EXTRACELLULAR ALKALINE XYLANASE FROM HALOPHILIC *BACILLUS CEREUS* IND-3 - A BIOTOOL FOR AN ENZYMATIC DEGRADATION OF PAPER PULP EFFLUENT

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Abstract

Paper pulp effluent degrading bacteria was isolated from hypersaline environment situated at beach road , Tuticorin, Tamil Nadu . The bacteria was characterized as *Bacillus cereus* - IND-3 by biochemical and 16 s r RNA sequencing . The bacteria was optimized by physico-chemical Parameters and nutritional sources such as carbon, nitrogen and metal ions for maximum xylanase production. At p^H 7.5, 35^0C temperature, 2.5mM NaCl concentration and 72 hours of incubation maximum xylanase and biomass production was recorded. Among the nutritional sources, carbon source rice bran showed maximum xylanase and biomass production (69.4 U / ml and 21.4 mg / ml respectively) followed by nitrogen source gelatin (74.3 U / ml and 29.2 mg / ml respectively) and metal ions - Ca Cl_2 (77.4 U / ml and 31.5 mg / ml respectively) . The extracellular xylanase enzyme obtained from the halophilic bacteria *B.cereus* -IND-3 was used for biodegradation of paper pulp effluent .The pretreated raw effluent showed high level of pH , temperature , BOD , COD , TDS and TH. But, the extracellular xylanase enzyme treated effluent recorded with reduction of impurities after the five days of incubation. The biodegradation studies revealed that the percentage of reduction was highest in TDS (67.70 %) followed by BOD (39.79 %) , TH (36.45 %) , COD (26.40 %) pH (4.172 %) and Temperature (2.32 %) in the xylanase enzyme treated effluent. The GC -MS analysis of raw and xylanase treated paper pulp effluent showed that many toxic compounds exist in the raw effluent was degraded and new compounds with less toxicity were generated due to the action of the extracellular alkaline xylanase enzyme.

Key words : Paper pulp effluent, *Bacillus cereus* -IND-3 , Hyper saline environment, Xylanase.

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1.0 Introduction

The pulp and paper industries generate various types of effluent composed of organic and inorganic toxic substances (Manuel Hernandez *et al*., 2001). These highly polluted brown coloured effluent is disposed into the environment without any treatment caused severe environmental problems (Shimp and Owns, 1993). Now a days various biological process of have been applied to degrade the highly toxic effluent in to lesser extent (Paice et al., 1996). The bacterial strains and their extracellular enzymes are played an important role in detoxification process of lignin content in the pulp paper mill effluent (Abhay Raj *et al.,* 2007). In general, the bacterial biodegradation systems consist of various specific extracellular enzymes which catalyse the production of many unique compounds (Masai *et al.,* 1999). Thus,the bacterial enzymes serves as an important tool for the transformation of highly toxic compound in to intermediate compounds(Chakar and Ragauskas, 2004).The bacterial strains *Pseudomonas putida, Serratia marcescens* and *Bacillus megatarium* are capable of degradation of the toxic compounds in the pulp paper effluents (Morri et al., 1995 : Pereestelo *et al.,* 1996). Therefore, in the present study, the bacterial strain *Bacillus cereus* IND-3 was optimized with physico-chemical parameter and nutritional sources, for extracellular xylanase production. Further, the xylanase enzyme was used for reduction of physico-chemical parameters in the pulp paper effluent. In addition , metabolic characterization by GC-MS was carried out in the raw and xylanase treated pulp paper effluent for safe discharge of effluent in to the environment .

2.0 MATERIALS AND METHODS

2.1 Collection and screening of bacterial strain for xylanase activity

The sediment sample was collected from the crystallizer ponds of solar salt pans situated at Thoothukudy, Tamilnadu. The collected sediment sample was serially diluted as per standard procedure explained by Bergey and Holt, 1994. From these, 1 ml of the sample (Dilution factor 10^3) was inoculated into the halophilic agar medium and incubated for 48 hours at 37°C. The isolated bacterial strains were sub cultured and preserved. The bacterial strain was identified by morphological and biochemical test and 16S rRNA sequence. Further, the isolated colonies with bacterial density 15×10^3 CFU ml^{-1} were screened for xylanase activity by using brich wood xylana agar medium (Chen *et al*., 2003).

