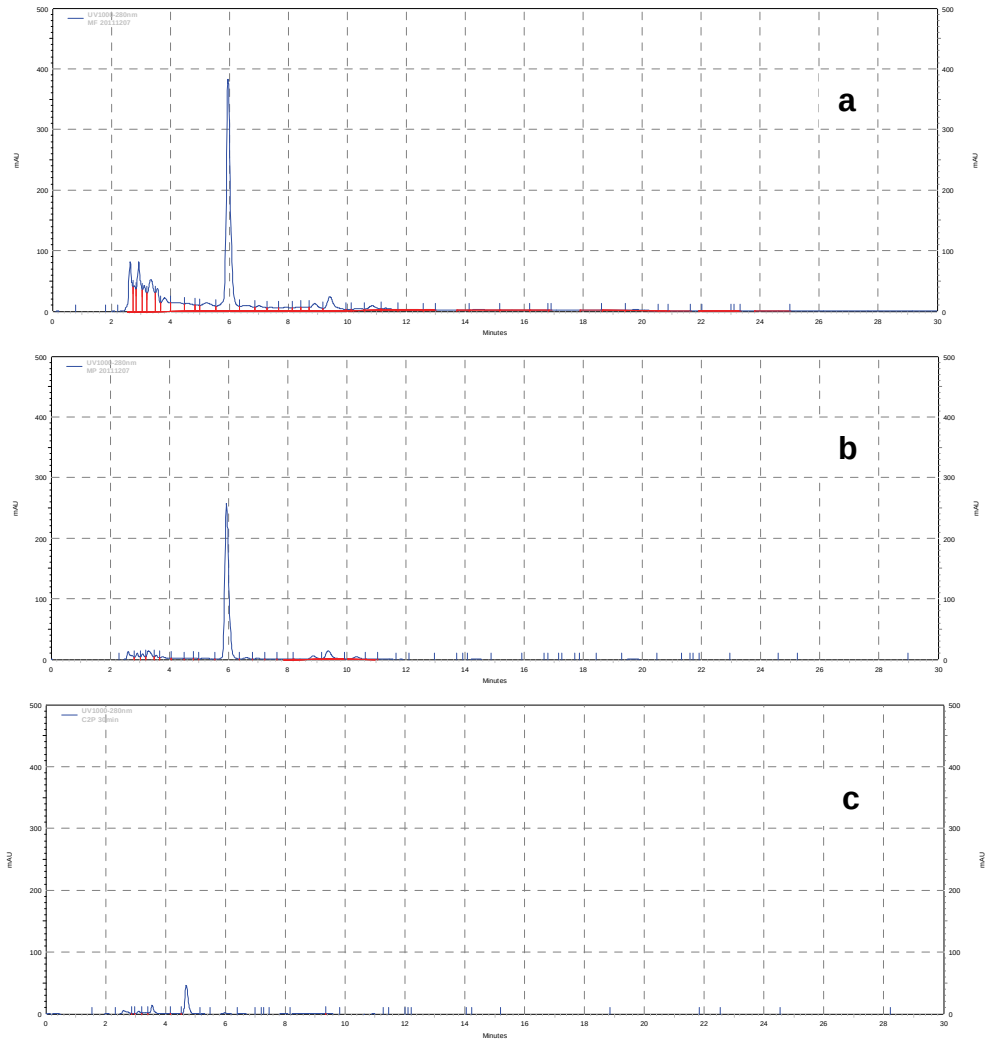

Figure 16: HPLC chromatograms of (a) wastewater feed sample, (b) permeate from the membrane system, (c) recovered wastewater. The major peak is hydroxytyrosol.

3.4 Operational cost

Table 4 shows estimated monthly operational cost for the production of crude extract (and concomitant production of purified water). These figures are estimated for an average monthly production rate of 1.4 kg (360 g per week). Capital costs are not included, and neither are the costs of further purification of extract or marketing and sales thereof. The cost of production of crude extract works out around R20/gram.

