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Bioremediation of Organic wastes arising from Drinking Water Treatment by white rot fungus

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Abstract: *The importance of maintaining high quality natural surface water sources, on both national and global scales, is well known as a major requirement for public supplies as well as for future industrial growth. The presence of natural organic matter (NOM) in raw water reduces drinking water quality and interferes with water treatment processes. NOM, a complex mixture of organic carbon compounds, is undesirable for several reasons, including its contribution to taste and odour of the water, as well as to the formation of disinfection by- products (DBPs) When NOM reacts with disinfectants such as chlorine. Many epidemiological studies have suggested that DBPs are carcinogenic and thus present a health risk to the consumer. Bioremediation utilizes living organisms such as bacteria or fungi, or isolated enzyme systems, to break down organic pollutants and transform them into harmless products or valuable by-products. Phanerochaete sp has been shown to remove 40-50% Non organic matter from solution, however, this was found to be mainly due to bioadsorption and to be partially metabolically linked. They found that environmental conditions such as carbon and nitrogen content, pH and Non organic matter concentration played an important role in the removal of colour by the fungus. Bioremediation technology is viewed as an attractive approach to the removal of Non organic matter as this environmentally friendly method is potentially more cost-effective, and limits by-product formation and associated mutagenic product generation.*

Key Words: *Bioremediation, Phanerochaete sp, Bioadsorption, chlorine, Non organic matter*

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I. Introduction

Natural organic matter (NOM) is ubiquitous and it plays an important role in the global carbon cycle. In aquatic ecosystems, NOM represents one of the largest active organic carbon reservoirs; the amount of NOM (commonly measured as dissolved organic carbon [DOC]) in aquatic systems is nearly equal to the amount of CO2-carbon that exists in Earth's atmosphere . Because NOM is a vital source of C, energy, and is important in mediating nutrient and trace metal availability, it has a substantial impact on aquatic food webs and nutrient dynamics[1,5,32]

There are many NOM removal processes available, such as coagulation, granular activated carbon (GAC) adsorption, ozonation, magnetic ion exchange resin (MIEX), and membrane filtration. However, these processes have been identified as having high operating cost and producing sludge and residuals, some of which contain NOM. These wastes can be problematic regarding disposal, as government legislation concerning the release of wastes has become more stringent. Bioremediation technology is viewed as an attractive approach to the removal of NOM as this environmentally friendly method is potentially more cost-effective, and limits by-product formation and associated mutagenic product generation. Besides playing such a vital role in supplying C and nutrients to microorganisms, NOM also has an effect on the fate, reactivity and transport of inorganic and organic pollutants [1,6,9]. Despite its importance and abundance in microbial food webs and its effect on pollutant transport, NOM remains incompletely characterized with respect to its chemical composition, bioavailability and reactivity.

NOM can be removed from water by a number of different treatment processes[14,16] . The most common and economically feasible processes to remove NOM are coagulation and flocculation followed by sedimentation/flotation and sand filtration. NOM with high molar mass (HMM) is removed effectively from water in the chemical coagulation process[26,22-24,31]. However, a part of the organic matter is passing through when this method is used. This part consists predominantly of intermediate molar mass (IMM) and low molar mass (LMM) organic compounds. The NOM remaining after coagulation can be further removed by advanced treatment processes, such as activated carbon (AC) filtration, biologically activated carbon (BAC) filtration and membrane filtration[13-14]. The intermediate molar mass (IMM) organic compounds and a part of the low molar mass (LMM) organic matter can be removed quite efficiently in the AC filtration process [23] . However, a part of the LMM organic matter fraction does not adsorb onto AC[29] . Membrane filtration achieves the highest

removal capacity of NOM, especially as a last step of treatment after conventional processes [20] . The costs of the membrane filtration processes, however, have been relatively high and its use, therefore, is restricted to special cases [14,20]

Bioremediation technology is viewed as an attractive approach to the removal of NOM as this environmentally friendly method is potentially more cost-effective, and limits by-product formation and associated mutagenic product generation. This process would be able to remove biodegradable organic matter (which is responsible for microbial regrowth in the distribution systems), increase disinfectant stability in the water delivery network, as well as reduce chlorine demand [4] . Furthermore, this biological treatment could be applied in drinking water treatment as well as for the treatment of concentrated NOM wastes such as those found in alum precipitation, regenerant wastes from the MIEX process, and retentates from membrane treatment plants.

