iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 5 **Issue:** IX **Month of publication:** September 2017

DOI:

www.ijraset.com

Call: 08813907089 | E-mail ID: ijraset@gmail.com

International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor:6.887
Volume 5 Issue IX, September 2017- Available at www.ijraset.com

Bioremidiation of Natural Organic Matter Wastes Arising From Water Reservation by White Rot Fungus Trametes Hirsuta MTCC 136

Jeyabharathi.S[1] and Stephan.R[2]
[1]*Assistant professor, Department of Microbiology, Cauvery College for Women,Thiruchirappalli,Tamil Nadu*
[2]*Assistant professor, Department of Botany, Govt Arts College,Ariyalur,Tamil Nadu.*

Abstract: ***The necessary for the requirement of pure water from natural reservoirs in need to satisfy the public water requirement for both domestic and personal uses. During large water reservation in dams and other artificial tanks the undesirable formation of NOM (natural organic matter wastes) consist of non humic and humic substances and consistent accumulation of NOM affect the taste and odour of the water that consider as organic pollutant which affect aquatic ecosystem. The safe removal of NOM was suggested in this present study. Here we fractionating the sample using DAX 8, XAD 4 and IRA 958 synthetic resins to obtain very hydrophobic acids (VHA), slightly hydrophobic acids (SHA), hydrophilic charged (CHA) and hydrophilic neutral (NEU) compounds. Then we measured the molecular weight of both whole NOM fractions using HPSEC (High- performance size-exclusion chromatography) and the results were compared before and after inoculation with the Basidiomycetes family fungi Trametes hirsuta MTCC 136 and there was a significant reduction in molecular weight of samples by their phenoloxidease enzyme Laccase and this was confirmed by Guiacol test.***
Keywords: White rot fungi, Laccase, VHA, SHA, CHA and NEU

I. INTRODUCTION

The importance of pure drinking water maintenance for both national and global scales consider as a major requirement for public supplies and also future industrial growth. The formation of NOM (Natural Organic Matter waste) in raw water during long term storage reduces quality and appearance of water and affects fish breeding ability.

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 & Pinhedo, 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 refractory to attack by micro-organisms (Motheo & Pinhedo, 2000). Furthermore, they are categorised into three classes based on their solubility characteristics: humin, fulvic acid and humic acid (Weber, 2001), Hence this Nom complex governs large active carbon reservoir gives taste and odour of the water but the undesirable reaction when contact with chlorine like disinfectants results the formation of dissolved by products which would consider as a carcinogen and leads to health risk to the consumer. The NOM in aquatic system is almost equal to that the amount of carbon exists in the earth's atmosphere. Besides playing such a vital role in reactivity it also act a good transport medium for organic and inorganic pollutants and the presence of this pollutants affect the aquatic food web hence Nom has to be safely removed from water without affecting aquatic environment.

In this present study focused on the degradation of NOM by White rot fungi belongs to the wood destroying Basidiomycetes and are the best known microorganism responsible for the mineralization of all wood polymer including lignin, cellulose and hemicelluloses. The ligninolytic system of white rot fungi a pool of enzymes like lignin peroxidase (LiP), manganese-dependent peroxidase (MnP) and laccase (Lac), which are highly effective in oxidizing and cleaving wood and lignin (natural components of the ecosystem), as well as various intractable xenobiotic pollutants structurally similar to lignin(Buswell et al.,1995) . Due to the similarity of parts of the structure of NOM with lignin, here we chosen the white-rot fungi Trametes hirsuta MTCC 136 to decolourise and degrade humic substances by adsorption and partial degradation.

II. MATERIALS AND METHODS

A. NOM Samples

The highly coloured MIEX NOM concentrate from Mettur, located in Salem Dt., was utilized 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 4^0C prior to treatment and analysis.

B. Microorganisms

Trametes hirsuta strain MTCC 136 was used in this study. *T. hirsuta* was retrieved on 2% (w/v) malt extract agar for 4-5 days and periodical sub culturing was done by using Wakesman medium and all the culture tubes were properly maintained at 4^0C prior use(Booth.,1971).

