Defouling of Ultrafiltration Membranes by Linkage of Defouling Enzymes to Membranes for the Purpose of Low Cost, Low Maintenance Ultrafiltration of River Water

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Report to the Water Research Commission
by the
Department of Biochemistry and Microbiology
Rhodes University

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DEFOULING OF ULTRAFILTRATION MEMBRANES BY LINKAGE OF DEFOULING ENZYMES TO MEMBRANES FOR THE PURPOSE OF LOW COST, LOW MAINTENANCE ULTRAFILTRATION OF RIVER WATER

Final Report to the

Water Research Commission

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Executive summary

Removal of colour arising from humic substances from surface waters for potable use using ultrafiltration as an alternative technology to sedimentation, adsorption and depth filtration has evoked considerable interest both locally and internationally. It is of particular relevance to the South African context since it offers the potential for small scale operation in rural environments which do not have access to municipal water purification facilities. However, rapid, safe and easily applicable technology for defouling of adsorbed humic substances would considerably extend the application of ultrafiltration.

A novel concept was thus proposed as a solution, called the defouling on demand strategy, using activatable enzymes. In this process, enzymes capable of the transformation of humic substances, but which are inactive during the operation of the ultrafiltration procedure are immobilised onto the surface of the membranes. These enzymes, in association with adsorbed humic polymers form a dynamic layer which enhances the ultrafiltration process. Once flux decline reaches a determined low point, the enzymes are "activated" by addition of a co-factor. The enzymes then act on the foulant layer, thereby restoring flux. The objective of this study was to evaluate this concept by choice of suitable enzymes and proof that this concept can be applied practically.

Based on available literature, three enzymes were chosen as candidates for the defouling process. These were horseradish peroxidase (HRP), soybean peroxidase (SBP) and manganese peroxidase (MnP). Peroxidases were chosen due to their known ability to transform humic substances and their requirement for hydrogen peroxide (and in the case of MnP, manganous ion) as a means of activation.

A laboratory experimental procedure was then developed to evaluate these enzymes using a stirred ultrafiltration cell with flat sheet membranes. No flux improvement was obtained after activation of immobilised SBP. Flux improvement was obtained from activated HRP and MnP, with MnP giving the best performance. Further experiments showed that flux improvement upon activation of MnP was considerable and rapid. Control experiments showed that this effect was due to enzyme action

and not artefactual. Photographic evidence was also obtained for the noticeable reduction of irreversible fouling using this process.

Due to the rapid action of this process, its ease of operation and its safety due to exclusion of harsh cleaning chemicals, it was recommended that further evaluation of this concept was warranted for application to compact units for surface water decolourisation.

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INTRODUCTION

Brown Water Clarification

Colour is a primary quality requirement for potable water supply for human consumption Humic substances present in soil and surface waters give rise to a yellow to brown colour in upland surface waters. These humic substances originate from contact with soil and vegetation such as Fynbos and constitutes a problem in the Southern and Western Cape where water colour is in the range of 1000mg/ml as Pt. World Health Organisation recommends a maximum of 15 mg/ml as Pt. (Swartz and de Villiers, 1996)

The Potential for Ultrafiltration in water purification

Membrane units, in terms of their operational costs, have been found to be highly competitive and effective in the treatment and provision of safe drinking water for general public consumption (Pervov *et al*, 1996). For this reason membrane filtration is now replacing conventional processes such as coagulation, sedimentation and sand filtration for potable water production. This is particularly relevant to the problem of brown water decolourisation. It has been shown by Jacobs *et al* (1996) that compared to conventional systems, membrane ultrafiltration is a low maintenance, simple to operate alternative for low volume water treatment. Typical procedures would include pre-filtration to remove particulate solids, followed by a single step clarification and sterilisation by UF and finally, chlorine treatment against contamination.

The WRC has supported research thus far on the development of ultrafiltration membrane modules for potable water production. The problem which pertains to all pressure-driven membrane systems, that of fouling, hampers application of this technology.

Various process improvement strategies can be utilised to minimise fouling. These include dynamic optimisation, the use of low-fouling membranes, occasional back-flushing (Noble and Stern, 1995), operation below critical flux (Fane, *et al*, 1996) or operation at constant C_{wall} (van Reis, *et al*, 1997). Irreversible fouling, however, still necessitates cleaning of the membrane

surfaces. Conventional technologies used for this purpose include:

- *Mechanical Physical methods*: these include the use of sponge balls or air sparging to physically disengage a foulant layer. These have the advantage of being non-polluting, but have all the other disadvantages of chemical cleaning agents (Noble and Stern, 1995).