White-rot fungi are recognised for their ability to degrade lignin and an array of persistent aromatic pollutants due to their non-specific extracellular ligninolytic enzymes and their ability to adapt to severe environmental constraints [5,8,21]. The lignin-degrading system of *Phanerochaete sp* has been shown to remove pollutants such as pesticides, it could be an option for the treatment of contaminated farmland. The wastewater effluents of textile, paper, printing and dye industries are highly coloured and contain toxic aromatic amines[15] in this study investigated the effects of environmental conditions such as pH, carbon source

II. Methodology

NOM Samples: The highly coloured MIEX NOM concentrate from Mettur, located in Salem Dt., was utilised as a source of organic matter throughout the experiments. The NOM concentrate was obtained from the regeneration process of the strong base magnetic ion exchange (MIEX) resin, a recently developed process for the removal of dissolved organic carbon (DOC) The concentrate was filtered (0.45 µm hydrophilic Millipore) and stored at 4^{o}C prior to treatment and analysis. The characteristics of the NOM concentrates used in this study, which were collected at different times, are tabulated in Table 2.1.

Table 1: Characterisation of MIEX NOM concentrates.

Description	**Units**	**MIEX NOM Concentrates**		
Batch		NOM 1	NOM 2	NOM 3
pH		7.63	7.15	8.30
Absorbance at 446 nm(1:100)[a]	cm^{-1}	0.079	0.024	0.728
Absorbance at 254 nm(1:2000)[b]	cm^{-1}	0.114	0.032	0.582
DOC	g C/L	6.5	2.0	31.0
SUVA[c]	$L.mg^{-1}.m^{-1}$	3.5	3.2	3.8

[a] Dilution factor of 100

[b] Dilution factor of 2000

[c]SUVA(SpecificUVabsorbance) = $\frac{A_{254}}{DOC}$

Micro-organisms: *Phanerochaete sanguinea* strain MTCC 1088 were used in this study. *P. sanguinea* was maintained by subculturing monthly on Waksman medium agar slants for 3-4 days. whereas.The composition of the Waksman medium agar slants for fungal maintenance is detailed in Table 2.2. Spore suspensions were prepared by washing agar plates with sterilized water and then filtering through sterile glass wool. Spore concentration was determined by measuring the absorbance at 650 nm and calculated on the basis that A_{650} = 1.0 cm^{-1} corresponds to 5.0 x 10^{6} spores/mL. All chemicals were of AR purity.

Table 2 Composition of Wakesman Medium.

Medium component	**Sources**	**Content (g/L)**
Agar	Himedia	25.0
Peptone	Himedia	5.0
D-Glucose	Himedia	10.0
NH_4Cl	SRL	2.0
KH_2PO_4	SRL	1.0
$MgSO_4 .7H_2O$	SRL	0.5

Phanerochaete sanguinea strains MTCC 1088 fungi were inoculated on Waksman medium as either a spore suspension or as plugs.

Analytical Methods

pH: Adjustment of pH was made with 0.1 M, 0.5 M or 1.0 M NaOH and H_2SO_4.

Dissolved organic carbon : DOC concentration was determined using a total organic carbon (TOC) analyser . Samples were filtered (0.45 µm hydrophilic, Millipore) and diluted as required with water prior to analysis.

Absorbance : Absorbance measurements were performed with a double beam scanning UV/vis spectrophotometer fitted with a cell of 1 cm pathlength. The absorbance of NOM solution was measured at both 446 nm (colour) and 254 nm (UV- absorbing components).

Determination of glucose concentration : Reducing sugar concentrations of samples were measured by the 3', 5'-dinitrosalicylic acid (DNS) method using D-glucose as a standard. A typical standard curve for glucose determination.

Dry weight of biomass : Dry weight of biomass for both fungal and yeast strains was determined at the end of fermentations by filtering the biomass on pre-weighed dried membrane filters (0.45 µm Whatman sterile membranes), washing with distilled water and then drying them in an oven at $90^{O}C$ to constant weight.

III. RESULTS AND DISCUSSION

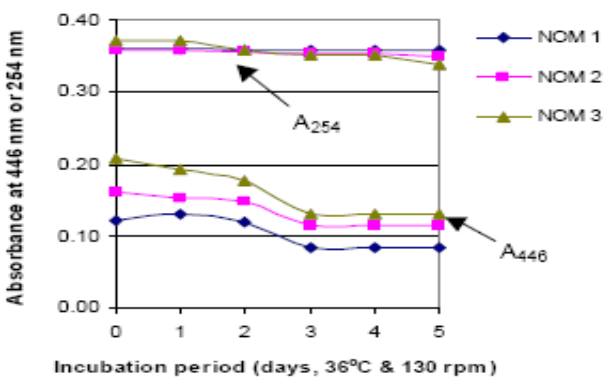
Decolourisation and Bioremediation of MIEX NOM

In the present study preliminary fermentations using *P. sanguina* MTCC 1088 for the removal of NOM from various batches of MIEX NOM concentrate were conducted to identify the effect of different characteristics of NOM on the process, and so the applicability of the process to the treatment of NOM waste arising from drinking water. The three NOM preparations (NOM 1, NOM 2 and NOM 3) were fractionated before the treatment to determine the natures of the organic compounds present. A comparison of the changes in colour (A_{446}), UV-absorbing components (A_{254}).The fermentations to established and the fractions removed by *P. sanguinea*.