Clearly the cost is high for wastewater treatment only, however, given the high value of the antioxidant product, the process becomes feasible. The process will probably have to be scaled up to become commercially viable, as downstream processing of the crude extract, and marketing and sales of the final products will contribute significantly to the overall cost of the final product.

Table 4: Estimated operational costs

Description		Rands
Salaries		12000
Petrol		3000
Consumables:	Ethanol	500
	Cleaning chemicals	200
	Analytical chemicals	500
Laboratory cost		2000
Equipment maintenance		300
Electricity & water		3000
Sundries:	Tel. & Internet	500
	Insurance	1000
	Security	500
	Bank charges	200
Equipment depreciation		2000
Contingencies		1000
Total		**26700**

3.5 Technical conclusions and recommendations

While operating the plant, a number of practical issues arose that need to be resolved for future operations:

The ethanolic extract obtained from the chromatography system needs to be distilled to obtain a crude antioxidant extract, and also so that it can be re-used for chromatography elution. This proved to be a major bottleneck of the overall process, as there was only a laboratory-scale 2 L rotary evaporator available, and thus evaporation was very slow (~ 1 $L.hr^{-1}$) for the amount extract produced by the rest of the system. Either a large scale evaporator needs to be acquired, or else processing of the extract needs to be outsourced.

Wastewater discharge from the factory is erratic and unpredictable. Better communication is required in order to divert primary waste brine discharge to the holding tank. The factory also discharges rinsing and washing water to the sump that it is not desirable to collect for processing. Additional storage tanks are also required. The discharge that is collected should ideally be adjusted to pH 3 directly after collection in the holding tanks, rather than in the 1 kL working tank. This would help to minimise variation of the wastewater during storage and prevent microbial action.

The membrane system is fully automated to perform backwashes and has programmed and manual fail-safes, therefore it is possible to run the system continuously (i.e. overnight). This would mean a 1 kL batch would take much less time to process, but unfortunately the container is situated near residential property,

so it is only possible to run the system during working hours because the high pressure pump is very noisy. The membrane process therefore takes several consecutive days to complete, which holds up the chromatography process. Alternative, quieter pumps are being investigated.

There were some instances of contamination occurring in the membrane permeate tank. This took the form of a thin white fungal-like mat on the liquid surface. The bulk of the liquid below the surface appeared to be unaffected, so this was processed through the chromatography system nonetheless. Caution is however necessary, as contamination could have a negative impact on the chromatography process, not so much in terms of health concerns (ethanol is run through the column in each cycle which sterilises it), but more in terms of clogging the resin bed and negatively affecting performance or absorption capability. None of the tanks are aseptically constructed, thus great care had to be taken to operate hygienically. The tanks had to be carefully cleaned and sterilised (using bleach) before use, and emptied immediately after. It would be a good idea to install an aseptic, sterilisable tank and piping between the membrane and chromatography system. The outside tanks, which are exposed to direct sunlight, were susceptible to algal growth if left standing for too long.

4. MARKET ANALYSIS FOR ANTIOXIDANT PRODUCT

A 3rd party specialising in the formulation of cosmetics and sports supplements was commissioned to produce a market research report on hydroxytyrosol, in addition to internet and other research during the project. The results have a strong correlation with the weight of academic research highlighting the beneficial properties of hydroxytyrosol. The most prominent obstacle to broad scale market acceptance appears to be cost.

A substantial amount of research has been performed into hydroxytyrosol's many beneficial properties. It has been reported in peer-reviewed scientific journals that hydroxytyrosol has protective effects against cardio-vascular disease and cancer, has anti-inflammatory, anti-microbial and anti-viral properties, confers protection to nerve and other cells against free radical attack, enhances mitochondrial function, and much more (recent research is listed in the References). It is therefore an ideal ingredient or additive for pharmaceuticals or nutraceuticals, foods and beverages, cosmetics, toiletries, sunscreens etc. It has been awarded Generally Regarded As Safe (GRAS) status; there are no known adverse effects from ingestion or topical application.