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

Glucose content varied according to the experiment.

C. 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.

D. 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 provided. Samples were centrifuged until the solution was clear and were diluted to 1:10 with Milli-Q water prior to A254 measurements.

E. 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.

F. Fractionation of NOM

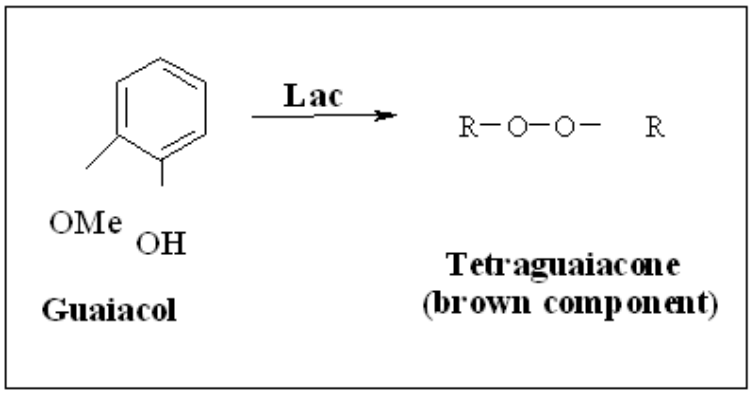
A NOM fractionation system as designed by Chow *et al.* (2004).The system allows fractionation of the NOM into four categories: very hydrophobic acids (VHA), slightly hydrophobic acids (SHA), hydrophilic charged (CHA) and hydrophilic neutral (NEU) compounds. The VHA, SHA and CHA fractions were absorbed 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.

G. Determination of Laccase activity

Laccase (Lac) activity was determined spectrophotometrically as described by Coll *et al.*, (1993) with guaiacol as a substrate. The oxidation of guaiacol (Sigma, 100%) to the polymer tetraguaiacone was monitored by increase in absorbance at 465 nm. The activity was expressed in U/mL where one unit (U) of Lac activity was defined as the amount of the enzyme that caused an increase of one absorbance unit per minute. The reaction (Figure 2.1) was started by addition of guaiacol.



where R represents the molecule after having donated H

Figure 2.1.Proposed mechanism for the oxidation of guaiacol by laccase .

H. Determination of Laccase Zone on agar medium with Guiacol :

The fungal isolate were screened for the production of lignolytic enzymes. The isolates were inoculated aseptically onto the pre solidified potato dextrose agar medium containing 0.05% guaiacol and incubated at room temperature for 7 days. Lignolytic positive organisms developed colored zone.

III. RESULTS

A. Biodegradation of NOM by Trametes hirusta

The results were ploted and the Plots of NOM removal (A_{446} and A_{254}, converted to mg), for the white-rot fungi was constructed. *T. hirsuta* attained the highest reduction in colour and UV-absorbing components.NOM removals in terms of colour and UV-absorption achieved by *T. hirsuta* was approximately 65% and 80% greater. It was observed that the *T. hirsuta* pellets were slightly lighter in colour(Figure3.1) .

HPSEC analysis was performed to determine any changes in the molecular weight distribution of the UV-absorbing species of the NOM remaining after treatment. A shift from high molecular weight towards lower molecular weight species was observed for both fungal species and was most marked for *T. hirsuta* gave greater degradation of the high molecular weight compounds *T. hirsuta* cultures was reduced by approximately 0.03 cm-1 and 0.10 cm-1.The absorbance of the low molecular weight compounds for both cultures was increased after the treatment, indicating that biodegradation of NOM had occurred to form a pool of low molecular weight compounds; this was greater for the *T. hirsuta* culture. There were two new peaks formed at 600 and 1000 Dalton after the treatment with *T. hirsuta*, again indicating the greater breakdown of the high molecular weight NOM, and suggesting a possible mechanism of sequential breakdown of the larger NOM molecules via molecules of intermediate size (Figure 3.2). The impact of the NOM-degrading enzymes on the molecular weight distribution of the UV- absorbing species for the NOM remaining after nine days treatment with T. hirsuta. A shift from high molecular weight towards lower molecular weight was observed.T. hirsuta was markedly more effective in removing and converting the high molecular weight UV-absorbing species to lower molecular weight compounds. The fungus at 30^0C was able to remove compounds of apparent molecular weight smaller