- *Chemical cleaning*. Chemical reagents with hydrolytic, oxidative or detergent properties are used to clean membrane surfaces. These are normally highly effective, but these chemicals tend to be highly aggressive, which limits the life expectancy of the membranes. They tend to be toxic and their discharge is normally not acceptable. Also, the risk of carry-over into product water can make their use in producing water for human consumption dangerous. For this reason, sophisticated rinsing and post-treatment regimes need to be put in place, which are not practical for use in rural situations where a lack of technical proficiency is the norm.

- *Enzymatic cleaning* has shown considerable promise in alleviating this problem. Enzymatic cleaning is advantageous because enzymes are biodegradable and do not cause additional pollution problems. Enzymes are also highly specific for the reactions that they catalyse. In addition, enzymes function under mild conditions in terms of pH, temperature and ionic strength and do not damage the membrane surface (Maartens *et al.*, 1996). Enzymes, however, do have several drawbacks as they are expensive and there are concerns that deleterious enzyme activity or degradation products may be carried into the material that is being processed (Tragardh, 1988; Coolbear *et al.*, 1992).

A solution to the cost problem is to immobilise the enzymes onto the active filtration layer of the membrane, so that they can be used for extended periods. The added advantage of such a system is that the foulant layer is degraded from the point of attachment, obviating the need to digest the whole cake. Various researchers have applied this approach successfully (Chen, *et al*, 1992 and references therein). The disadvantage of using immobilised enzymes in this fashion is that they degrade the very macromolecules which are to be retained by filtration

The "defouling on demand by activatable enzymes" concept

We have therefore developed a new approach to the use of immobilised enzymes for the cleaning of ultrafiltration membranes for water purification. This system involves the use of immobilised activatable enzymes. The term "activatable" refers to enzymes that are not functional during the normal operation of the ultrafiltration process, i.e. they are dormant until activated. The activation occurs when a chemical co-factor or co-substrate is introduced to the enzyme on demand. Thus the process would operate as follows:

1. Immobilisation of the inactive enzyme.:

The enzyme was immobilised by physical adsorption in research carried out so far, but other methods of immobilisation such as covalent bonding to the membrane surface could also be used.

2. Operation of the UF process.

The water purification process can be operated as required, either by dead-end or cross-flow filtration. Eventually cake/gel layer formation at the membrane surface causes flux decline, resulting in an unacceptably low process productivity.

3. Activation of the defouling Enzymes - Defouling on demand

At a state of low trans-membrane flux, the enzymes can be activated as required (on demand) by addition of the activator solution containing the necessary co-factors for enzyme action. This can be achieved by addition of a slug of this solution in the feed or by back-diffusion from the outside of the membrane. Minuscule amounts of this solution are required and flux restoration was shown to be instantaneous due to the catalytic efficiency of the enzyme (see results). The flux restoration occurs because of transformation of the humic substances (and certain other organic polymers) by the enzyme action, allowing the foulant layer to be removed at its point of attachment and lifted off. Once the activator solution is exhausted, the enzyme returns to its inactive state and the filtration process can begin again. This process is depicted schematically in fig.1:

Although this technology has major application for industrial effluents, the focus of this work is to evaluate it for potable water, a national development priority in South Africa.

