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Drinking Water Treatment of Natural Organic Wastes Matter with HPSEC Profile Using White Rot Fungus

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Abstract: ***The large water reservation of water bodies in dams and municipal tanks will have many undesirable formations that cause the organic pollution and lethal effect to aquatic ecosystem. The present study was made to remove safely the natural organic matter (i.e. undesirable formation) consisting non-humic and humic substances , since the accumulation of those gives an unpleasant odour and taste, the investigation was done using the samples from mettur dam where the sample undergoes three different dilution followed by fraction study using DAX8,XAD4 and IRA958 synthetic resins. The result shows the presences of VHA, SHA, CHA and NUE components whose molecular weight as a whole and also in diluted state was using HPSEC, since HPSEC requires relatively small volume of samples with or without pre concentration. Then we inoculated the entire sample with basidiomycetes family fungi Phanerochaete sanguinea MTCC 1088 a promising white rot fungi shown greater results in breakdown of fulvic acid and humic acid which were present in NOM.***

Keywords: Basidiomycetes, Ecosystem, *Phanerochaete* s*anguinea*, Synthetic resins, White rot fungi

1. Introduction

The essence of maintaining the purity of drinking water both in national and global scenario is considered as a major requirement for public supply and industrial growth. The long term storage of raw water leads to the formation of NOM which affects the quality of water and also the breeding ability of aquatic lives.NOM is a complex mixture of organic carbon compounds that are responsible for the taste and odour of the drinking water. But when it come in contact with disinfectants like chlorine it leads to the undesirable formation i.e. dissolved by products consider as a carcinogen that causes health hazards to the consumer. Besides this NOM act as a carrier for organic and inorganic pollutants in the large water reservoirs that affects the aquatic food web. Hence safe removal of NOM i.e. nonhumic and humic compounds using White-rot fungi belong to the wood-destroying basidiomycetes and are best known as the only micro-organisms responsible for the mineralization of all major wood polymers, including lignin, cellulose and hemicelluloses (Crawford, DL & Crawford, RL 1980) is undertaken in this investigation.

NOM consists of non-humic (hydrophilic) and humic (hydrophobic) substances (Hood *et al.*, 2003). The non-humic hydrophilic fractions are composed predominantly of well-defined chemical structures such as hydrophilic organic acids and low molecular weight compounds (carbohydrates, carboxylic acids, amino acids, lipids, proteins etc.), which are easily attacked by micro-organisms (Motheo, AJ & Pinhedo, L 2000).

On the other hand, humic substances, which originate from microbial or chemical conversion of bacteria, plants and other living organism residues, are naturally occurring heterogeneous organic substances that are based on N-containing polymers. Humic substances comprise both aliphatic and aromatic high molecular weight components (Liao *et al.*,1982). They have a complex chemical structure with no defined chemical and physical properties, which are generally repellant to the microbes (Motheo, AJ & Pinhedo, L 2000).

The mechanisms for their biodegradation ability are dependent on the fungal species and culture conditions,(Baldrian, P & Gabriel, J 2002, De Souza *etal.*, 2002, Fujian *etal.*,., 2001& Mehna *etal.*, 1995)The ligninolytic system of white-rot fungi a pool of enzymes like lignin peroxidase (LiP), manganese-dependent peroxidase (MnP) and laccase (Lac) (Sunil *et al* 2011)these promising enzymes have high oxidation and cleavage ability of wood and lignin (natural components of the ecosystem), and various intractable xenobiotic pollutants structurally similar to lignin (Buswell *etal.*, 1995& Tuor *et al.*, 1995).they are also able to bio-transform hazardous compounds including xenobiotics; reduce ammonia, iron and manganese levels and bio-oxidise assimilable organic carbon (AOC) that allows production of biologically stable water (Carraro *et al* 2000, Robinson *et al.*, 2001, Wricke *etal.*, 1996). Recently many studies have demonstrated the importance of cultivating lignocellulose residues for enhancing the production of phenoloxidase enzymes (Pooja *et al* 2016).

Due to the similarity of parts of the structure of NOM with lignin, here we had choose white-rot fungi *P. sanguinea* MTCC 1088 .the results shows the safe removal of NOM by adsorption and partial metabolic biodegradation when compared to whole and fractions by their molecular weight peaks (HPSEC) and fractionation (synthetic resins)respectively.