2.2 Xylanase enzyme assay

The xylanase activity of the bacterial strain *B. cereus* IND- 3 was measured by using 1% brich wood xylan as substrate (Bailey *et al*., 1992). Xylanase activity was assayed in 3.0ml of a reaction mixture containing 1.0 ml of crude extracellular enzyme source, 1 ml of 1% birch wood xylan (prepared in 0.05 MNa-citrate buffer, pH 5.5) and 1 mlof 0.05 M citrate buffer. The mixture was incubated at 55°C for 10minutes. The reaction was stopped by the addition of 3.0 ml of 3,5-dinitrosalicylic acid (DNS) and the contents were boiled for 10 min (Miller, 1959). After cooling, the colour developed was read at 540 nm. The amount of reducing sugars liberated was quantified using xylose as standard. One unit of enzyme activity is defined as the amount of enzyme which releases 1 μmol of xylose in 1 minute under assay conditions (Khan *et al*., 1986).

2.3 Optimization of bacterial strain with physico-chemical parameters

The bacterial strain *B.cereus* IND-3 (Accession no: MZ089446 IND-3) was optimized with various physico-chemical parameters for maximum xylanase production. The effect of pH on xylanase production was studied in pH ranged from 5.5 to 9.5(1 pH interval) under different temperature ranged from 25°C to 45°C (5°C interval) using brich wood xylan as substrate. By using the same substrate, sodium chloride (NaCl) concentration between 0.5mM to 2.5mM (0.5mM interval) with optimum pH and temperature, maximum xylanase production was recorded. Similarly, at 24 hours interval, incubation time ranged from 24 to 120 hours, maximum xylanase production was observed with optimum temperature and NaCl concentration using brich wood xylan as substrate.

2.4 Biomass determination

The bacterial biomass was determined with the method followed by Bakri *et al*., 2009. The dry weight of bacterial biomass was determined by filtering 1 ml of cell medium through pre-weighed whatman filter paper number 44. Then, it was dried at 80°C for an overnight in a hot air oven and reweighed. The difference in weight showed the biomass of bacteria and it was denoted as dry weight per milli litre.

2.2 Paper pulp effluent collection

The samples were collected from paper manufacturing industry located at Cheranmahadevi, Tirunelveli district. Samples were collected in sterilized glass bottles aseptically and transported to the laboratory in an ice pack condition. The

collected samples were preserved at 4°C in refrigerator for further analysis.

2.6 Physico-chemical characters of raw and treated paper pulp effluent

The physico-chemical parameters such as pH, Temperature, BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand), TDS (Total Dissolved Solids), TH (Total Hardness) and chlorides were determined in the raw paper pulp effluent. Simultaneously, 5 ml of crude xylanase enzyme obtained from bacterial strain *B. cerus* IND -3 was mixed with 100 ml of raw paper pulp effluent in a conical flask. Then, the setup was kept for 5 days in an incubator at 35°C. After the incubation period, the physico-chemical parameters of xylanase treated paper pulp effluent were determined. The samples were analyzed as per the standard methods given in APHA, 2005.

2.7 Percentage of biodegradation

The percentage of biodegradation of paper-pulp effluent was determined by using the following formula.

$$\text{Degradation}(\%) = \frac{\text{Initial value} - \text{Final value}}{\text{Initial value}} \times 100$$

Initial value : Physico-chemical parameters of raw paper-pulp effluent (Control). Final value: Physico-chemical parameters of bacterial strain (Experimental).

2.8 Gas Chromatography - Mass Spectrometry analysis (GC - MS)

The Gas Chromatography - Mass Spectrometry analysis (GC - MS) of metabolites was carried out by using PerkinElmer (UK) equipped with a PE auto systeXL gas chromatograph and a PE-5 MS capillary column (20 m × 0.18 mm internal diameter, 0.18 mm film thickness). Helium was used as a carrier gas (flow rate: 1 mL min-1) using split less injector (injector temperature was 280 °C). The column temperature was programmed as 50 °C (5 minutes); 50-300°C (10 °C min-1, hold time: 5 minutes). The MS transfer line and ion source temperatures were keptat 200 and 250 °C, respectively. A solvent delay of 3.0 min was selected. In full-scan mode, the electron ionization (EI) mass spectra were recorded in range of 30-550 (m / z units) at70 e V. The metabolites were identified by using National Institute of Standardsand Technology (NIST) library available with instrument and by comparing the retention time (RT) and fragmentation pattern.