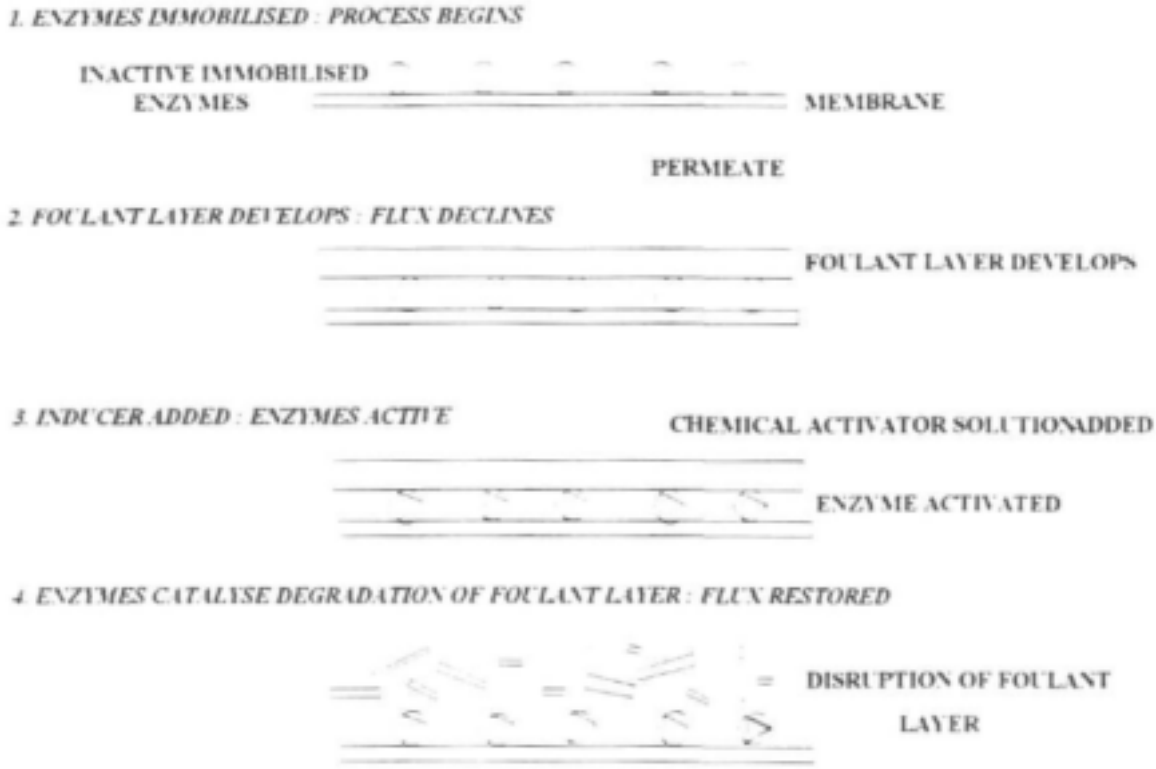

Fig. 1: Schematic depiction of the defouling on demand concept

RESEARCH OBJECTIVES

The objectives of this study were:

1. To develop techniques for attachment of enzymes to membranes

2. To evaluate the use of different enzymes in terms of ease of attachment to membranes and ability to degrade the foulant layer.

3. To prove the concept can be shown experimentally.

EXPERIMENTAL APPROACH

UF system:

Of the various ultrafiltration systems available (plate- and frame, spiral wrap, hollow fibre, tubular, etc.), the stirred ultrafiltration cell was used since it is well characterised, easy to model and operate and requires small volumes of enzyme and water to be filtered.

Experiments were performed in a stirred ultrafiltration cell (Amicon) with a working volume of 200ml. A flat sheet polysulphone membrane (MWCO 30 000) with a total surface are of 27.5cm^2 was used for each experiment. Pressure was supplied by nitrogen gas and the permeate flux was measured volumetrically every minute for the duration of the experiment. All ultrafiltration batch experiments were carried out at 160kPa, 700rpm and 22^{0}C for 45 minutes. The experimental setup is shown in figure 2.

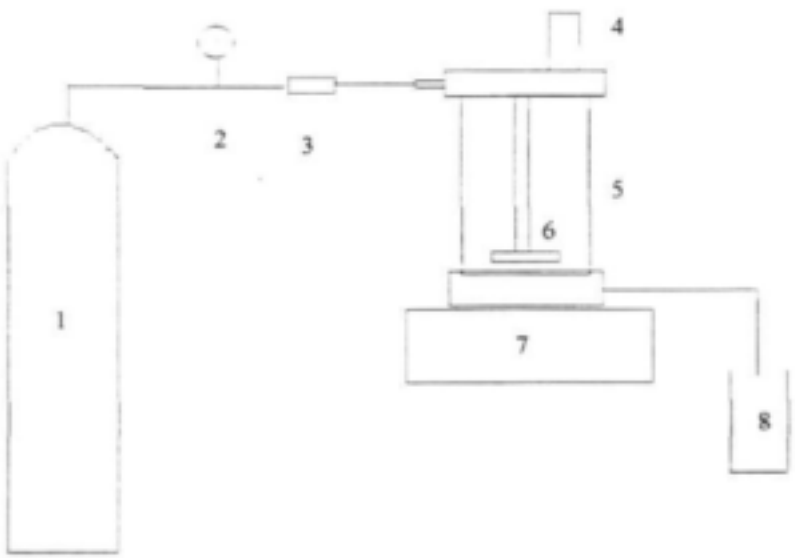

Fig. 2: Experimental stirred cell UF system. 1. Nitrogen gas. 2. Pressure gauge. 3. Induction port. 4. Pressure release valve. 5. Polycarbonate vessel (200ml. capacity). 6. Agitator. 7. Magnetic stirrer table. 8. Permeate collection device.

Choice of enzyme

The criteria for the choice of enzyme to be used for this process can be summarised as follows:

1. The enzyme of choice needs to be capable of degrading humic substances which were identified in earlier work as major contributors to fouling by river water (Ed Jacobs, pers. comm.).

2. It needs to be inactive under the physico-chemical conditions at which the ultra-filtration process operates.

3. It needs to be activatable by some means for defouling.

4. It needs to be fairly stable for extended use.

5. It should preferentially have a broad enough substrate range to also degrade some of the other organic materials that contribute to fouling, e.g. polysaccharides, lipids and proteins.