Uptake of hydroxytyrosol as an ingredient by manufacturers has however been relatively slow. This is because of limited availability and ignorance, and in certain instances because of high cost. Pure hydroxytyrosol (> 90%) costs between R15,000-R50,000 *per gram* because of the purification processes employed; this essentially limits the use of such to medicinal and scientific research. Extracts containing hydroxytyrosol (at concentrations from 2-12%) cost from R20-R70 per gram. Table 5 shows a list of other hydroxytyrosol producers, the type of product, and the cost thereof. These are gradually finding their way into various products and the uptake is expected to increase, although there is always concern from the users' viewpoint about the other components in these extracts, which make up a substantial percentage and are not generally very well characterised.

It is difficult to estimate the potential size of the market for hydroxytyrosol because of the relatively recent and limited availability of the product, and the limited product offerings. The value of natural products being used in the cosmetics industry in Europe alone in 2009 was estimated to be Euro 3.6 billion per annum; extracts containing standardized concentrations of hydroxytyrosol are already used in some cosmetic products. "Functional" foods, sports drinks and supplements are large markets where hydroxytyrosol can also be exploited as an ingredient. There is a popular Australian sunscreen that contains hydroxytyrosol as an active ingredient. Some varieties of Pringles potato crisps contain "olive oil extract". Hydroxytyrosol has also been used in topical creams for the treatment of burns, and in skin whitening creams to ameliorate the free radical damage caused by bleaching.

The chemically pure products are expensive, while the extracts are of dubious quality. There is a gap in the market for partially purified extracts of greater purity than that listed above. These would be highly competitive if they could be sold at similar prices to the less pure extracts.

Customers are likely to be manufacturers of consumer items in the upper living standards measure (LSM) categories. Local market potential in the following industries was investigated:

- Food and beverage (wine and other premium perishable foods)
- Nutraceuticals
- Sports supplements
- Pharmaceuticals

Given that the end consumers of products containing HT are likely to be from the high LSM categories it is anticipated that they are likely to be quality sensitive and relatively price insensitive. The marketing report that was commissioned stated that this is the prevalent situation in markets in the developed world. Based on the market research it is anticipated that around 5 anchor customers could be secured. These customers are all based in South Africa.

Table 5: Commercial hydroxytyrosol prices in 2012

Supplier	Purity	Amount	Cost	Rands/gram		Notes
Chemically pure						
Extrasynthese	? 90%	100mg	Euro 148	15,060		No bulk discount
Cayman	> 98%	5mg	R 3 633.27	726,654		
		10mg	R3 755.43	375,543		
		50mg	R4 566.13	91,323		
		100mg	R 5 365.72	53,657		
Carl Roth	> 98%	25mg	R4,617	184,610		
Chromadex	> 98%	5mg	R3 115	623,000		from Industrial Analytical
Chengdu Biopurify	> 98%	0.1g - 1000kg				No response
Seprox	>98%	1g	Euro 12	123		Cant be right (communcation
						problems)
Extracts						
Hytolive	12%	1kg	Euro 275	23		
Hidrox	12%	1 - 9 kg	US625/kg	40		Total polyphenols not HT
		10 - 100kg	US563/kg	36		
		> 100kg	US530/kg	34		
		> 1000kg	US500/kg	32		
	6%	1 - 9 kg	US386/kg	49		
		10 - 100kg	US368/kg	47		
		> 100kg	US350/kg	45		
		> 1000kg	US315/kg	40		
	2%	> 100kg	US193/kg	74		
DB fine	7%	> 1 kg	R3000	42		

A trial internet advertisement was run using Google Adwords. The keyword tag was “hydroxytryrosol” and the advertisement received 39 hits within 4 days. This is an indication that there is also a broad, accessible market beyond South Africa. It is difficult to quantify this market but there is much internet activity related to searches for “olive extract” and “hydroxytyrosol”.