White-rot fungi play a significant role in the recycling of lignin, capable of completely mineralising lignin to CO2 and H2O due to their non-specific extracellular ligninolytic enzyme system. Degradation of the complex irregular aromatic structure of lignin polymer by white-rot fungi enables them to access cellulose and hemicellulose, which they then utilise as carbon and energy sources [11,17-18,25]

The three NOM preparations were similar, and the plot for NOM 3 (Figure1) is shown as it gave the highest reduction in A_{446} and A_{254}. The pH dropped markedly over the first two days of incubation and then plateaued. The change in pH occurred concurrently with the decrease in glucose content. The drop in pH was probably due to the accumulation of organic acid as metabolite or by the freeing of hydrogen ions in substrate transfer by the fungus [12] . This may support biosorption as the bioadsorptive capacity of a fungus increased with decreasing pH for humic acids as reported by Zhou and Banks[33] . The fungus consumed only approximately half of the glucose (1.0 g/L) provided over the incubation period. This is consistent with the results found by Rojek (2003), where the fungus consumed only 1-1.2 g/L glucose even though higher initial glucose concentrations (4 and 10 g/L) were supplied. A_{446} decreased for the first three days and then plateaued whereas A_{254} decreased gradually during the whole fermentation. This is contrary to the findings of Blondeau [3] , where the decolourisation of humic acids by *P. sanguinea* only started after an initial lag phase of four days and continued up to day 15 and suggested that the lignin-degrading system played a role in the humic acid decolourisation.

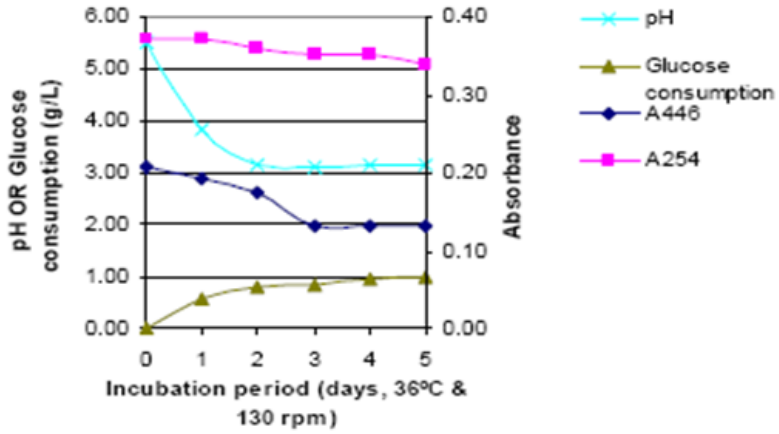
Figure-1 A446 and A254 of NOM 1, NOM 2 and NOM 3, 100 mg C/L initial NOM concentration, *P. sanguinea M*TCC 1088. (A254 represents readings of 1/10 dilution of culture medium)



The lignin-degrading enzyme system of white-rot fungi has been widely studied. A number of white-rot fungi and their enzymes have been used successfully in various configurations in different types of industrial applications. Since a diverse range of recalcitrant organic pollutants contain a chemical structure similar to lignin, the ligninolytic system and oxidative mechanism is considered to be involved in the degradation of the pollutants[10,28]as well as humic acids and so of naturally occurring organic matter due to the similarity between the structures of lignin and NOM.