6. It needs to be readily available.

To this end the peroxidases were considered good candidates, since they are known to have wide substrate ranges, which include phenolic compounds (such as tannins and other humic substances). Also, they require hydrogen peroxide as a co-factor for their action, so they would be inactive during normal ultrafiltration applications.

Mechanism of action of peroxidases

The peroxidases all follow a basic mechanism of action. The ferric form of the enzyme, the resting form, is initially oxidised by two electrons from the hydrogen peroxide to produce a form of peroxidase known as compound I. Compound I can be reduced by one electron by chemicals such as aromatic pollutants having a suitable reduction potential. The enzyme is reduced to a form called compound II whereas the chemical is oxidised by one electron to form a radical. A second chemical then donates a further electron to compound II to return it to its resting state (Aust

1995). In the process, the aromatic reducing substrate is oxidised to aryl cation radical (Gold and Alic, 1993). This catalytic cycle is depicted schematically in figure3.

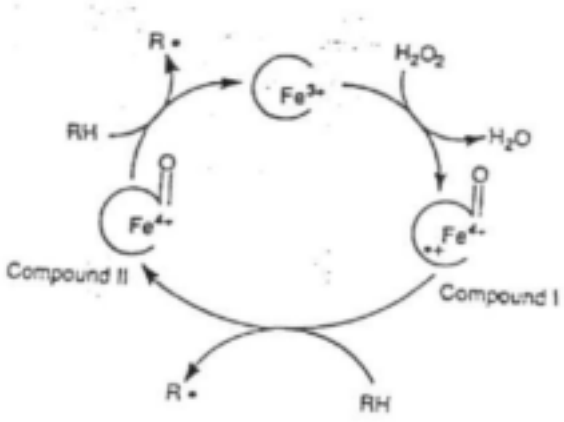

Fig. 3 The catalytic cycle of most peroxidases

Out of this large group of enzymes, the following were chosen because of their potential action on the tannins and availability:

Lignin peroxidase and manganese peroxidase

Lignin peroxidase is different from other peroxidases in that it has a higher oxidation potential (~ -1.35V) than other peroxidases such as horse radish peroxidase (~ - 0.8V) giving it a greater range of chemicals that it can oxidise.

Lignin and manganese peroxidase (LiP and MnP) are produced extracellularly by white rot fungi for the degradation of lignin. Blondeau (1989) showed that the fungus *Phanerochaete chrysosporium* is capable of the degradation humic substances and that this is due to its extracellular enzymes. LiP is capable of the degradation of 90% of the most recalcitrant of wood (lignin) substructures, while MnP is capable of the degradation of the more labile phenolic components (Jensen, *et al* (1996) and references therein). Subsequent work by Sayadi and Ellouz (1995) showed that with cultures of *P. chrysoporium*, conditions which stimulated the production

of lignin peroxidase led to a greater extent of decolourisation of olive mill waste (which gets its colour from humic substances such as tannins, catechins and anthocyanins) than conditions which stimulate MnP production. This implies that LiP would be the enzyme of choice for cleavage of humic acids, although they show that LiP on its own caused further polymerisation of humic substances and not cleavage.

They go on to show that cultures with high MnP activity effectively removed low molecular weight fractions while LiP removes high molecular weight fractions. From this it is evident that the role of these enzymes in the cleavage of polyphenolic compounds is not clear. For example, it was reported that high molecular weight chlorolignins (MW > 30 000) from bleach plant effluents were depolymerised by MnP (Paszczynski and Crawford (1995) and references cited therein). Wariishi *et al* (1991) showed that MnP was responsible for cleavage of lignin model compounds, but that re-polymerisation reactions also occurred. Therefore, an empirical approach to screening of suitable enzymes was taken. MnP has a different mechanism of action to other peroxidases in that it uses hydrogen peroxide to oxidise Mn(II) to Mn(III). The catalytic cycle of this enzyme is depicted in fig. 4.

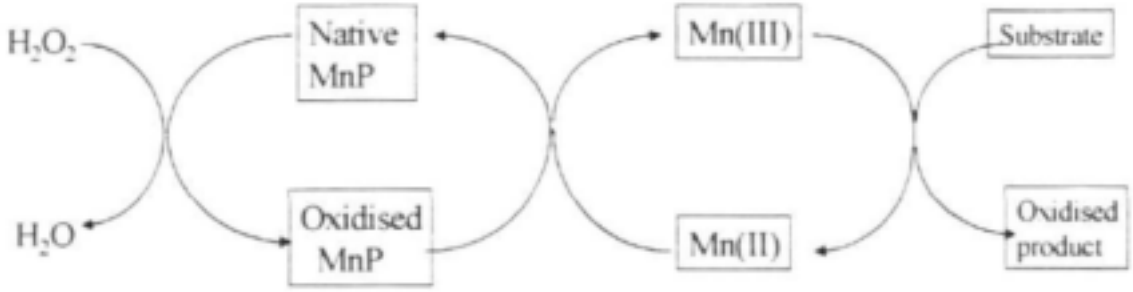

Fig. 4: Catalytic cycle of MnP (Aust 1995)

Although LiP appeared to be a good candidate for this application, based on theoretical considerations, practical experience with this enzyme has shown it to be unstable.