3.0 Result and discussion

The bacterial strain *B.cereus* IND-3 was optimized with different incubation time for xylanase and biomass production. The activity of xylanase and biomass production were gradually reduced from

72 hours of incubation. Minimum xylanase and biomass production (52.1 U/ml and 16.3 mg/ml respectively) was observed at 120 hours of incubation and maximum (75.6 U/ml and 28.4 mg/ml respectively) were recorded in 48 hours of incubation (Figure-1).The growth of bacterial strain was gradually increased from 24 hours of incubation and at 120 hours very less growth was recorded. The bacterial strain *B.vallismortis* RSPP-15 showed maximum growth at 48 hours of incubation was in agreement wirh present findings (Nagar *et.al.,* 2011).

The bacterial strain *B.cereus* IND-3 was optimized with different pH, temperature and NaCl concentration for xylanase and biomass production. At pH 8.5, 45ºC temperature and 2.5 mM NaCl concentration, minimum xylanase activity (11.4 U / ml) and biomass production (2.5 U / ml) were recorded and maximum xylanase activity (30.4 U / ml), and biomass production (9.6 mg / ml) were observed at pH 7.5, 35ºC temperature and 1.5 mM NaCl concentration (Table-1).In these respects, the bacterial strain resembled with the extremely halophilic bacteria *Holorhabdusutahensis* (Waino *et.al.,*2003) and in *Chromohalobacter* sp TPSV101 (Prakash and Veeranagouda 2009).

The bacterial strain *B.cereus* IND-3 was optimized by nutritional sources such as carbon, nitrogen and metal ions for xylanase and biomass production.The bacterial strain *B.cereus* IND-3 prefered rice bran, sugarcane bagasse and jackfruit seed for enzyme and biomass production. The maximum xylanase and biomass production was observed in rice bran (69.4 U / ml and 21.4 mg / ml) followed by sugarcane bagasse (64.2 U / ml and 20.8 mg / ml) and jackfruit seed (58.6 U / ml and 17.7 mg / ml). Among the substrate used for xylanase production, rice bran reported maximum production of xylanase. Among the nitrogen sources utilized for the xylanase and biomass production gelatin showed maximum production of xylanase and biomass (74.3 U / ml and 29.2 mg / ml) followed by casein (68.7 U / ml and 29.2 mg / ml) and beef extract (57.4 U / ml and 16.8 mg/ml). The metal ions such as calcium chloride, magnesium sulphate and ferrous sulphate were involved in enzyme and biomass production in *B.cereus* IND-3. Among the ions tested for xylanase and biomass production $CaCl_2$ enchanced maximum enzyme and biomass production (77.4 U / ml and 31.5 mg / ml) followed by $MgSO_4$ (63.9 U / ml and 21.6 mg/ml) and $FeSO_4$ (34.6 U / ml and 8.6 mg / ml). Rice bran act as an important carbon source which

induced the microbial strain *Cladas porium* KF5 sp for production of maximum xylanase was inagreement to the present finding (Alexo poulus, 1952). The nitrogen source, Gelatin was involved the maximum xylanase production in *Achromobacter xylosoxidans* inagreement was to the present findings (Mahalakshmi and Jeyalakshmi, 2016). The metals like calcium chloride enhanced the xylanase production in *Bacillus* sp. AP_4 was in accordance with the present study (Khasin *et.al*.,1993) (Table-2).

The physico-chemical parameters of the pre-treated and bacterial strain *B.cereus* IND-3 treated paper pulp effluent was recorded on the 5^{th} day of incubation. The results showed that the pH of the treated effluent was reduced from 7.9 to 8.24 with 4.172 % of biodegradation, temperature was decreased from 30.1 ºC to 29.4 º C with 2.32 % of biodegradation. BOD was decreased from 362.6 mg/ml to 218.3 mg/ml with 39.79 % of biodegradation. COD was decreased from 652.2 mg/ml to 480.0 mg/ml with 26.40 % of biodegradation. TDS was decreased from 887.0 mg/ml to 286.5 mg/ml with 67.70 % of biodegradation and TH was reduced from 755.7mg/ml with 480.2 mg/ml with 36.45 % of biodegradation.Similar observations of microbial degradation of paper pulp effluent was reported in *Bacillus* sp (Hernandez *et.al*.,2001; Dlez *et.al*.,2002). The results of present study showed that the reduction BOD and COD were observed when the effluent was treated with *B.cereus* IND-3. The reduction of these parameters due to the degradation of chlorinated organic compounds and lignin present in the effluent. (Singh and Thakur,2006 ; (Latorre and Malmqvist,2007). The bacterial strain *Pseudomonas aeruginosa* DSMZ 0.3504 has been successfully reduced TDS of paper pulp effluent was resembled to the present findings (Tiku *et.al.,*2010). In the present study TH was decreased due to the influence of bacterial strain *B.cereus* IND-3. The total hardness of the effluent may due to the presence of heavy metals. Similar result also reported by earlier workers on the biodegradation (Shail singh and Chandra patel, 2008) (Table-3).