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2. Materials and Methods

2.1 NOM Samples

The highly coloured MIEX NOM concentrate from Mettur, located in Salem District., was utilized as a source of organic matter throughout the experiments. The samples were processed further in Microbiology Laboratory, Cauvery College for women, Tiruchirappalli. 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 4^0C prior to treatment and analysis.

2.2 Microorganisms

P. sanguinea MTCC 1088 was used in this study. *P. sanguinea* MTCC 1088 was retrieved on 2% (w/v) malt extract agar for 4-5 days and periodical sub culturing was done by using Waksman medium(table:1) and all the culture tubes were properly maintained at 4^0C prior use(Booth, C 1971).

2.3 Preparation of Inoculum

The fungi were inoculated as a spore suspension .Spore suspension was prepared by washing agar plates with sterilized water. 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 (Kirk *et al.*, 1978). Spore suspension (10 mL) was then added to the culture media to attain a concentration.

2.4 Absorbance

Absorbance measurements were performed with a UV/vis spectrophotometer fitted with a cell of 1 cm path length. The absorbance of NOM solution was measured at both 446 nm (colour) and 254 nm (UV- absorbing components). The correlations between NOM concentration for the three different preparations and absorbance at 446 nm and 254 nm are provide. Samples were centrifuged until the solution was clear and were diluted to 1:10 with Milli-Q water prior to A254 measurements.

2.5 High performance size exclusion chromatography

The molecular weight distribution of samples was determined using high performance size exclusion chromatography (HPSEC). The analysis was conducted using a Waters 2690 Alliance system with a temperature controlled oven at 30^0C and a Shodex KW 802.5 glycol functionalized silica gel column with a Waters 996 Photo Diode Array detector set at 260 nm. The column was calibrated with polystyrene sulphonate standards and the apparent molecular weight (Dalton) of NOM was calculated from the linear regression of the relationship between the retention time (t, minutes) and the logarithm of molecular weight of the standards (log (Mw)):log(M_w) µ 0.399 µt 7.205.

2.6 Fractionation of NOM

A NOM fractionation system was adopted here designed by Chow (Chow *et al.*, 2004). The system allows fractionation of the NOM into four categories: very hydrophobic acids (VHA), slightly hydrophobic acids (SHA), hydrophilic charged (CHA) and hydrophilic neutral (NEU) compounds. The VHA, SHA and CHA fractions were absorbed by DAX-8 resin, XAD-4 resin and IRA-958 resin respectively; and the NEU fraction was the effluent from the IRA-958 column. Three 20 cm glass columns for DAX-8, XAD-4 and IRA-958 resins respectively were set upon series, after exhaustive cleaning of resins with methanol and Milli-Q water. Resin-water slurries were added to give bed volumes of approximately 14.7 mL, 14.1 mL and 20.6 mL respectively. Each bed was backwashed with 2-3 L Milli-Q water to classify the resin particles and to remove air bubbles and debris (Chow *et al.*, 2004).Before fractionation, samples were filtered through a 0.45 µm hydrophilic PVDF (Millipore) and acidified to pH 2.0 with concentrated HCl. The pH-adjusted samples were then passed through the DAX-8 column at the rate of 0.2 bed volumes/min. The first two bed volumes were discarded before collecting the effluent. A sub sample of 100 mL was stored for TOC, A446 and A254 assays. The remaining effluent was then passed through the second (XAD-4) column. The same procedures were followed except that the effluent from the XAD- 4 column was adjusted to pH 8.0 with NaOH solution before pumping through the last (IRA-958) column.

3. Result and Discussion

3.1 Fractionation of the MIEX NOM Preparations

The three NOM (100 mg C/L) preparations were separated into four fractions: VHA, SHA, CHA and NEU to establish the types of organic compounds present. The relative proportions of DOC, A_{446} and A_{254} in each fraction were determined. The proportion of each fraction in Figure 1 is an average of duplicate determinations, and the values of the duplicates varied only by ±1%.The three preparations were dominated by hydrophobic acids such that VHA > SHA > CHA > NEU (Fig:1). NOM 1 and NOM 2 exhibited almost identical proportions of these fractions, but those in NOM 3 were markedly different. NOM 3 had the highest hydrophobic content with 61% VHA and 23% SHA. The hydrophilic neutral fraction was relatively small for all the preparations. This is because the MIEX NOM concentrates were obtained from the resin regeneration process, and constitute only approximately 80% of the NOM originally present in the water since the MIEX resin is an anionic exchanger and so cannot remove the neutral components of NOM.