Soybean peroxidase (SBP)

It is for this reason that soybean peroxidase was chosen for evaluation. Soybean peroxidase is an anionic peroxidase isolated from soybean seed coat with a pI of 4.1 and a molecular weight of

37000 Da (Enzymol Corps). Soybean peroxidase has been shown to have the same substrate range as LiP, but has the advantages of better general- and thermal stability and a wider pH range. Also, since soybeans are widely available and inexpensive, the cost of harvesting would be low.

Horseradish peroxidase (HRP)

This enzyme is one of the most thoroughly characterised of all enzymes and is widely used in medical applications and has been evaluated for use in the bioremediation of foundry effluents which have a high phenolic content (Cooper and Nicell, 1996). Because of its widespread application it is readily available in bulk and the largest producer worldwide is a local company, Seravac. Ferrer, *et al* (1991) showed that HRP was capable of the decolourisation of Kraft effluent, hence it should be applicable to the humic substances present in river water.

Sources of enzymes

Manganese Peroxidase

MnP was produced by the fungus *P. chrysosporium*. Stock cultures of *Phanerochaete chrysosporium* ATCC 24745 were maintained on malt agar slants as described by Gold and Cheng, 1978. Agitated cultures were grown in 1- litre Erlenmeyer flasks containing 375ml media as described by Bonnarme and Jeffries (1990). Cultures were inoculated with *P. chrysosporium* and flushed with oxygen every 24 hours. Manganese peroxidase production is stimulated by high manganese concentrations which also functions to suppress lignin peroxidase (Bonnarme and Jeffries, 1990). The agitated cultures where therefore induced with manganese sulphate after 72 hours after which manganese peroxidase activity peaked within 24-48 hours thereafter.

Manganese peroxidase activity was determined spectrophotometrically at 420nm where ABTS was used as a substrate as described by Gold and Glenn (1988). One unit of activity is defined as 1µmol of substrate oxidised in 1 minute. In each experiment 0.5 U of MnP (and the other enzymes) was immobilised onto the membrane surface, by adsorption, unless otherwise stated

Soybean Peroxidase

A crude extract of soybean peroxidase was prepared as follows. 20g Soybean seed coats were soaked and homogenised in 200ml. of 25mM potassium phosphate buffer, pH 7.5. Another 300ml of the above extraction buffer was added to the homogenate and stirred for 2-3 hours at room temperature. This was then filtered through a sieve, then filter paper. The filtrate was subsequently cut with 0-30% ammonium sulphate, stirred for 30min. in an ice bath, and thereafter centrifuged for 15min. at 18000g. The centrifugate is cut again with 30-80% ammonium sulphate, stirred for 2 hours in an ice bath and centrifuged again. The precipitate was suspended in the extraction buffer and dialysed against water.

Horseradish peroxidase

A commercial preparation of HRP was used (Sigma).

Chemical Activation

The immobilised enzymes were induced by a chemical activator solution containing the necessary co-factors for enzyme action. The pressure was dropped and the activator solution was added through the induction port after 15 and 35 minutes in each experiment.

The activator solution used for MnP induction was 0.475mg/l $MnSO_4$ and 0.05% (v/v) H_2O_2 in 5ml of distilled water.

The activator solution used for both HRP and SBP was 1ml of 30% H_2O_2 in 120ml of H_2O adjusted to 0.4 absorbance units at 240 nm.

Brown Water

The water used in these experiments was obtained from the municipal water purification plant in George and has a turbidity of approximately 7 NTU, and a colour index which ranges from 700- 1200 Hazen units (Pretorius, pers.comm).These readings vary according to the season.

RESULTS AND DISCUSSION

Choice of enzyme

The data for flux restoration using the three enzymes chosen are shown in figures 5-7

In each of these, flux decline over time in the experimental system described is shown with the enzyme immobilised without induction and compared to flux decline with the enzyme induced with the appropriate activator solution.