The GC-MS analysis of the raw of paper pulp effluent and the xylanase enzyme treated effluent showed presence of various compounds.The compounds such as 4-Hexenoic acid-amino-6-hydroxy, 4-methyl (RT 38.780), Trans2,3-Epoxynonane (RT 33.5878), N(Epsilon)-methyllysin (RT 30.600), 1H-Azepint,1-amine hexahydro (RT 29.954), 3-Amino-1,2-propanediol (RT 28.129), 4-Ethylamino-1-butanol (RT 27.063), 8-

Azabicyclo (3.1.0) octane (RT 23.866), 2-cyclohexane-1,4-dione,5-bromo 2,6-dimethyl-1-oxime,o(4-nitrobenzoyl) (RT 21.721) and N-(3-(N-Aziridyl (propylidene) hexylamine) (RT 9.627) ,were detected in the raw paper pulp effluent the xylanase enzyme treated effluent showed the presence of compounds such as 1,3-Bis-T-butylperoxyphthalan (RT 28.619), 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4yl) methylester, cis (RT 28.214), 12,15-octade cadiynoic acid, methyl ester (RT 28.168), cholesta-8,24-dien-3-ol,4-methyl-(3.beta,4.alpha) (RT 27.848), propanoic acid, 2-(Aminooxy) (RT 27.603), 2R,3S-9-[(1,3-dihydroxy-4-fluro-3-butoxy)(methyl)guanine (RT 27.353), Hexade cane1-chloro (RT 27.273), Beta carotene (RT 27.188), Pterine-6-carboxylic acid (RT 26.988), 2-Carbamyl-9(beta-d-ribo-furanosyl) hypoxanthine (RT 26.508) and propanoic acid 2-(aminooxy) (RT 26.413). The earlier results of Madan sankar *et al*., 2019 revealed that the novel bacterium *Bacillus* sp. IITRDVM5, involved in the pulp and paper effluent was comparable to the present finding.The detection of compound such as 4-Nitrophenyln-(5-bromo-2-hydroxybenzyl)-n-methylcarbamate,8-Azabicyclo (3.1.0) ocane, 3-Amino-1,2-propanediol and 4-Hexenoic acid 2-amino 6-hydroxy 4-methyl were formed in the pretreated paper pulp effluent (Yadav and Chandra, 2018).The detection of transformed chemical compounds in the xylanase treated sample indicated that the xylanase enzyme obtained from *B.cereus* IND-3 mediated the degradation of paper pulp effluent (Chandra *et al*., 2009). After treatment several new peaks of low molecular weight compounds were detected in the effluent also reported by earlier workers (Chandra and singh,2012). During paper pulp degradation four intermediate compounds such as 9-Octadecenoic acid, (2-phenyl-1, 3-dioxolan-4-yl)methyl ester, CIS, 2R, 3S-9-((1,3-didydroxy-4-fluoro-3-butoxy(methyl)) guanine, propanoic acid, 2-(Amino oxy) and 12,15- octade-cadiynoic acid, methyl ester were generated aiso comparable to the previous studies (Kumar *et al*., 2015). The results indicated that the xylanase enzyme obtained from *B.cereus* IND-3 degraded the high molecular compounds into low molecular compounds in the paper pulp effluent (Table 4).

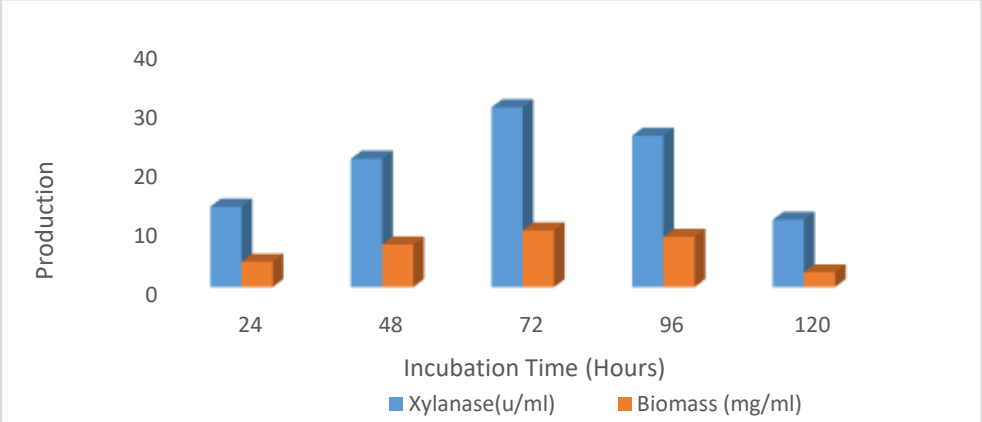


Figure.1 Effect of incubation time on xylanase production in *B.cereus* IND-3

Table-1 : Effect of pH, temperature and NaCl concentration on xylanase enzyme production in