than 1000 Dalton, and the first peak at 350 Dalton remained similar or reduced slightly, indicating that either the low molecular weight species were produced (from the breakdown of the high molecular weight molecules) and were removed simultaneously probably by metabolism or, that the low molecular weight species were not UV-absorbing. As noted previously, the ability of T. hirsuta to degrade VHA fractions NOM was greater due to its greater production of NOM-degrading enzymes, especially MnP and Lac, where MnP is presumably responsible for the removal of low molecular weight species. Thus, MnP and Lac may act synergistically in the enzymatic breakdown of medium molecular weight (500-2000 Dalton) fulvic acids and high molecular weight (2000-5000 Dalton) humic acids are examples for VHA compounds.it has a less biodegrading activity on SHA,CHA compounds(fig 3.3). It was considered that the extracellular phenoloxidase enzymes (LiP, MnP and Lac) may have been involved in the biodegradation of NOM by
T. hirsuta, consequently, a spot test on a colony on an agar plate was undertaken. A reddish brown zone was visible on the T. hirsuta colony when 0.02% guaiacol was added indicating the presence of laccase as laccase catalyses the oxidative polymerization of guaiacol to form brown tetraguaiacone(fig 3.4).

IV. CONCLUSION

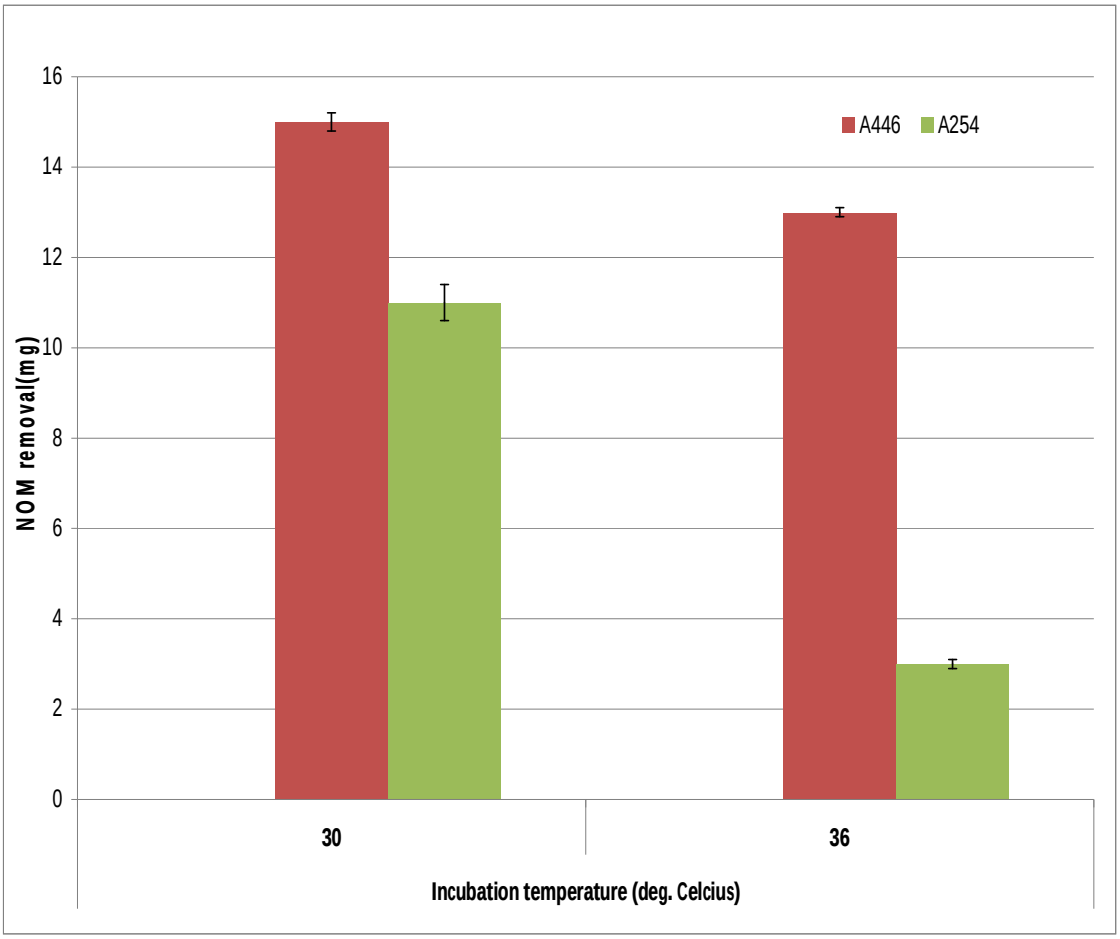
The rate of NOM removal initial slow and then higher rate of with *T. versicolor* at 30^{o}C was attributed to the production of oxidative enzymes during secondary metabolism. The lack of brown colouration of the fungal pellets supports this premise of NOM removal by biodegradation rather than adsorption. The trends in reduction in A_{446} and A_{254} can be related to the activities of the extracellular phenoloxidase enzymes. Although two studies (Fenice *et al*., 2003) reported that LiP and MnP were associated with humic acid degradation, the resistance of humic substances to microbial decolourisation is largely related to the differences in their chemical structures and is microbial species dependent, as suggested by Yanagi et al. (2002), and is dependent upon the culture conditions.