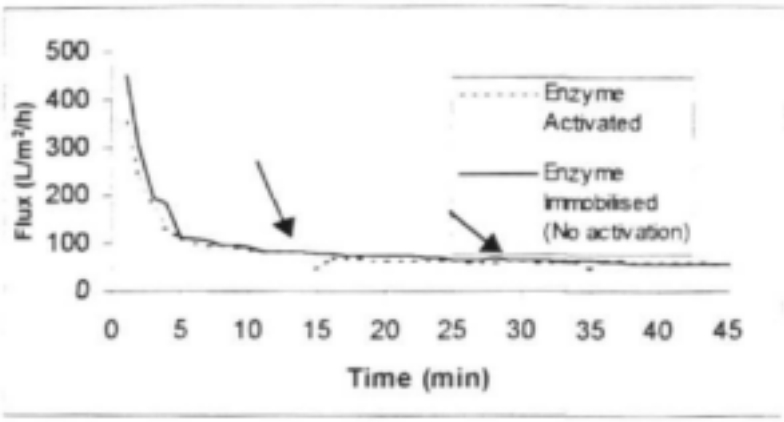

Fig.5: Flux recovery using immobilised soybean peroxidase. Arrows indicate where the activator solution was added. Clearly no flux improvement was observed

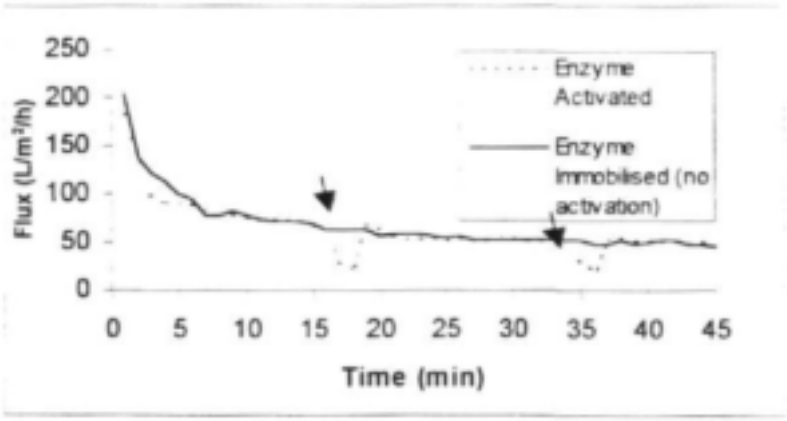

Fig. 6. Flux recovery using immobilised horseradish peroxidase. Arrows indicate induction. A slight flux improvement can be observed

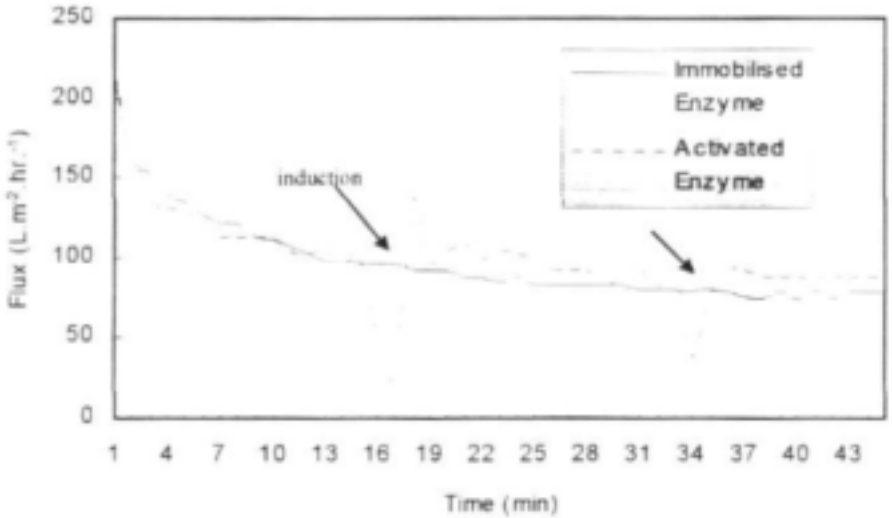

Fig.7: Flux recovery using immobilised Manganese Peroxidase from *P. chrysosporium*. Clear flux improvement was observed immediately after induction. Greater improvement was observed after the first induction (15 min.) Compared to the second (35 min.). This is expected since retentate concentration increases during batch filtration.

From the above it can be observed that MnP gives the best flux recovery. HRP provides a barely noticeable improvement, while no flux recovery is observed using soybean peroxidase. Some further evidence to the difference in activity between the 3 enzymes is obtained from spectrophotometric evaluation of the permeate fig. 8 and the retentate, fig..9

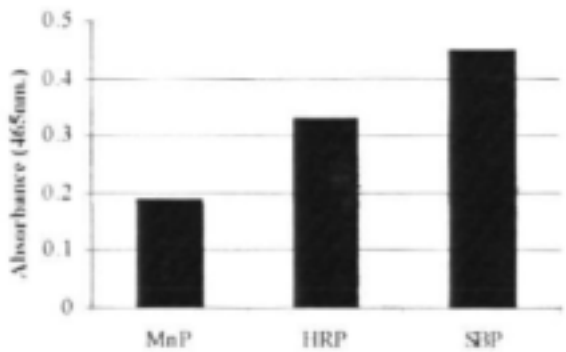

Fig. 8: Spectrophotometric analysis of the permeate by absorbance at 465nm, the wavelength range of humic substances. MnP and HRP caused a reduction in colour of the permeate.

