

Decolourisation of aquatic NOM with the white rot fungus *Phanerochaete chrysosporium* : a thesis submitted in the fulfilment of the requirements for the degree of Master of Engineering

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SUMMARY

NOM has an impact on all water treatment processes: from the removal of particles through alum coagulation to chlorine disinfection and interference in activated carbon adsorption (Newcombe and Drikas, 1997). The presence of NOM in water is unaesthetic in terms of colour and odour, and may lead to potentially carcinogenic disinfection byproducts (Slunjski et al., 2000b). Conventional water treatments are usually effective but they are also expensive, time consuming, based on chemical addition and usually generate wastes (Hrubec, 1995). Liquid concentrated NOM waste has also become a concern since alternative drinking water processes like ion exchange, i.e. MIEX, and membrane filtration were introduced on large scale.

The white rot fungus *Phanerochaete chrysosporium* possesses a non-specific enzymatic system that enables it to degrade a wide range of recalcitrant compounds including lignin (Kirk and Farrell, 1987).

As NOM contains a large range of constituents of different elemental composition and chemical properties, mostly refractory (Marhaba, 2000), *P. chrysosporium* was investigated as an alternative means of NOM removal. *P. chrysosporium* ATCC 34541 grown in modified Waksman medium containing NOM had been observed to cause rapid (within 7 days of inoculation) reduction in NOM content. The influence of various factors on this process was investigated in this study.

Agitated cultures exhibited superior performance over the static cultures for both extent and rate of decolourisation. In the most cases the decolourisation process was completed within 3 days in agitated cultures and maximal colour removal was 55%.

Glucose was necessary to initiate fungal growth and the decolourisation process. At greater than the optimal concentration (determined as approximately 1.0 g/L for 50 mg/L NOM) there was no further improvement in decolourisation with increased glucose content. The requirement for glucose increased with increased NOM concentration, and increased NOM concentration stimulated glucose consumption and fungal growth. The presence of NOM up to 480 mg/L did not inhibit its removal, approximately 40% of NOM was decolourised in the range of 120-480 mg/L, provided that sufficient glucose was present in the medium. The amount of NOM removed (mg/L), and the amount of NOM removed per unit biomass, increased with increased NOM concentration showing that fungal efficiency increased with increased NOM concentration (over the concentration range of NOM studied).

The colour removal process did not seem to be triggered by either glucose or nitrogen starvation, furthermore increased nitrogen content had a positive effect on the degree of decolourisation, signifying that the ligninolytic enzyme system may not be involved in the decolourisation process. However, increased glucose consumption and biomass production with NOM addition indicated that the removal mechanism depended on the metabolic activity of *P. chrysosporium*. The increased requirement for glucose at increased NOM concentration could be related to glucose being used in the decolourisation process for the generation of H₂O₂ required by oxidative enzymes or in the synthesis of veratryl alcohol, a fungal metabolite that acts as a substrate in LiP catalysis (Schick-Zapanta and Tien, 1997).

The addition of Mn²⁺ did not improve decolourisation therefore either manganese peroxidase was not involved in the decolourisation process or sufficient available Mn²⁺ was present in the medium. The degree of decolourisation of NOM by *P. chrysosporium* cultures grown in nitrogen-limited medium with added trace elements was lower than for those grown in Waksman medium, indicating that the modified Waksman medium provided better conditions for NOM decolourisation.

When the pH was controlled with buffers, sodium tartrate appeared to be efficient to maintain the pH of media and the greatest decolourisation of NOM was obtained in cultures with pH maintained at around 3. When the pH was manually controlled with NaOH and H₂SO₄, the control cultures (without pH adjustment and so final pH 3) exhibited greater NOM decolourisation. Increased pH (pH 4-6) appeared to stimulate glucose use and biomass production, however, no clear trend was found between synthesis efficiency and pH. Low pH affects the charge of biomass and possibly greater NOM binding occurred in low pH.

Increasing ionic strength by addition of up to 10 g/L NaCl had little effect on the decolourisation process, but increasing the salt content to 40 g/L NaCl and above caused lag phases in glucose consumption and decolourisation. Decolourisation slightly declined with increased salinity up to 50 g/L NaCl and then declined greatly with further added salt such that no decolourisation occurred at 80 g/L NaCl. The colour removal per unit biomass increased with increased salt content (≤50 g/L NaCl).

Decolourisation with inactivated fungal pellets (≤13%) was less than for live pellets (approximately 25%) and varied for the two methods of inactivation used, Colour removal by autoclaved cultures was almost double that observed for sodium azide-inactivated cultures. This increase, and the higher NOM removal per unit biomass, was attributed to the disruption of the autoclaved biomass allowing the cell interior to be exposed for adsorption. As NOM was decolourised to a greater extent in NOM-medium rather than in NOM-water systems, and viable biomass showed greater decolourisation, some aspects of the decolourisation process must be metabolically dependant.

Live pellets decolourised less NOM compared with mycelium grown from spores in medium containing the same NOM concentration. The NOM decolourisation process appears to accompany growth and NOM can be accumulated by the fungus as it grows.

The results showed that removal of NOM by *P. chrysosporium* is a complex process and involves different mechanisms such as: chemical and physical sorption, metabolically dependant

sorption and accumulation, and biodegradation. The molecular weight distribution of NOM remaining after treatment indicated that for fungus incubated in media, NOM removal could be attributed to both biodegradation and biosorption as reduction in the highest molecular weight fractions was accompanied by an increase in the smaller molecular weight fractions. However, the molecular weight distribution of NOM after viable fungal pellet treatment showed that reduction in the high molecular weight fractions did not coincide with increase in lower molecular weight fractions, suggesting that removal could be attributed solely to biosorption. The removal of NOM by non-viable biomass can be related to physical and chemical sorption only.

Sorption appeared to be the predominant mechanism in the removal of NOM by the fungal cultures, estimated as approximately 60-70% of the removal, 50% of which was attributed to metabolically dependant biosorption while the other half was attributed to physical and chemical sorption. The NOM appeared to be refractory to fungal treatment in the conditions studied as only 30-40% of the removal was estimated as biodegradation, however, it could be an over estimate and metabolically dependant biosorption and bioaccumulation can have greater contribution.