Hydroxytyrosol products and extracts are an emerging market. There is currently only a niche market for the research grade product. Beyond that there are many speculative suppliers who are unable to supply product when requested. As shown in the References, there is lots of interest within academic circles about the benefits of hydroxytyrosol. The volumes of positive academic literature are driving manufacturers to look for opportunities to develop products which use hydroxytyrosol as an ingredient.

The biggest barrier to market entry is most likely product cost. The current market price for pure hydroxytyrosol is too high to make it cost effective for inclusion in an end user product. Where there are “affordable” product offerings the purity is circa 10%. At this level of purity the benefit of using hydroxytyrosol is questionable, due to the other components in the extract. There appears to be a gap in the market for partially purified extracts with HT concentrations of > 20%, if these can be produced cost-effectively.

Depending on which manufacturing process is used, the cost and complexity of processing feedstock is potentially an issue. Internationally, most of the annual olive harvest is used to produce olive oil. The cost of producing hydroxytyrosol from this olive oil waste feedstock is suspected to be significantly higher than the technology developed during this project for the olive brine wastewater feedstock.

Anticipated hurdles to market penetration are that hydroxytyrosol as an isolated extract is an emergent product. Potential customers are going to be amongst early adopters of the product. There is thus a risk that downstream products fail and that there is an adverse market reaction to it as an additive to these products.

It is difficult to estimate the potential market size given that the market is still emerging. Competitors do not readily reveal production and sales data. Discussions are currently being held with a local fine chemicals distributor who sells imported hydroxytyrosol extracts, to determine what current and future expected market volumes are. There are no known local producers of hydroxytyrosol, so it is likely that a local company started soon will be the first to market.

“Natural”, “antioxidant”, and “sustainable”, in addition to the many beneficial properties of hydroxytyrosol, are expected to be strong market drivers for the uptake thereof into various products, if competitively priced.

5. COMMERCIAL IMPLEMENTATION STRATEGY

It is desirable to create an SMME to take the project forward, as sufficient research has been performed and the technology now needs to be commercialised. The overall objective is the establishment of an expanded processing facility on-site at the Buffet Olive farm in order to produce crude extract and purified antioxidant, and the subsequent marketing and sales of these. What is proposed is continued and improved operation at the current production level of crude extract, while specific focus is given to downstream processing and packaging, marketing, and sales of product. This firstly involves modifications to the existing plant and addition of extra capacity, particularly in terms of downstream processing of the antioxidant extract.

Currently downstream processing and analysis happens in the CeBER laboratories in Chemical Engineering at UCT. This situation is unsatisfactory; a dedicated processing and analytical facility is required. What is envisioned is the addition of 2 more containers, such that: one may be used for the membrane and chromatography processes that treat the wastewater and produce the ethanolic extract; one may be used for distillation of the extract and subsequent purification and formulation of the antioxidant product; and the third to be used as a combined analytical lab and office. A concrete slab needs to be cast upon which the containers will be arranged in a U-shape, with a basic floor layout as shown in Figure 17.

A business plan is being developed in order to apply for funding for the following specific developments:

- modification and improvement of the current pilot-scale unit into a dedicated extraction and water purification system
- the addition of another dedicated unit, on-site and adjacent, for downstream processing of the extract into a market ready product, including distillation and further purification processes
- the addition of a third unit, as above, as an on-site analysis laboratory/office
- Development of a marketing and sales drive
- Operations until such time as the business becomes cash-flow positive

Key challenges include locating the expanded facility: it needs to be near the current factory wastewater outlet, however the extra space required will necessitate some earthworks and moving some olive trees. Once the site has been located, the concrete slab has been cast and the containers have been installed, the additional equipment will be acquired. The system will then be constructed and commissioned, after which expanded operations and troubleshooting can begin.