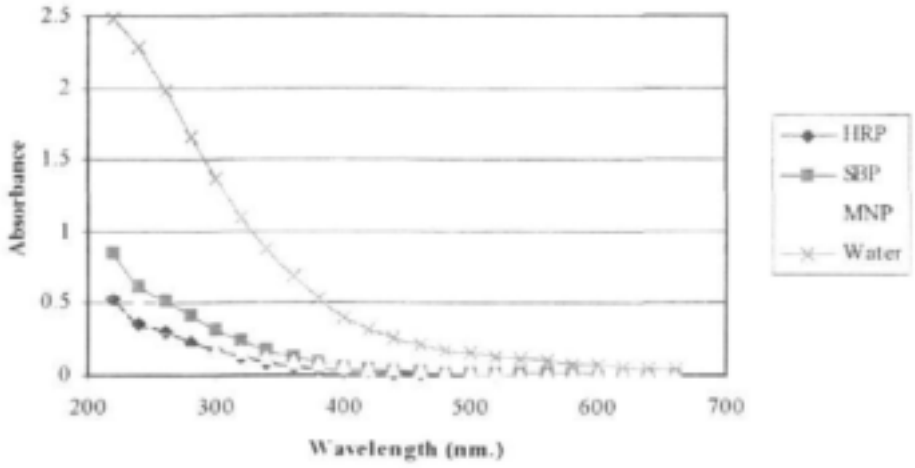

Fig. 9: Photometric analysis of the retentate after enzyme induction Once again the MnP and HRP had a greater effect on the river water than SBP. These are the same enzymes that affected flux upon induction

From the above it is evident that the colour component of the river water is reduced by the action of MnP and to a lesser degree, HRP. This indicates that humic substances are transformed by enzyme action. The nature of this action could be a polymerisation reaction or a cleavage reaction. If the humic polymers were cleaved to smaller oligomers or monomers, these would no longer be retained by the membrane and an increase in absorbance at lower wavelengths would be observed. If the humic polymers were further polymerised, larger, less soluble and possibly more stable particulate matter could be formed which would improve retention of such compounds. More knowledge is needed about the size distribution and composition of the humic substances in the river water. Knowledge of the nature of the enzymatic action on such a complex matrix is required before more rational evaluations of the mechanisms of flux recovery can be made.

Based on the above, only MnP was studied further. First, it was necessary to determine the impact of an immobilised enzyme layer on the performance of a batch ultrafiltration system, since it was expected that the enzyme layer would provide resistance to flux on its own. The results from this experiment are depicted in table 1

Table 1 - Flux profile for immobilised, non- induced MnP compared to filtration of river water without immobilised enzyme

Time	Ultrafiltration of river water	Effect of enzyme immobilisation
Initial flux ($L./m^2.hr$)	930.8	211.6
Flux during experiment ($L./m^2.hr$)	95.99	95.99
Flux at end of experiment ($L./m^2.hr$)	34.9	78.35

Table 1 shows a set of experiments where the enzyme is not induced and shows the flux decline profile of an ultrafiltration system for brown water decolourisation and the effect of enzyme immobilisation on flux. Initially the presence of immobilised enzymes resulted in the formation of an intermediate layer increasing trans- membrane permeability resistance. This initial difference in flux only lasts for approximately 5 minutes. It is encouraging to note that the steady state flux is the same and that, in fact, an improvement in flux is obtained at 45 minutes in the case where an immobilised enzyme layer is present. In comparison flux was significantly lower (34.9 $L/m^2/h$ compared to 78.35 $L/m^2/h$) in the absence of the immobilised enzymes at the end of the experiment. The reason for this is unknown, at present.

Table 2-Flux values obtained upon induction of the enzyme compared to relevant controls for artefacts. Control Experiment 1: To determine if an increase in flux is a result of the catalytic action of manganese peroxidase, the enzyme was denatured and immobilised onto the membrane surface and induced as normal. Control 2: To determine if the activator solution has an oxidative effect on the foulant layer, the induction solution was added to the system but no enzymes were immobilised onto the membrane surface. Control 3: In normal operating procedure the pressure is dropped to add the activator solution, thus to test if this decrease in pressure has an effect on flux, water was added to replace the activator solution.