B.cereus IND-3

S.NO	pH	Temperature (^{0}C)	NaCl (mM)	Xylanase production (U/ml)	Biomass (mg/ml)
1	6.5	25	0.5	13.6	4.3
2	7.0	30	1.0	21.7	7.2
3	7.5	35	1.5	30.4	9.6
4	8.0	40	2.0	25.6	8.5
5	8.5	45	2.5	11.4	2.5

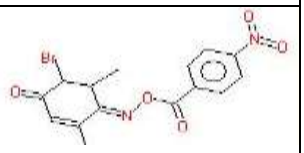
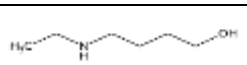
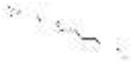
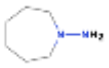
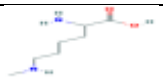
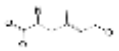
Table -2 : Effect of nutritional sources on xylanase production in *B.cereus* IND-3

Carbon sources (1%)	**Xylanase production (U/ml)**	**Biomass (mg/ml)**
Rice bran	69.4	21.4
Sugarcane bagasse	64.2	20.8
Jack fruit seed	58.6	17.7
Nitrogen sources (1%)	**Xylanase production (U/ml)**	**Biomass (mg/ml)**
Gelatin	74.3	29.2
Caesin	68.7	24.8
Beef extract	57.4	16.8
Metal ions (1mM)	**Xylanase production (U/ml)**	**Biomass (mg/ml)**
$CaCl_2$	77.4	31.5
$MgSO_4$	63.9	21.6
$FeSO_4$	34.6	8.6

Table – 3 : Physico-chemical parameters of raw paper pulp effluent and xylanase enzyme treated effluent

S.NO.	Parameters	Pretreated effluent	Treated effluent	Biodegradation (%)
1	pH	7.9	8.24	4.172
2	Temperature (^{0}C)	30.1	29.4	2.32
3	BOD (mg/l)	362.6	218.3	39.79
4	COD (mg/l)	652.2	480.0	26.40
5	TDS (mg/l)	887.0	286.5	67.70
6	TH (mg/ml)	755.7	480.2	36.45

Table-4: GC-MS analysis of raw paper pulp effluent

Sl. No.	**Name**	**Retention time (minutes)**	**Area (%)**	**Molecular weight (g/mol)**	**Molecular formula**	**Structure**
1	2-Cyclohexane1,4-dione,5-bromo 2,6-dimethyl,1-oxime, o(4-nitrobenzoyl)	21.721	7.110	380	$C_{15}H_{13}BrN_2O_5$	
2	8-Azabicyclo(3.1.0)octane	23.866	7.238	111	$C_7H_{13}N$	
3	4- Ethylamino-1-butanol	27.063	21.787	117	$C_6H_{15}NO$	
4	3-Amino-1,2-propanediol	28.129	5.577	91	$C_3H_9NO_2$	
5	N-(3-(N-Aziridyl(propylidene)hexylamine)	9.627	22.390	182	$C_{11}H_{22}N_2$	
6	1H-Azepin 1-amine,hexahydro-	29.954	3.571	114	$C_6H_{14}N_2$	
7	N(Epsilon)-methyllysin	30.600	17.956	160	$C_7H_{16}N_2O_2$	
8	Trans 2,3-Epoxynonane	33.578	13.147	142	$C_9H_{18}O$	
9	4-Hexenoic acid2-amino6-hydroxy 4-methyl	38.780	43.917	159	$C_7H_{13}NO_3$	