Moreover, Dehorter and Blondeau, (1992) established that MnP rather than LiP was the major enzyme involved in the microbial degradation of different concentrations of humic acid with T. hirsuta. The high levels of MnP and Lac observed for T. hirsuta at 30 ^{o}C are consistent with the findings of Galliano et al., (1991), who reported that these enzymes worked synergistically in the degradation of lignin. They reported that when these two enzymes were isolated and purified from Rigidoporus lignosus, neither was able to solubilise lignin. However, degradation of lignin occurred when the two enzymes were added to the reaction medium simultaneously.
These findings support the suggestion that removal of NOM by T. hirsuta incubated at 30^{o}C was mainly due to enzymatic breakdown whereas at 36^{o}C it was probably removed by different mechanisms such as: chemical and physical sorption, metabolically dependent sorption and accumulation, and biodegradation as reported by Rojek, (2003) in relation to the NOM removal with P. chrysosporium. Rancane et al., (2003) illustrated a relationship between humic acid degradation and extracellular enzyme activity of P. chrysosporium and T. versicolor, and showed that the production of extracellular LiP and MnP increased with increasing concentrations of humic acids. The surfactant properties of the humic acids were suggested to be responsible for the increase in enzyme activities. However, 1% or higher concentrations of humic acids were found to be inhibitory to growth and enzyme induction. This is in an agreement with the present findings, where LiP and MnP activities decreased with NOM concentrations from 300 mg C/L (equivalent to 1.0%) to 700 mg C/L (equivalent to 2.3%).
From this investigation *T. hirsuta* was the most effective for the biodegradation of NOM and gave the greatest NOM removal. Therefore, further investigation was undertaken into the conditions to improve growth and enhance the extracellular enzyme activities of *T. hirsuta* to enhance NOM removal. In present study suggested that the lignin-degrading system required high O_2 demand and so the impact of dissolved oxygen on NOM removal may play a role in the biological treatment.