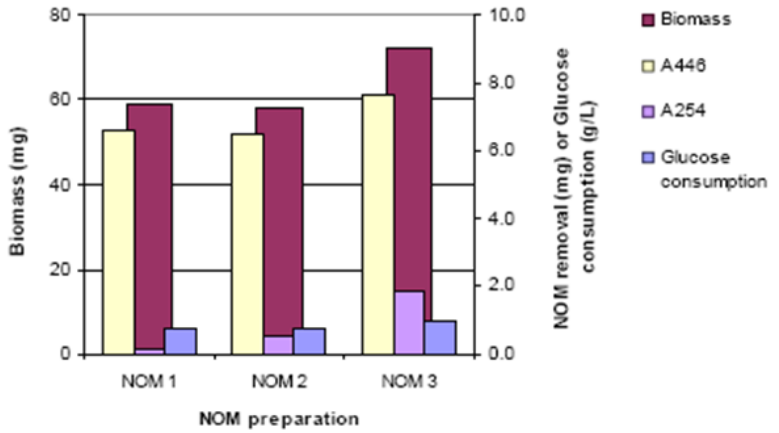
The initial and final colour (as A_{446}) of NOM 3 was much higher than for the other two NOM preparations even though the initial NOM concentrations in terms of total carbon were the same. The greatest decolourisation for all NOM preparations was obtained on day 3: 37% (NOM 3), 31% (NOM 1) and 29% (NOM 2). Losses in A_{254} of 9% for NOM 3 and 3% and 1% for NOM 2 and NOM 1, respectively, occurred after five days (Figure2).

Figure -2 History Plot Showing pH, Glucose Consumption, A446 and A254 for NOM Incubated With *P. Sanguinea* MTCC 1088 at 36°c and 130 rpm For Five Days.



To elucidate NOM removed in terms of total carbon, NOM removals (converted to mg), measured at 446 nm and 254 nm, were calculated (Figure3) . The extent of NOM removal followed the trends: NOM 3 > NOM 1 > NOM 2 for A_{446}, and NOM 3 > NOM 2 > NOM 1 for A_{254}. The low reduction in A_{254} indicates that the removal of conjugated double bonds (unsaturated aldehydes, phenols, aliphatic and aromatics) by these systems was negligible; consequently, little if any chemical change to the UV-absorbing components due to fungal activity or removal by adsorption occurred. The addition of NOM 1 and NOM 2 led to similar NOM removal, glucose consumption and biomass generation. In contrast, the fungus caused the removal of more NOM, higher glucose consumption and produced more biomass for NOM 3.

Figure -3 NOM removals (as mg, converted from A446 and A254), glucose consumption (g/L) and dry weight of biomass generated (mg) for the three NOM Preparations on day 5, 100 mg C/L initial NOM concentration, *P. sanguinea* MTCC 1088.



It was observed that the fungal pellets appeared brownish in colour. This indicated that NOM molecules were bonded to the fungal mycelium and so adsorption of NOM to the biomass seemed to play a role in colour removal as found by Rojek[27]. As the removal of colour was greatest for NOM 3, for which the biomass was greatest, and the fungal pellets were uniform in colour for all three NOM preparations, the major mechanism for NOM removal appeared to be via adsorption. This concurs with the previous proposal that NOM removal by biosorption occurred due to the pH drop [27] as low pH supports the binding of humic acid components to the fungal cell wall surface [33]. In addition, the degree of removal of NOM was directly related to the amount of biomass produced due to the greater number of adsorption sites.

IV. CONCLUSION

In this present study discussed with several available processes for the removal of NOM in waste and drinking water treatment industries. However, all have advantages and disadvantages. Conventional water treatments have been identified as having low removal efficiency and high operating cost, are mostly based on chemical addition and applicable to a limited concentration range, as well as producing sludge and residuals[29]. The NOM-containing wastes generated from alum precipitation, membrane process plant and the anionic exchange MIEX process can be problematic regarding disposal. Conventional coagulation, which requires the addition of a coagulant such as alum, ferric salts or polyaluminium chlorides, has low NOM removal efficiency (10-50%) and generates sludge disposal problems [14,30] . In addition, the control of coagulant addition and adjustment in pH are necessary, as these must be adjusted with any change in the raw water [14,30]. Some studies on coagulation suggested that lower molecular weight, hydrophilic, uncharged and fulvic acid-like components still remain in natural waters after the treatment [7,9] . This is consistent with the proportion of polysaccharide-derived compounds (i.e., of hydrophilic character) generally increased after alum treatment, indicating that these compounds are refractory to alum coagulation. Adsorption by granular or powdered activated carbon (GAC or PAC), which is widely used in the United States, has limitations since its adsorption capacity is limited and may be exhausted after a short period. Consequently, frequent reactivation or replacement of activated carbon is necessary [14] . Ozonation, which is effective in transformation of refractory NOM to biodegradable dissolved organic carbon (BDOC), has some limitations. Consequently, reduction of treatment costs and development of energy-efficient waste treatment processes are needed as much sludge, some of which contains NOM, is generated in NOM removal processes. Biological treatment can be an option for the breakdown of the NOM, as the treatment would overcome some problems associated with conventional treatment processes, including chemical usage and sludge management. Bioremediation technology is viewed as an attractive approach to the removal of NOM as it is 'natural' and a potentially chemical-free process, which should have good public acceptance, and leads to minimal waste production.

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