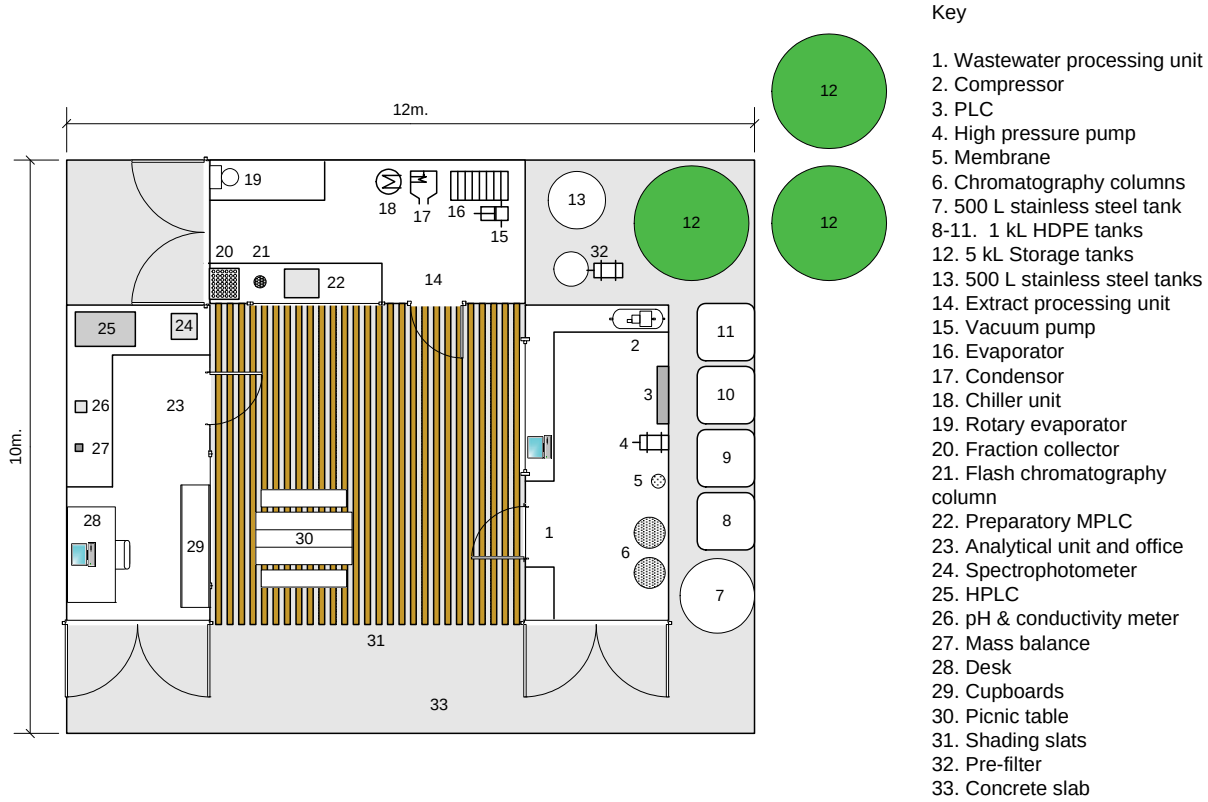

Figure 17: Floor plan for proposed development of wastewater treatment plant

The membrane and chromatography parts of the process are reasonably well characterised at this stage and are not going to change significantly, however, the scaled-up distillation and purification stages are potentially more problematic in that a different type of equipment is to be used for distillation, and purification has not been attempted at such a large scale yet.

Wastewater purification and extraction, distillation, monitoring and control, rudimentary analysis, and record keeping all happen in the pilot-scale plant. The operations therein are somewhat cramped. The distillation system, made from a vintage lab-scale rotary evaporator (Figure 18), is inadequate for the amount of ethanolic extract produced. Barrels of extract containing the antioxidants are being stockpiled until a more suitable distillation apparatus can be acquired, or a suitable contract manufacturer can be found.