It has been shown that NOM fractions contain a wide range of compounds and the types of compounds in each fraction are dependent on the water source. The typical classes of compounds in each fraction are summarized. The hydrophobic fractions are inclined to possess greater aromaticity than the hydrophilic fractions; therefore NOM 3 had the highest proportion of conjugated aromatic and high molecular weight compounds as

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supported by the HPSEC chromatograms (Fig:2). To better understand the components of the three NOM preparations, they were fractionated according to the method of Hess (Hess *et al*., 2000). This was to provide information on which types of compounds are removed in the treatment, as was mentioned by Yanagi (Yanagi *et al*., 2003) where the extent of decolourisation of humic acids varied with different chemical properties.

3.2. Comparison of molecular weight distribution of the NOM fractions

The molecular size distributions of the UV-absorbing components of the 'whole' NOM and each NOM fraction for the three NOM preparations were investigated (Fig: 2). NOM 1 and NOM 2 had very comparable molecular weight distribution with a large peak at ~1200 Dalton and another at 300 Dalton (Fig:2A). NOM 3 had peaks at almost similar apparent molecular weights, with an additional peak at 1000 Dalton, and it exhibited some higher molecular weight compounds.

The UV-absorbing species in all the NOM preparations were mostly in the hydrophobic fractions (VHA and SHA). The hydrophobic fractions contained molecules of apparent molecular weight >1500 Dalton for the three NOM preparations, but this was not so for the CHA fractions. The molecular weight distribution of each fraction was very similar for NOM 1 and NOM 2where as NOM 3 had a very different pattern for all fractions. It should be noted that the NEU fractions were very small for all and so no trends were apparent.

The VHA fraction gave two peaks for all the NOM preparations: at apparent molecular weights of ~1200 Dalton and 200 Dalton (Fig:2B). The absorbance at the peak of ~1200 Dalton for NOM 3 was lower than for NOM 1 and NOM 2, however, it contained more compounds with apparent molecular weight >2000 Dalton. The SHA fraction comprised markedly fewer high molecular weight UV-absorbing compounds than the VHA fraction for the three NOM preparations. NOM 3 possessed two peaks in the molecular weight range of 1000-2000 Dalton but with lower absorbance compared with the VHA fraction. There was only one peak in the SHA fraction for NOM 1 and NOM 2, which was at ~1200 Dalton (Fig:2C).

The CHA fraction for NOM 3 was relatively lower than for NOM 1 and NOM 2. All NOM preparations had a peak at ~1500 Dalton and an additional peak at 1000 Dalton for NOM 3 (Fig:2D). Their UV-absorbing contents were very small compared to the hydrophobic fractions, especially in NOM 3.There was little, if any, UV-absorbing species in the NEU fraction for all the NOM preparations (Fig:2E) as the most UV-absorbing compounds in the hydrophilic fractions (CHA and NEU) were in the CHA fraction.

The three NOM preparations contained less non-humic (hydrophilic) than humic (hydrophobic) substances due to the lower contents of the UV-absorbing species in the hydrophilic fractions, especially in NOM 3, than the hydrophobic fractions. Possible constituents of the hydrophilic fractions would be hydrophilic base compounds such as amphoteric proteinaceous materials containing aliphatic amino acids, amino sugars, peptides and proteins, Polysaccharides, hydrophilic neutral compounds, were unlikely to be present in the NOM preparations as the NEU fraction was very small (Fig :2). All the NOM preparations most likely contained fulvic acids and humic acids with NOM 3 having the highest humic acid content as it was highly alkaline and dark brown in colour, and had more high molecular weight compounds (>2000 Dalton).

3.3.Molecular Size Distribution of the NOM after Treatment with *P. sanguinea* MTCC 1088

The molecular weight distribution of the UV-absorbing species for the three NOM preparations after five days treatment with *P. sanguinea* was determined using HPSEC (Fig:3). A small shift from higher to lower molecular weight for the NOM remaining after treatment with *P. sanguinea* was observed for all NOM preparations (Fig:3). NOM 3 showed a slightly greater shift to lower molecular weight following the treatment. The removal of the high molecular weight compounds was accompanied by the accumulation of the low molecular weight species, presumably due to some breakdown by the fungus, especially for the NOM 3 preparation. All the NOM preparations exhibited a decrease in the UV absorbance for the molecular weight fraction >2000 Dalton. This was largely due to the decrease in the high molecular weight hydrophobic fractions and was most marked for NOM 3 as only the hydrophobic fractions exhibited apparent molecular weight >2000 Dalton (Fig:3A & B). For the cultures with NOM 1 and NOM 2 there was almost no shift in the apparent molecular weight range 1000-2000 Dalton, and there was a similar reduction in absorbance for the peak at ~1500 Dalton.