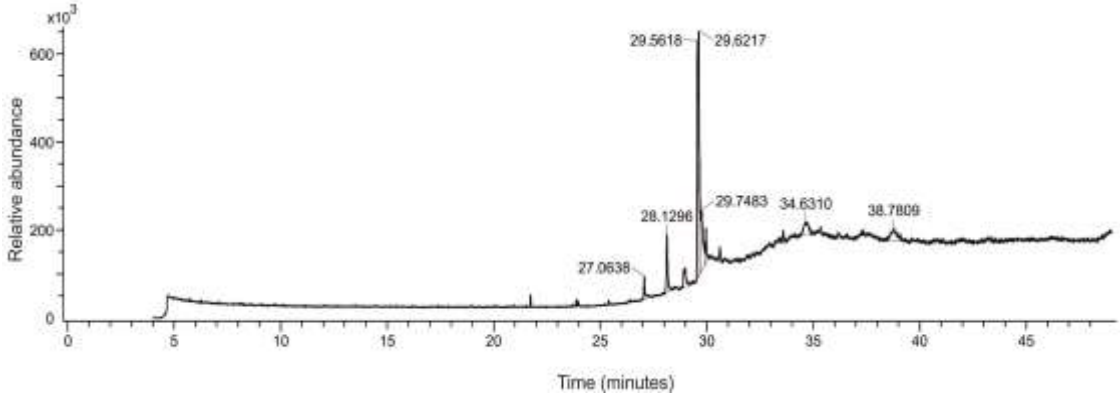
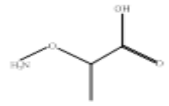
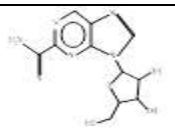
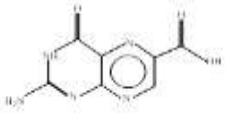
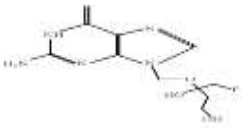
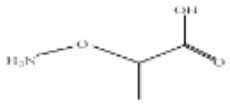
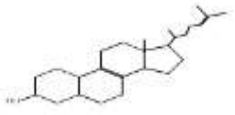
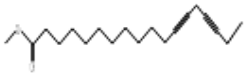
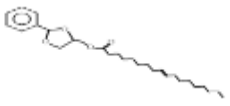
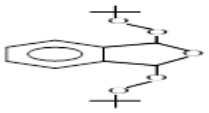


Figure :2 GC-MS spectrum detected in the raw paper pulp effluent

Table- 5 GC-MS analysis of the extracelluar xylanase enzyme treated paper pulp effluent

SL No.	Name of the compound	Retention time (minutes)	Area (%)	Molecular weight (g/mol)	Molecular formula	Molecular structure
1.	Propanoic acid, 2-(aminooxy)	26.413	4.879	105	$C_3H_7O_3N$	
2.	2-Carbamyl-9-(beta-d-ribo-furanosyl)hypoxanthine	26.508	5.957	295	$C_{11}H_{13}O_5N_5$	
3.	Pterin-6-carboxylic acid	26.988	9.636	207	$C_7H_5O_3N_5$	
4.	Beta carotene	27.188	14.278	536	$C_{40}H_{56}$	
5.	Hexadecane,1-chloro	27.273	15.073	260	$C_{16}H_{33}Cl$	
6.	2R,3S-9-[[1,3-dihydroxy-4-fluoro-3-butoxy]methyl]guanine	27.353	5.737	287	$C_{10}H_{14}O_4NSF$	
7.	Propanoicacid,2-(Aminooxy)	27.603	4.851	105	$C_3H_7O_3N$	
8.	Cholesta-8, 24-dien-3-ol,4-methyl-, (3.beta.,4.alpha.)	27.848	5.524	398	$C_{28}H_{46}O$	
9.	12,15-octade-cadiynoicacid, methyl ester	28.168	13.929	290	$C_{19}H_{30}O_2$	
10.	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, CIS	28.214	5.800	444	$C_{28}H_{44}O_4$	

SL No.	Name of the compound	Retention time (minutes)	Area (%)	Molecular weight (g/mol)	Molecular formula	Molecular structure
11.	1,3-βis-T-butylperoxy-phthalan	28.619	4.669	296	$C_{16}H_{24}O_5$	