The container itself is positioned next to the discharge sump from the factory, from where the wastewater brines originate. It is placed upon an earthen berm, built by the farmers, which is slowly and disconcertingly subsiding due to water erosion, and winter is coming. The farmers have agreed to a more permanent structure in the same general location, ideally a 10 x 12m concrete slab, and are prepared to relocate some olive trees for this purpose. After earthworks and waterworks they would like to see clean water for re-use being generated consistently by the modified plant over the period of a year, after which further talks could be held. The current

pilot-scale system is capable of treating only 2-3% of the annual discharge, thus full-scale operations are not to be considered for a while, as substantial investment will be required.

Figure 18: Current distillation system

The best business strategy for initial operations after funding has been acquired and operations have been suitably established is probably to market and sell a hydroxytyrosol extract, at a greater percentage purity than competitors' products, to manufacturers of consumer products, either directly or through agents/distributors. The rationale for this approach is as follows:

- A below-the-line (BTL) marketing strategy is possible with a direct approach to potential customers and little or no associated advertising costs
- It simplifies the number of customers needed to build and maintain relationships with
- It makes for easy distribution of product
- It focuses on core competence (i.e. the production of hydroxytyrosol)

Once a core market base of customers has been established, it makes sense to investigate venturing further up the value chain, by producing pure hydroxytyrosol for the niche market. This market (pharmaceuticals and research) has lower anticipated volumes but much higher margins. The cost of production will need to be established at laboratory scale first, but if competitive it could be sold directly through the internet at low associated sales cost.

Further down the line the development of actual consumer products can be considered. In this regard new market sectors should be investigated in addition to those where hydroxytyrosol is already used. Two intriguing possibilities have already

been identified: 1) using it as an alternative to sulphites for the preservation of wines. Hydroxytyrosol has been identified in trace quantities in both red and white wines, therefore there should be no significant complications to using it as an additive. 2). It should be investigated as a natural alternative to currently used (synthetic) products for the prevention of melanocytosis, or blackening, of shellfish after harvest. Both these would obviously require substantial research, but potential markets in both cases are large.

Quality assurance of product will have to be performed both in-house and externally. Certified external laboratories have been identified that can perform quantitative analysis (for hydroxytyrosol), antioxidant activity, and microbial and heavy metal analysis.

6. CONCLUSIONS

A modular treatment system was successfully designed, constructed and operated in order to process wastewater brines from the table olive industry. The system was comprised of two main unit operations: membrane separation and chromatographic adsorption. These two sequential unit operations were able to simultaneously produce purified brine for recycling back into the table olive process, and recover high value antioxidants which would otherwise have been discarded. In addition, the volume of wastewater for final disposal was significantly reduced. The process can be considered to be green, as no environmentally harmful or toxic chemicals were used or produced.

Despite lower than anticipated yields and productivity in terms of antioxidant recovery, the project was considered overall to be a success. The membrane system operated adequately in terms of separating the darkly coloured high M_w phenolic components from the brine, which was then treated by the chromatography system to produce purified brine for recycle. The purified brine was good quality and was deemed to be suitable for re-use.

Operation of the membrane system in terms of volumetric throughput could be improved by removing suspended solids from the wastewater before filtration. The suspended solids negatively affected permeate flux due to fouling of the membrane, resulting in the need for rigorous backwashing and chemical cleaning.

Recovery of antioxidants from the chromatography column could be improved in two ways: firstly, pure ethanol should be used for elution. The ethanol used in this project was denatured by 5% ethyl acetate (a legal requirement for non-registered business). The ethyl acetate is suspected of interfering with the subtle electrochemical interactions which allow the antioxidants to adsorb and desorb from the column. Secondly, the aspect ratio of the column was too low (it was constructed from a beer keg). The column needs to be longer and thinner such that a better flow distribution pattern is achieved. It is suspected that channeling occurred through the short wide column, resulting in inefficient use of the resin therein.

Overall process performance and economic feasibility of the system was evaluated. While not optimal, based on market analysis there is a strong case to be made for continued and improved operation of the system, and for subsequent increase to full-scale operation. In this regard a spin-out company is to be created in order to exploit the technology developed during the course of the project.

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