BIBLIOGRAPHY

[1] Booth, C 1971, 'Fungal culture media', In Methods in Microbiology, 4th edn, Academic Press, New York.
[2] Buswell, J.A., Y. Cai and S. Chang. 1995. Effect of nutrient nitrogen and manganese on manganese peroxidase and laccase production by Lentinula (Lentinus) edodes. FEMS Lett. 128: 81-88.
[3] Chow, CWK, Fabris, R & Drikas, M 2004, 'A rapid fractionation technique to characterise natural organic matter for the optimisation of water treatment processes', Journal of Water Supply Research and Technology-Aqua, 53(2), pp. 85-92.
[4] Coll, PM, Fernandezabalos, JM, Villanueva, JR, Santamaria, R & Perez, P 1993, 'Purification and characterization of a phenoloxidase (laccase) from the lignin-degrading Basidiomycete Pm1 (Cect-2971)', Applied and Environmental Microbiology, 59(8), pp. 2607-2613.
[5] Dehorter, B & Blondeau, R 1992, 'Extracellular enzyme activities during humic acid degradation by the white-rot fungi Phanerochaete chrysosporium and Trametes versicolor', FEMS Microbiology Letters, 94(3), pp. 209-215.
[6] Fenice, M, Sermanni, GG, Federici, F & D'Annibale, A 2003, 'Submerged and solid state production of laccase and Mn peroxidase by Panus tigrinus on olive mill wastewater-based media', Journal of Biotechnology, 100(1), pp. 77-85.