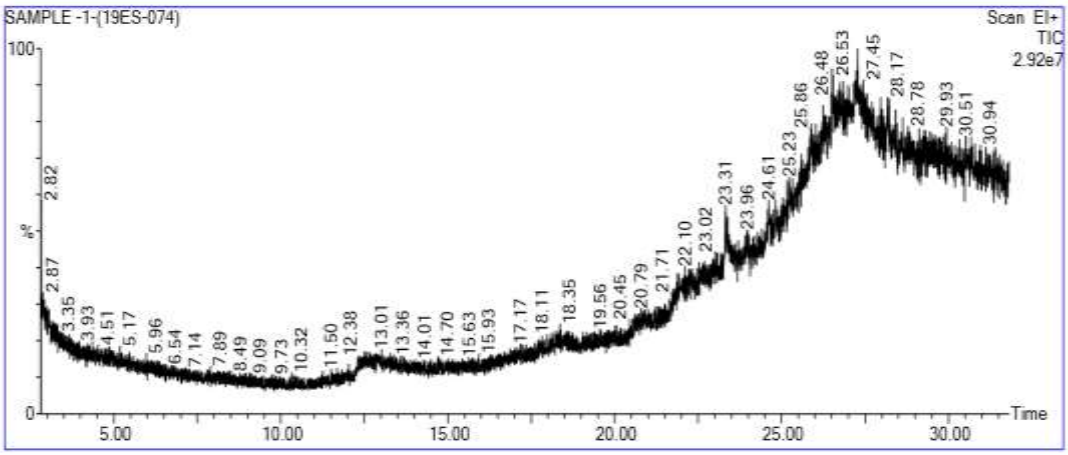


Figure:3 GC-MS spectrum detected in the alkaline xylanase enzyme treated paper pulp effluent

CONCLUSION

The findings of the present study revealed that the bacterial strain *B. cereus* -IND-3 produced maximum extracellular xylanase enzyme at an alkaline pH 7.5. This enzyme significantly reduced the pH , BOD, COD , and TH of the paper pulp effluent within five days . The GC-MS analysis of raw paper pulp effluent and xylanase enzyme treated effluent showed the degradation of chemical compounds of high toxicity to the less toxicity. Thus, the extracellular xylanase enzyme was a suitable candidate for the degradation of highly toxic pollutants present in the paper pulp effluent.

Reference

Abhay RAJ,Ram Chandra, Mohan M. krishna Reddy. Hemant J.Purohit.Atya kapley,2007, 'Biodegradation of kraft lignin by a newly isolated bacterial strain,

Aneurinibacillus aneurinilyticus from the sludge of apulp paper mill ‘, world j microbial biotechnol , vol. 23 pp 793-799.

Alexopoulus, CJ 1952, Introductory my cology, Mac. Willey Inc. London, Sydney.

APHA, 2005, Standard methods for the examination of water and waste water,21st edition, American public Health Association/American water works Association/water Environment, Federation washing ton DC.

Baily, M .J ,Beily, 1992, poutanen, Inter laboratory testing and methods for assay of xylanase activity.*Biotechnol.j.,23,257 , 70*

.Bakri, Y, Magali, M, Thonart, P 2009, Isolation and identification of a new fimgal strain for amylase biosynthesis. Polish J. Microbiol., 58, 269-273.

Bergey. D.H and Holt, JG 1994, Bergey’s manual of determinative bacteriology, 9th edition, Williams & Wilkins. biotrans formation of pentachlorophenol and pulp paper mill effluent decolorisation by the bacterial strains in a mixed culture Bioresource Technology, vol. 99, pp. 5703-5709.

Chandra, R & Sing, R 2012, ‘Decolourisation and detoxification of rayon grade pulp paper mill effluent by mixed bacterial culture isolated from pulp paper mill effluent polluted site’, Biochem. Eng., vol. 161, pp. 49-58.

Chandra, R, Raj, A, Yadav, S& Patel, DK 2009, ‘Reduction of pollutants in pulp paper mill effluent treatedby PCP-degrading bacterial strains’, Environ. Monit.Assesment, vol. 155, pp. 1-11.

Charkar FS,Ragaukas AJ,2004,’ Review of current and future soft wood kraft lignin present chemistry indust crops prod , ‘ vol.20 pp131-141

Chen, YH, Miller, JR, Francis, JA, Russell, GL & Aires, F 2003, ‘Observed and modeled relationships among Arctic climate variables’, *J. Geophys. Res.*,vol. 108, no. D24, p. 4799.

Diez, MC, Castillo, G, Aguilar, L, Vidal, G & Mora, ML 2002, ‘Operational factor and nutrient effect on activated sludge treatment of pinus

radiato kraft mill waste water', Bioresour. Technol., vol. 83, pp. 131-138.

Hernandez, M, Hernandez-coronado, MJ, Montiel, MD, Rodriguez, J & Arias, ME 2001, 'Analysis of alkali lignin in a paper mill effluent decolorized with two Streptomyces strains by gas chromate graphy-mass spectrometry after cupric oxide degradation', J. chromatogrA vol. 919, pp. 389-394.

Khan, AW., Tremblay, D and LeDuy, A 1986, Assay of xylanase and xylosidase activities in bacterial and fungal cultures, Enzyme microb. Technol, 8, 373-377.