Hou (Hou *et al*.,2004) obtained reduction in the high molecular weight range but without accumulation of low molecular weight species after the fungal treatment. However, adsorption also contributed to the removal of the coloured high molecular weight fractions as the fungal pellets turned brown (Rojek, K 2003).

All the NOM preparations exhibited a decrease in the UV absorbance for the molecular weight fraction >2000 Dalton. This was largely due to the decrease in the high molecular weight hydrophobic fractions and was most marked for NOM 3 as only the hydrophobic fractions exhibited apparent molecular weight >2000 Dalton. For the cultures with NOM 1 and NOM 2 there was almost no shift in the apparent molecular weight range 1000-2000 Dalton, and there was a similar reduction in absorbance for the peak at ~1500 Dalton. In contrast, there was a slight shift in apparent molecular weight in the range 1000-2000 Dalton and some decreases in absorbance for the culture with NOM 3. *P. sanguinea* seemed to preferentially remove the VHA fraction, and so was most effective for the NOM preparation with the highest VHA content. However, this could not be demonstrated by fractionation of the NOM-containing medium after growth of the fungus as

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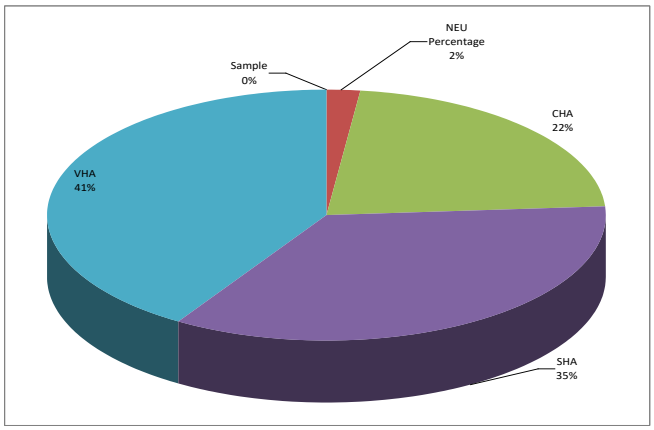
it contained glucose, metabolic products and salts, which would have interfered with the function of the resins (Yuan *et al.*,2005, Hou *et al.*,2004)

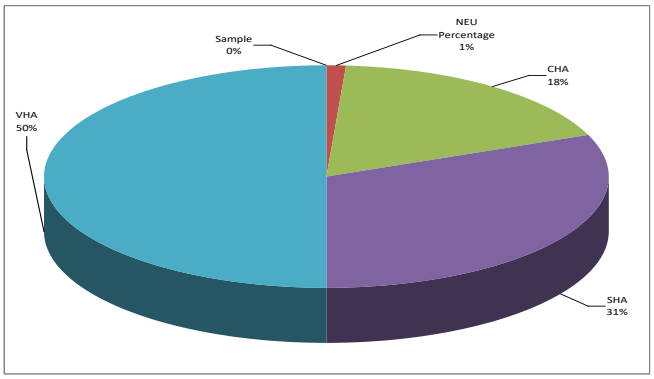
Table 1: Composition of growth media

Medium	Composition (g/L)				
	Glucose	NH_4Cl	NH_4NO_3	KH_2PO_4	$MgSO_4$
Waksman	2.0 or 5.0[a]	0.5	-	1.0	0.5

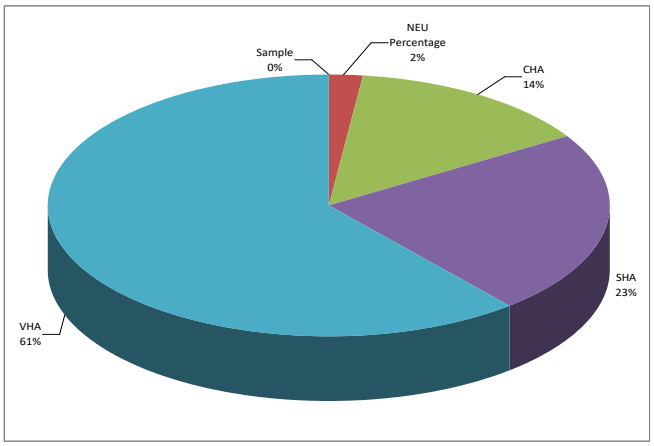
[a] Glucose content varied according to the experiment.



(a) NOM1

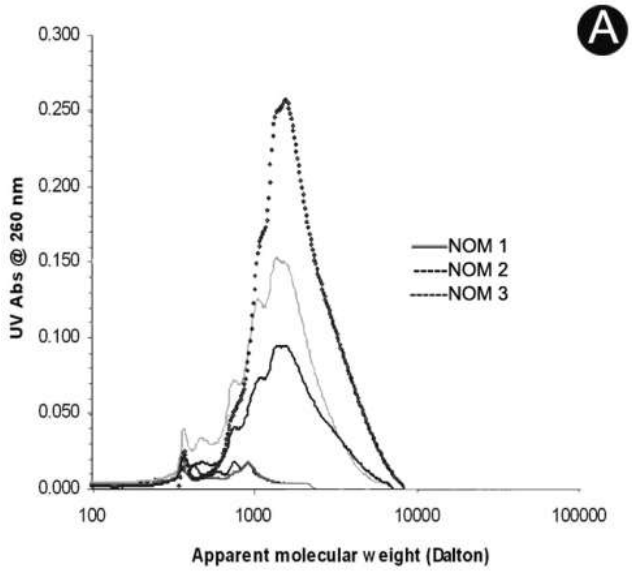


(b) NOM2



(c)NOM3

Figure 1: The preparations of each fraction in the three NOM preparations



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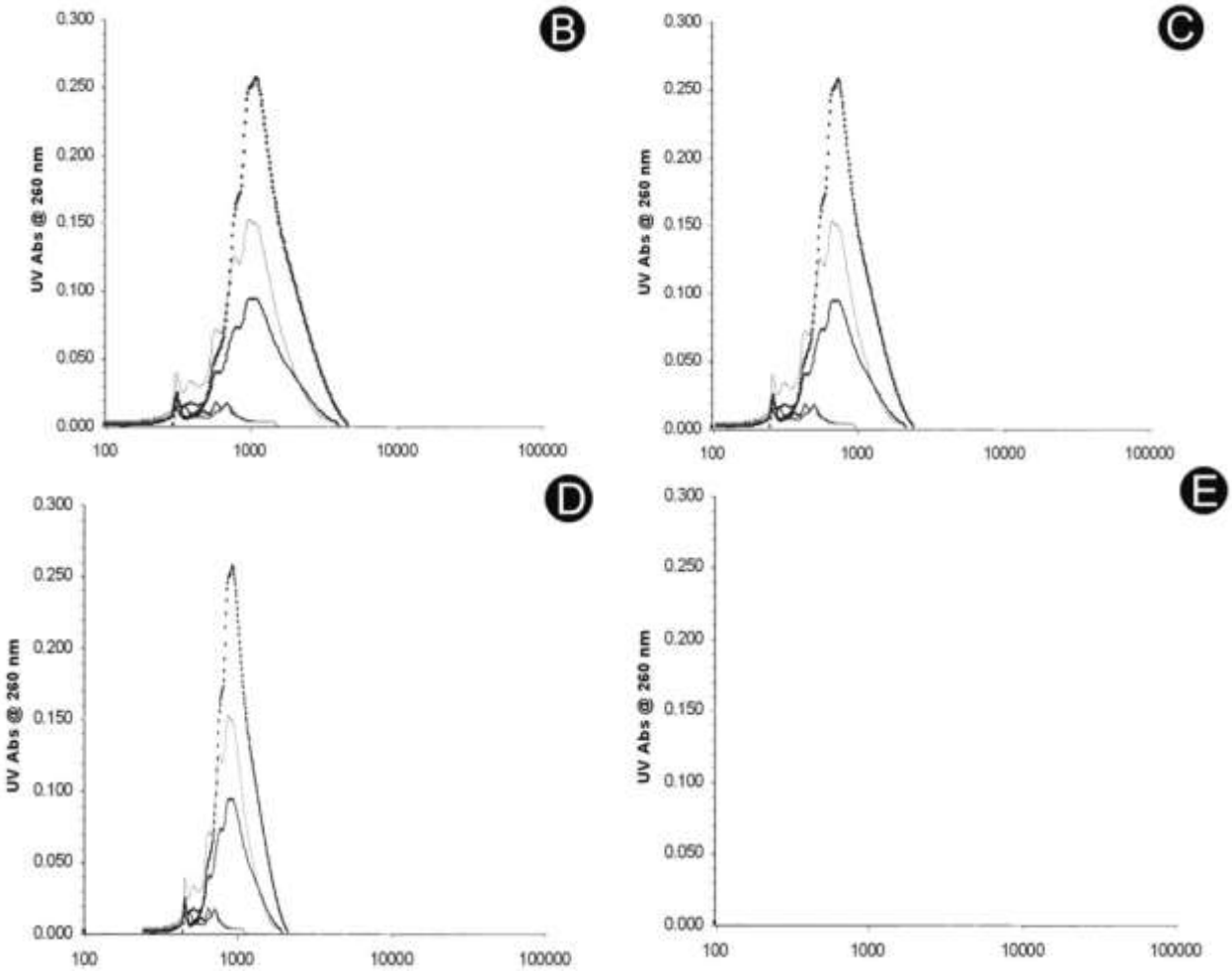
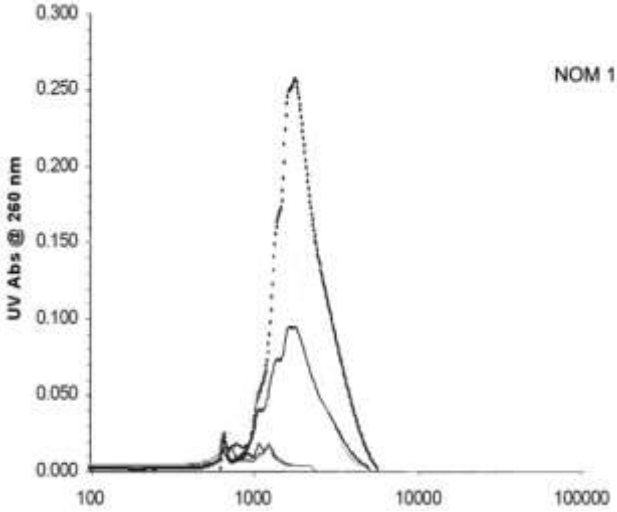


Figure 2: HPSEC chromatograms for the (A) whole NOM, (B) VHA, (C) SHA, (D) CHA and (E) NEU fractions for all NOM preparations.



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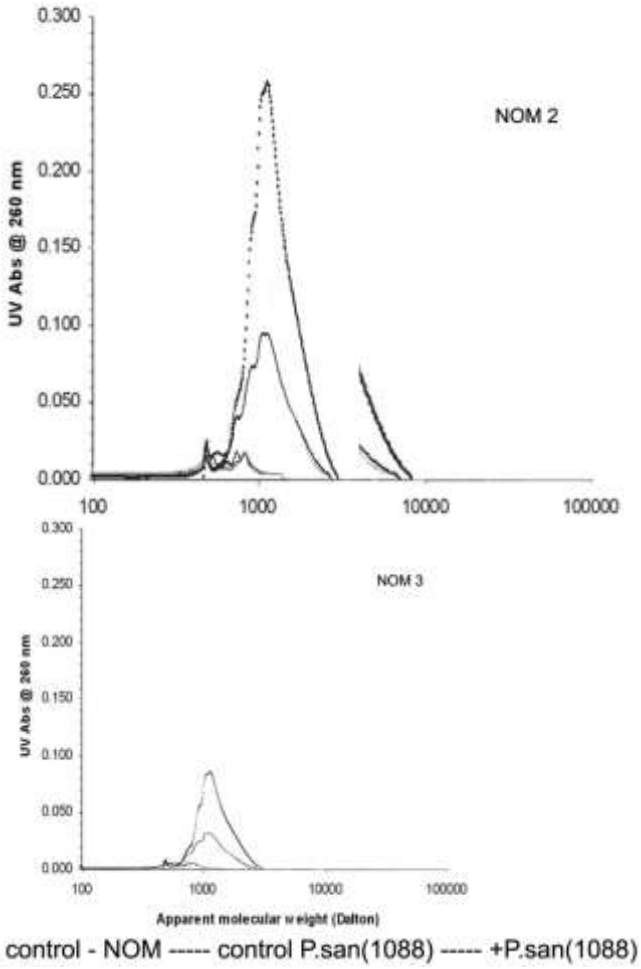

Figure 3: HPSEC chromatograms for the three NOM preparations incubated with *P. sanguinea* in Waksman medium, control – NOM (medium plus NOM), control – *P. sanguinea* 1088 (*P. sanguinea* 1088 grown without NOM).

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