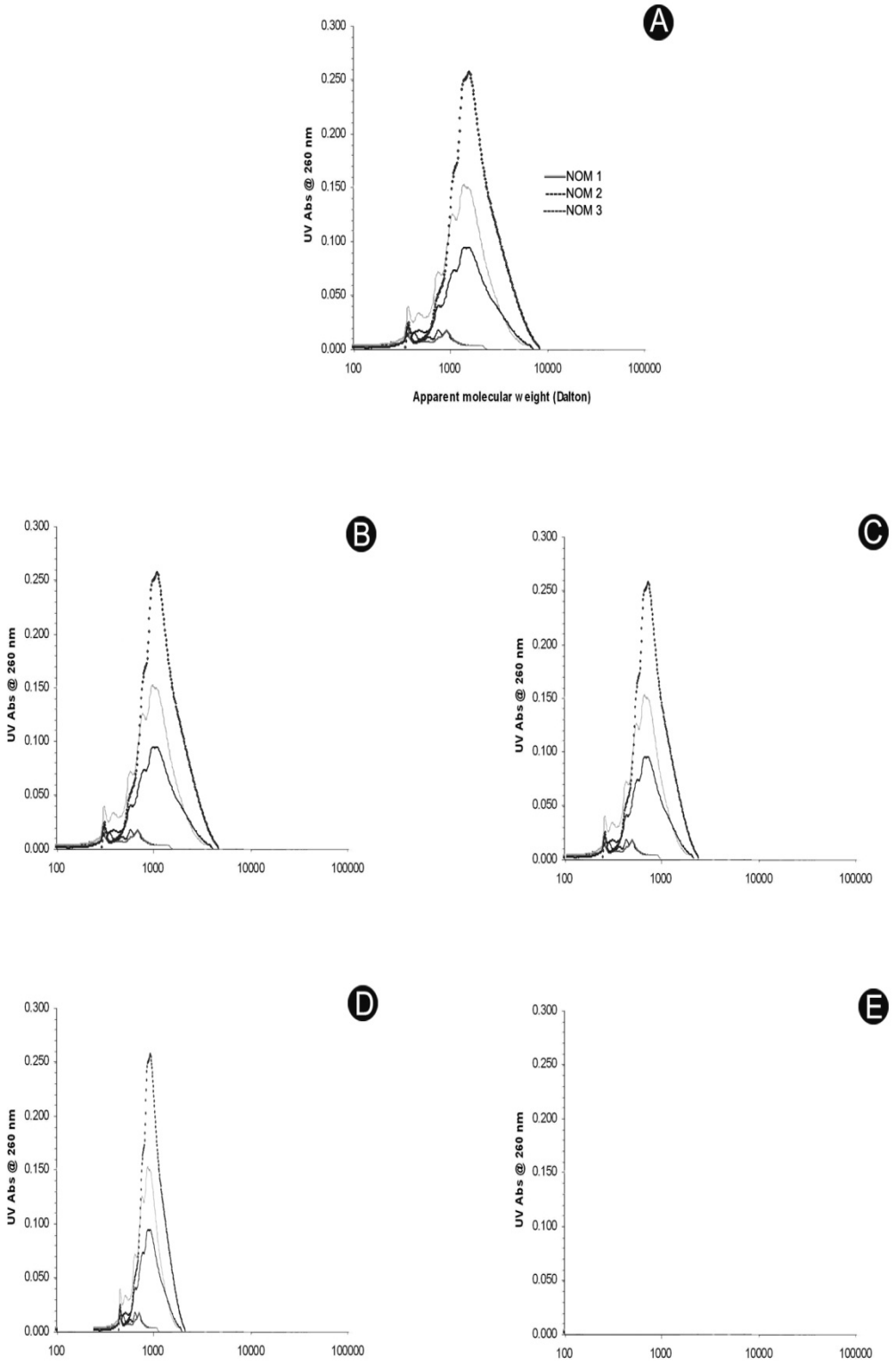
[7] Galliano, H, Gas, G, Seris, JL & Boudet, AM 1991, 'Lignin degradation by Rigidoporus lignosus involves synergistic action of 2 oxidizing enzymes - Mn peroxidase and laccase', Enzyme and Microbial Technology, 13(6), pp. 478-482
[8] Hood, E, McKnight, DM & Williams, MW 2003, 'Sources and chemical character of dissolved organic carbon across an alpine/subalpine ecotone, Green Lakes Valley, Colorado Front Range, United States', Water Resources Research, 39(7), pp. 1-12.
[9] Kirk, TK, Schultz, E, Connors, WJ, Lorenz, LF & Zeikus, JG 1978, 'Influence of culture parameters on lignin metabolism by Phanerochaete chrysosporium', Archives of Microbiology, 117(3), pp. 277-285.
[10] Liao, W, Christman, RF, Johnson, JD, Millington, DS & Hass, JR 1982, 'Structural characterization of aquatic humic material', Environmental Science & Technology, 16(7), pp. 403-410
[11] Motheo, AJ & Pinhedo, L 2000, 'Electrochemical degradation of humic acid', The Science of the Total Environment, 256(1), pp. 67-76.
[12] Rancano, G., Lorenzo, M., Molares, N., Rodrı´guez Couto, S. and Sanroman, A . (2003) Production of laccase by Trametes versicolor in an airlift fermentor. Process Biochem 39, 467–473.
[13] Rojek, K 2003, 'Decolourisation of aquatic NOM with the white-rot fungus Phanerochaete chrysosporium', Master of Engineering thesis, School of Civil and Chemical Engineering, RMIT University.
[14] Weber, J 2001, Definition of soil organic matter, Humintech, viewed 7 June 2005 <http://www.humintech.com/001/articles/article_definition_of_soil_organic_matter.html>.
[15] Yanagi, Y, Hamaguchi, S, Tamaki, H, Suzuki, T, Otsuka, H & Fujitake, N 2003, 'Relation of chemical properties of soil humic acids to decolorization by white rot fungus-Coriolus Consors', Soil Science and Plant Nutrition, 49(2), pp. 201-206.

Figure 3.1.NOM removals (as mg converted from A_{446} and A_{254}) in *T. hirsuta* cultures at 30° C and 36°C