Khasin, A., Alchanati, I &Shoham, Y 1993, 'Purification and characterization of a thermostablexylanase from Bacillus stearothermophilus T-6', Appl. Environ. Microbiol., vol. 59, pp. 1725-1729.

Kumar, M, Singh, J, Singh, MK, Singhal, A & Thakur, IS 2015, 'Investigating the degradation process of kraft lignin by β-protes bacterium, pandoraea sp., STKB', Environ sci. Pollut. Res., vol. 22, pp. 15690-15702.

Latorre, A, Malmqvist, A, Lacorte, S, Welander, T *& Barcelo, D 2007, 'Evaluation* of the treatment efficiencies of paper mill whitewaters in terms of organic compostion and toxicity', Environmental Pollution, vol. 147, pp. 648-655.

Madan sonkar, Madan kumar, Dharm Dutt, Vivek, 2019, ' Treatment of pulp and paper mill effluent by anovel bacterium *Bacillus* sp IITRDVM-5 through a sequential batch process' , biocatalysis and Agricultural Biotechnology, vol.20,pp101232.

Mahalakshmi, N & Jayalakshmi, S 2016, 'Amylase, cellulase and xylanase production from a novel bacteria isolate achromacter xylosoxidans isolated from marine environment

Manual Hermandez, Maria Jesus Hermandez-Coronado, Maria Dolores Montiel, Jana Rodriguez, Maria Enriqueta Arias, 2001, 'Analysis of alkali – lignin in a paper mill effluent decolourised with two *streptomyces* strain by gas chromatography-mass spectrometry after cupric oxide degradation ' , Journal of chromatography A, vol.919, pp 389-394.

Masai, E, Shinohara,S, Hara,H, Nishikawa,s, Katayana,Y, Fukuda.M, 1999, 'Genetic and biochemical characterization of a 2-pyrone-4, 6-dicarboxylic acid hydrolase involved in the protocatechuate 4,5-cleavage pathway of Sphingomon aspaucimobilis SYK-6, ' J Bacterial vol.181 pp 55-62.

Miller G L. Anal.chem., 1959;31 (3) : 426-428

Morii, HK, Nakamiya & Kinoshita, 1995, 'Isolation of lignin decolouring bacterium', J. Ferment. Bioeng., vol. 80, pp. 296-299.pp

Nagar, S, Mittal, A, Kumar, D, Kumar, L, Kuhad, RC. &. Gupta, VK 2011, 'Hyper production of alkali stable xylanase in lesser duration by Bacillus pumilus SV-85S using wheat bran under solid state fermentation', New Biotechnology, vol. 28, no. 6, pp. 581–587.

Paice ,M.G, Archibald, F.S, Bourbonasis, R,Jurasek,l, Reid, I.D, Charles ,T, Dumonceaux, in,T,

Jeffries,T.W.viikari (Eds).L,1996,' Enzymes for pulp and paper processing , 'ACS Washington DC,j, ASC symp.vol.655pp 5.

Perestelo F, Rodriguez A, perez R, Carnicero A, de la Fuente G, Falcon MA 1996 , ' Isolation of a bacterium capable of limited degradation of industrial and labelled , natural and synthetic lignin ., ' World j Microbial Biotechnol, vol.12 pp111-112.

Prakash, S, Veeranagouda, Y, Kyoung, L & Sreeramulu, K 2009, 'Xylanase production using inexpensive agricultural wastes and its partial characterization from a halophilic chromohalo bauter sp. TPSV vol. 101.

Shail Singh, R, Chandra, Patel, DK, et.al.,2008.Bioresource Technology vol.99 (13):pp5703-57090.

Shimp, R.J , Owns, JW, 1993 , 'Toxicol.Environ. chem.vol.40, pp 213.

Singh, P & Thakur IS 2006, 'Colour removal of anaerobically treated pulp and paper mill effluent by microorganisms in two steps bioveactor', Bio resource Technology, vol. 97, pp. 218-223.

Tiku, DK, Kumar, A, Chaturvedi, R, Dayal, S, Manoharan, A, Kumar, K 2010, 'Holistic bioremediation of pulp mill effluents using

autochthonous bacteria', Int. Biodeterior. Biodegrad., vol. 64, pp. 173-183.

Wainer, M. Ingvorsen, K 2003, 'Production of β-xylanase and β-xylosidase by the extremely halophilic archaeon Halornabdus utahensis', Extremophiles, vol. 7, p. 87-93.

Yadav, S& Chandra, R, 2018, 'Detection and assessment of the phytotoxicity of residual organicpollutants in sediment contaminated with pulp and paper mill effluent', Environ. Monit.Assess. 190,581.