	Experiment	Control 1	Control 2	Control 3
nitial flux	213.8	381.78	263.91	610.84
Flux before nduction L/m²/h.)	98.17	113.44	102.54	106.89
Flux after nduction L/m²/h.)	135.26	113.44	104.72	95.99
Flux 5 min after nduction L/m²/h.)	104.71	102.54	95.99	95.99
Flux at end of xperiment L/m²/h.)	81.26	63.26	71.14	52.35

Table 2 shows the initial experiments where MnP is immobilised onto the membrane surface and induced with the activator solution after 15 minutes. An immediate improvement in the flux is evident after induction. At the end of each experimental run the flux was noticeably higher than any of the control experiments. In all three control experiments indicated in Table 2 no increase in flux was observed after induction indicating that any increase in flux is a result of enzymatic action on the foulant layer.

Visually (fig. 10) there are distinct differences and significant improvements in the density, colour and dispersion of the foulant layer when MnP is immobilised and activated. This method of cleaning membranes using activatable enzymes has proved to be effective and shows great potential as an effective biological cleaning method for polysulphone membranes during brown water decolourisation.

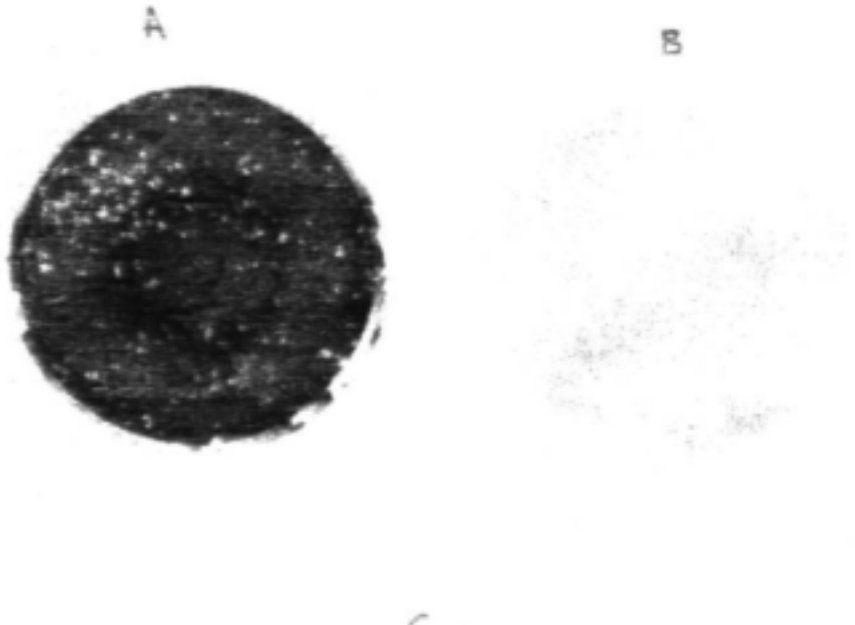

Fig. 10: Photographic depiction of the extent of irreversible fouling of polysulphone membranes after ultrafiltration of river water. (A) shows an evenly distributed dense foulant layer resulting form river water ultrafiltration. (B) shows a membrane surface with a disengaged foulant layer due to induction of MnP (0 5 U). (C) shows a cleaner membrane surface due to the induction of MnP (4 5 U).

CONCLUSIONS AND POTENTIAL FEASIBILITY

From the above experimental evidence, it is clear that the MnP of *P. chrysosporium* can, upon induction, provide noticeable flux restoration in membranes fouled by river water particulates. The defouling action is instantaneous and easy to accomplish technically. However, since this was a batch ultrafiltration study, the stability of the enzyme in this environment could easily be quantified. These are preliminary studies and proper characterisation of the mechanism of action of the enzyme under experimental conditions are still to be carried out. Once this characterisation has been performed, an optimisation study followed by scale-up to a larger system would be required before an economic assessment can be made.

Hence, few predictions can be made at present about the competitiveness of this technology, when compared to existing technology, such as the use of chemical cleaning agents. Its simplicity and speed, on the other hand make it a very attractive for small scale system for river water purification in rural environments, either for individual use or for small groups where municipal sterilisation and purification processes are not yet in place or not yet feasible. Water for personal use could be produced using a hand pump (this is not possible with reverse osmosis) and a small ultrafiltration system. The ultrafiltration membrane would be able to sterilise river water, while a dynamic cake layer could remove finer particle and possibly some heavy metal- or algal toxin-like contaminants by ion exchange. Cleaning a blocked filter or replacing a saturated dynamic layer is easily accomplished by addition of an activator solution. This does not involve tampering with the membrane in any way so its integrity is not compromised.

Although, subjectively speaking, it appears that small scale application is the major potential application area of this technology- it must be remembered that this is a new approach to membrane defouling. Different activatable enzymes can be found which are active on a wide range of foulants and this technology may well provide a unique or competitive solution to some industrial scale problem. Also, this technology is not limited to ultrafiltration, but other membrane systems as well.

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