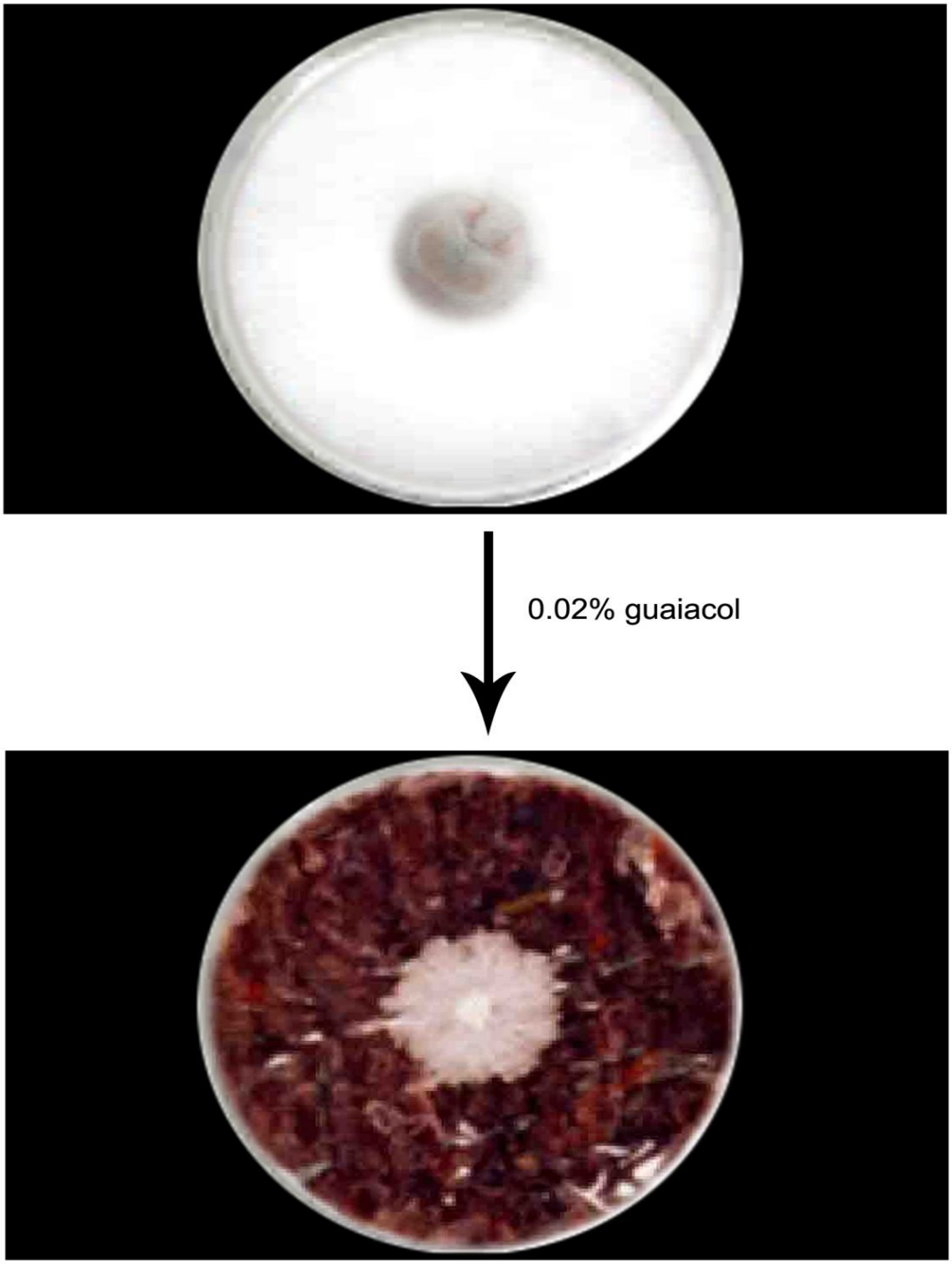
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor:6.887
Volume 5 Issue IX, September 2017- Available at www.ijraset.com

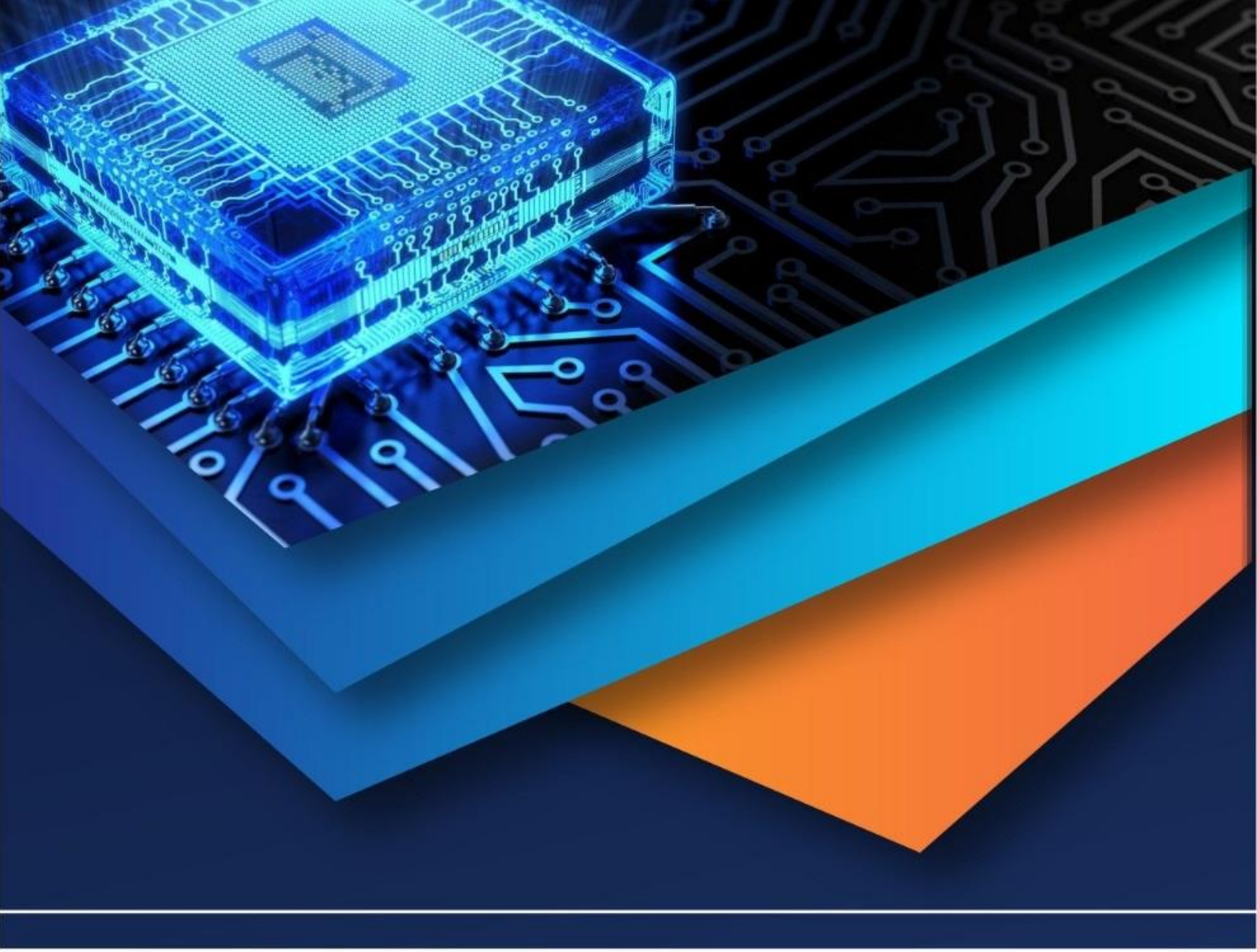
Figure 3.2. HPSEC chromatograms for the (A) whole NOM, (B) VHA, (C) SHA, (D) CHA and (E) NEU fractions for all NOM preparations.



ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor:6.887
Volume 5 Issue IX, September 2017- Available at www.ijraset.com

Figure 3.4.Reaction of guaiacol on *T. hirsuta* agar plate colony indicating presence of the laccase enzyme





10.22214/IJRASET

45.98

IMPACT FACTOR: 7.129

IMPACT FACTOR: 7.429

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY