Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 24, No. (2) : 2022 : 314-321

ISSN-0972-3005

DOI No.: http://doi.org/10.53550/AJMBES.2022.v24i02.017

BACTERIAL TREATMENT OF MELANOIDIN CONTAINING POST METHANATED DISTILLERY SPENT WASH

PRITI MAHLA AND NIKHIL BHATT*

Department of Biogas Research and Microbiology, Gujarat Vidyapith, Sadra 382 320, Gujarat, India

(Received 1 December, 2021; Accepted 7 March, 2022)

Key words: *Melanoidin, Distillery spent wash, Phytotoxicity*

Abstract– Untreated or primary treated distillery spent wash is lethal for the environment due to its highly acidic and other physicochemical impurities. An attempt to screen out potent bacterial consortium for melanoid in containing post methanated distillery spent wash (PMDSW) was carried out. Developed bacterial consortium was subjected to treat PMDSW for decolorization and degradation purpose. BBD design was projected and achieved significant variable was founded as 0.2% urea concentration, 20% PMDSW concentration and 40 h incubation in static with 99.99% decolorization. Enzymes involved in the degradation process (manganese peroxidase, lignin peroxidase and laccase) was assayed as well as analyzed with HPTLC and FTIR techniques for confirmation of degradation as reduction in peak height and area. Toxicity removal was also being proceed as phytotoxicity with *Vigna radiata* and achieved 95% seed germination and measured other plant growth parameters. The consortium was isolated (3.2022) on solid medium and identified with 16S rRNA sequencing.

INTRODUCTION

The most important environmental problems faced by the world are management of wastewater (Chaudhari *et al.*, 2012). The pollutants from distilleries creates a threat to all organisms (Santal *et al.*, 2016). Sugar industry produces sugar and molasses (by product) from sugarcane. Molasses is further processed in the distillery to produced alcohol and large amounts of high strength wastewater is produced called distillery spent wash (DSW). It is highly coloured and acidic in nature with high biochemical oxygen demand (BOD), and chemical oxygen demand (COD) (Mahla and Bhatt, 2020). India has around 298 distilleries producing 3.2 billion l of alcohol and 45 billion l of spent wash annually. Distillery industry among 17 top polluting industries in India was categorized by the central pollution control board (CPCB) and 2.3 billion l production of alcohol, approximately 40 billion l of effluent was generated in India (Patel and Jammaludin, 2018). Various physical, chemical and biological methods were reported for treatment of DSW since last three decades but biological techniques are feasible and eco-friendly (Khandekar and Shinkar, 2020). For the reduction of high COD and other impurities there was monostage and multistage sequential rectors and techniques were in practice since long (Acharya *et al.*, 2011). The reported bacterial strains for decolorization *Bacillus sp., Lactobacillus sp., Alkaligenes sp., Pseudomonas putida, Pseudomonas fluorescens*, etc can decolorize and degrade major component of spent wash via enzymatic degradation (Boopathy, 2014). Various statistical treatment strategies were implied for biological methods (Singh *et al.*, 2016). The present study was focused on bacterial treatment incorporated with statistical approach to achieve decolorization and degradation of spent wash.

MATERIALS AND METHODS

Sample collection

Distillery spent wash (DSW) was collected from Madhi Sugar and Distillery Industry, Surat, Gujarat, India. DSW was treated under biphasic anaerobic fixed film bioreactor containing first acidogenic phase and last methanogenic phase. Effluent of the bioreactor is termed as post methanated distillery spent wash (PMDSW). The PMDSW was collected from laboratory of Department of Biogas Research

and Microbiology, Gujarat Vidyapith, Sadra, Gujarat. Soil, sludge, decaying organic waste and liquor contaminated soil was collected from deferent region of South and North Gujarat. Sterile containers were preferred to collect samples and preserved at 4 °C. Examination of DSW and PMDSW by the physicochemical parameters mentioned in Table 2 and followed methods of APHA, (2012).

Development of bacterial consortium, isolation and identification

For the screening of spent wash degrading and decolorizing bacteria Bushnell Hass medium with 7 pH amended 15% PMDSW was utilized, incubated at 35°C temperature in static condition. Decolorization of PMDSW was measured at every 8 h interval up to 48 hrs. Centrifuge at 10,000 rpm for 15 min and take OD in UV-Visible spectrophotometer at 475nm (Bhargava *et al.*, 2009). Potent consortium was selected for furthers study. Selected potent consortium was isolated on same medium composition mentioned above added with 3% agar. Isolated bacteria were submitted for 16S r RNA sequencing for identification at GBRC, Gandhinagar, Gujarat, India.

Optimization parameters of decolorization with BBD design

For BBD design five important parameters were selected as independent variable as per Table 1. and the dependent response variable was decolorization. Three different level (1, 0, -1) of each variable was carried out with total 46 experiments. All experiments were executed at 30 ± 2 °C temperature under static conditions. Effect of different ranges of each variable was corresponding to decolorization (%), selected specific range is mentioned in Table 1.

Design Expert Version 11.0 (PA, USA) statistical software was used as a statistical model and interpreted as a cubic model. ANOVA was utilized to set up the essentialness of the model parameters.

Enzyme assay

Manganese peroxidase and laccase activity was assayed from the obtained best significant experiment. Thrice centrifugation was done at 10,000 rpm for 15 min to obtained supernatant as a crude enzyme. Substrate for MnP and laccase activity was phenol red and ABTS, respectively (Bharagava *et al.*, 2009). Experiment mixture was contained for MnP activity; five milliliter of reaction mixture contained 1 ml sodium succinate buffer (50 mM, pH 4.5), 1 ml sodium lactate (50 mM, pH 5), 0.4 ml manganese sulphate (0.1 mM), 0.7 ml phenol red (0.1 mM), 0.4 mL H_2O_2 (50 mM), gelatine 1 mg/ml and 0.5 ml of enzyme extract. The reaction was initiated at 30°C by the addition of H_2O_2 and the rate of $MnSO_4$ malonate complex formation was monitored at every minute by measuring the increase in absorbance at 610 nm. One unit of enzyme activity is equivalent to an absorbance increase of 0.1 U/ml. While, laccase reaction mixture containing 2.8 ml sodium acetate buffer (10 mM, pH 5), 1 ml ABTS (2 mM) and 0.2 ml of enzyme extract was incubated at 25°C for 2 h. The absorbance was read at 420 nm. Laccase activity has been expressed as unit U/ml (Yadav and Chandra, 2012).

Phytotoxicity test

Healthy seeds of *Vigna radiata* sterilized by 0.1% $HgCl_2$ for 2 minutes were cultivated in fertile soil for 10 days. The phytotoxicity of treated (supernatant of incubated PMDSW with consortium), untreated (20 % PMDSW) and control (tap water) was irrigated during the study. Study includes % Germination, Shoot length (cm/seed), Root length (cm/seed), Wet shoot weight (mg/seed), Wet root weight (mg/seed) and Dry shoot weight (mg/seed), Dry root weight (mg/seed), number of leaves.

Analytical techniques for degradation confirmation

For the confirmation of the melanoidin degradation from the PMDSW by potent consortium, untreated

Table 1. Selected specific range for BBD

Selected variables	Code	Range and level		
		-1	0	+1
Sucrose concentration	A	0.2	0.5	1.0
Urea concentration	B	0.2	0.8	1.0
PMDSW concentration	C	10	15	20
pH	D	6.5	7.0	7.5
Incubation period	E	32	40	48

and treated samples were taken and centrifuged at 10,000 rpm, 15 min thrice. Syringe was washed by 0.5 ml of AR grade methanol for high-performance thin layer chromatography analysis. Untreated and treated samples were applied for HPTLC analysis using silica gel, application system (Linomat-5), 1butanol, acetic acid, water (2:4:1 v/v) as mobile phase, and TLC scanner at 254 nm for observation (Chavan *et al.*, 2013). The change occurred in the functional group of compounds present in distillery spent wash was studied through FTIR spectral analysis by associating treated samples with the untreated samples. Samples were prepared as treated and untreated; 5 mL samples were centrifuged at 10,000 rpm for 15 min three times. Supernatant was collected as sample and submitted at (MNIT, Jaipur, Rajasthan, India) and the spectra were recorded in the region from 400 to 4000 cm^{-1} with a spectral resolution of 4 cm^{-1} (Zhao *et al.*, 2018).

RESULTS

Physicochemical characterization of DSW and PMDSW

Physicochemical analysis was carried out as soon as possible for more accuracy and results obtained are mentioned in Table 2. It is clearly indicated that after treatment in anaerobic biphasic bio reactor there were still impurities.

Development of bacterial consortium, isolation and identification

Development of bacterial consortium study was selected PN1 to PN35 number of consortiums for decolorization study and obtained PN27 as a potent consortium which was isolated as six different culturable bacterial strains identified as *Paracoccus pentotrophus*, *Pseudomonas balearica*, *Paracoccus sp*., *Paracoccus pantotrophus*, *Paracoccus denitrificans* and *Ochrobactrum ciceri* was developed by the screening and enrichment culture method. PN27 consortium can decolorize 15% PMDSW with BHM within 72 h. Higher concentration of PMDSW takes time and can't get complete decolorization before death phase indicated in Figure 1. Thus, the study conducted statistical approach to achieve maximum decolorization at higher concentration.

Table 2. Physicochemical parameters of DSW and PMDSW

Parameters	Results	
	DSW	PMDSW
pH	4.3 ± 0.9	8.66 ± 0.22
mV	-	-090 ± 0.3
TS (mg/l)	112400 ± 24.6	31.61± 4.3
TVS (mg/l)	81400±11.5	6 ± 2.1
TDS (mg/l)	15931 ± 15.4	11 ± 2.9
TSS (mg/l)	87732 ± 18.9	8.8± 1.2
Phosphate (mg/l)	45.60 ± 1.06	1,443 ± 20.6
Sulphate (mg/l)	1736± 14.6	21 ± 2.1
Nitrogen (mg/l)	6224 ± 21.4	2,128 ± 40
BOD (mg O_2/l)	48520 ± 19.4	13,800 ± 200
COD (mg/l)	182000 ± 29.5	23,000 ± 300

Optimization parameters of decolorization with BBD design

Study conducted 46 run of BBD in five variables as shown in Table 3 resulted PMDSW decolorization varied under conditions experimented. PMDSW decolorization varied from 59.71 to 99.99 %. The lowest decolorization noted with run number 20 (0.2% w/v sucrose; 0.8% w/v urea; 15% v/v PMDSW concentration; 7 pH and 32 h incubation period). The maximum decolorization 99.99% was exhibited with run numbered 28 (0.5% w/v sucrose; 0.2% w/v urea; 20% v/v PMDSW concentration; 7 pH and 40 h).

For more clarification Tables formed by Design Expert Version 11.0 (PA, USA) statistical software for sequential model sum of squares clearly show that quadratic vs 2FI has 1759.95 sum of squares, 5 df, 351.99 mean square with F value 6.35 and P value 0.0006 was suggested, also values from ANOVA for quadratic model and coefficients in terms of coded factors are mentioned in Table 3.

The graphical explanation defines the relation between the response and levels of experiments of each variable and type of interactions between experimented variables. Fig. 2 (a) and (b) and Fig. 3 exhibited that the PMDSW decolorization was varied when variables were altered. The experiment was successfully significant because before this approach it was actioned full decolorization could gain at long incubation period with less PMDSW concentration (15%) but with this statistical approach it was completely altered as high concentration of PMDSW (20%) could be decolorized within 40 h.

For validation of experimental model, some confirmative runs were experimented using obtained optimum variables. The highest decolorization of PMDSW found with the

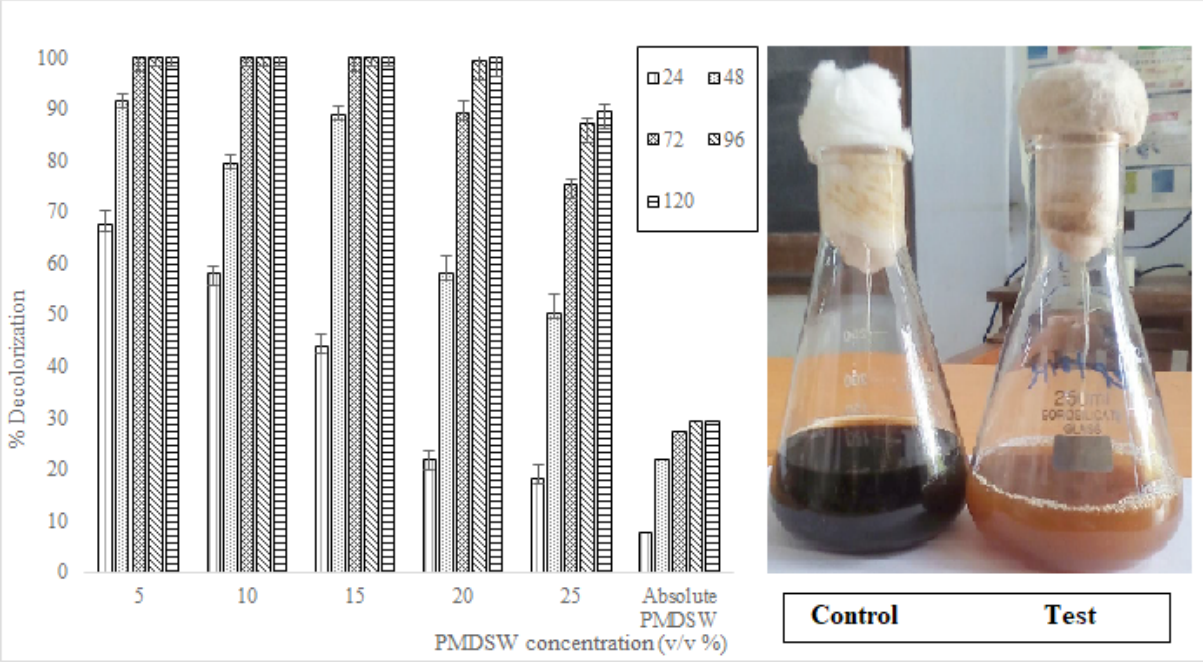

Fig. 1. PMDSW decolorization by PN27

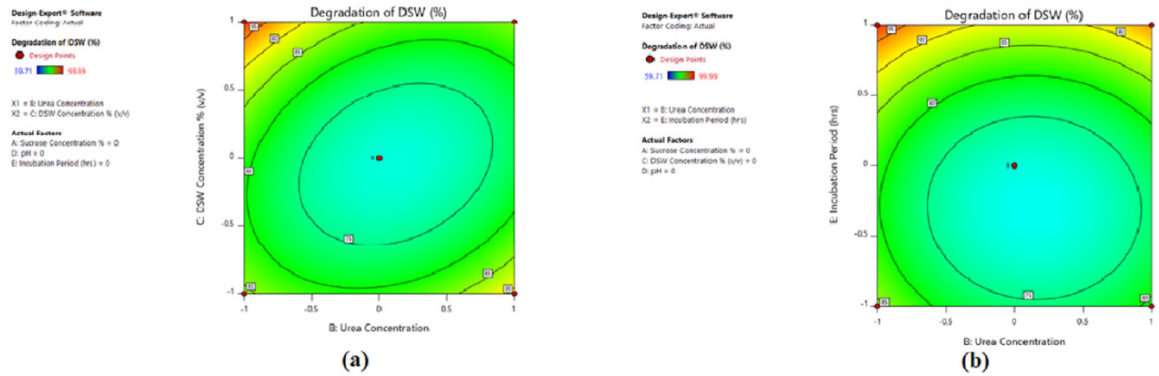

Fig. 2. Contour showing (a) interaction between urea concentration and DSW concentration (b) interaction between urea concentration and incubation period

combination of sucrose concentration (0.5%, w/v); urea (0.2%, w/v); PMDSW was concentration (20% v/v/), pH (7) and incubation period (40 h). The outcome of the BBD statistical design is potently validated the conditions value which were also achieved as optimum during one factor at a time approach with difference in concentrations only. Thus, this combination was used in further experiments.

Enzyme assay

The mechanism of decolorization of post methanated distillery spent was determined by manganese peroxidase (MnP) enzyme activity since, MnP consider as a key enzyme to degrade melanoidins. As mentioned in Fig. 4 the MnP activity shown by PN27 consortium was maximum 37.5 ± 0.3 U/ml/h at 30 h.

The laccase enzyme was also included as prime enzyme to break melanoidins, monophenols, polyphenols, aromatic amines and their derivatives. Laccase enzyme assay was performed and the maximum enzyme activity 18.98 ± 0.5 U/ml/h was achieved at 42h by PN27 consortium.

Phytotoxicity test

Phytotoxicity study was carried out on mung bean (*Vigna radiata*) plant and obtained 95% of seed

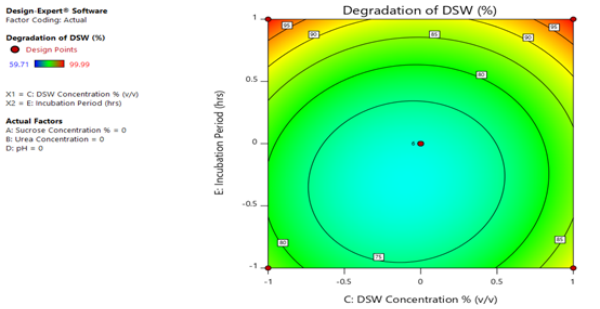

Fig. 3. Contour showing interaction between incubation period and DSW concentration

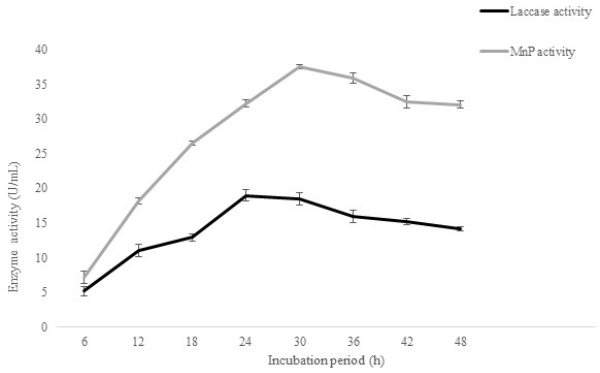

Fig. 4. Enzyme activity (U/ml)

germination and plant growth within 10 days irrigation with control (tap water), treated and untreated PMDSW. The results detailed in Table 4.

Analytical techniques for degradation confirmation

The degradation of PMDSW was confirmed by high performance thin layer chromatography and fourier transform infrared spectroscopy analysis. Resulted spots with different R*f*-values observed as compared to untreated PMDSW in UV-light. The PMDSW was observed in UV light at 254 nm. The results indicated that decolorization was due to PMDSW degradation. Mentioned figure 3 clearly shows that R*f* values and % area of treated sample was lower than untreated sample which clearly shows that the degradation of melanoidin linked PMDSW was successfully carried out.

Fig. 5 (a) and (b) show obtained peaks of untreated and treated sample, respectively. The peak was gradually decreased very well as well as metabolite compound was representing another peak in treated sample which proved that degradation was carried out by MnP enzyme activity. Chavan *et al.*,(2013) recorded HPTLC spectra and they also got decrement in peak number and peak hight. FTIR analysis of untreated sample

Table 3. ANOVA for quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2994.18	20	149.71	2.70	0.0100	***significant***
A-Sucrose Concentration %	73.32	1	73.32	1.32	0.2610	
B-Urea Concentration	69.10	1	69.10	1.25	0.2748	
C-DSW Concentration % (v/v)	40.70	1	40.70	0.7343	0.3996	
D-pH	69.22	1	69.22	1.25	0.2744	
E-Incubation Period (hrs)	674.70	1	674.70	12.17	0.0018	
AB	9.00	1	9.00	0.1623	0.6904	
AC	66.99	1	66.99	1.21	0.2821	
AD	17.18	1	17.18	0.3099	0.5827	
AE	83.27	1	83.27	1.50	0.2318	
BC	84.82	1	84.82	1.53	0.2276	
BD	2.94	1	2.94	0.0531	0.8197	
BE	0.4489	1	0.4489	0.0081	0.9290	
CD	14.25	1	14.25	0.2571	0.6166	
CE	8.70	1	8.70	0.1570	0.6953	
DE	19.58	1	19.58	0.3532	0.5576	
A^2	226.63	1	226.63	4.09	0.0540	
B^2	503.09	1	503.09	9.08	0.0059	
C^2	1059.20	1	1059.20	19.11	0.0002	
D^2	94.66	1	94.66	1.71	0.2032	
E^2	1026.77	1	1026.77	18.52	0.0002	
Residual	1385.90	25	55.44			
Lack of Fit	1385.90	20	69.29			
Pure Error	0.0000	5	0.0000			
Cor Total	4380.08	45				

was analyzed which obtained peaks 690.08, 834.82, 1038.99, 1107.99, 1319.20, 1627.07, 1922.49, 2339.92, 2625.63 and 3244.09 cm^{-1} which represents C-H deformation as in benzene and C-Cl stretching in ring, C-OH stretching, C-O stretching, C-N stretching, –N=N– stretching as in azo compounds, C=O stretching (corresponds to C=O stretching frequency of aldehydic group), Ca–C, O-H stretching respectively with % transmission attached in Fig. 6(a). The treated sample was analyzed which obtained peaks 533.95, 842.17,989.20, 1092.42, 1358.80, 1455.24, 1626.67, 1659.63, 3218.2, 3351.30 and 3444.4 cm^{-1} which represents S-S aryl disulphide stretching, C-O stretching, C-N stretching, –N=N– stretching as in azo compounds, C=O stretching (corresponds to C=O stretching frequency of aldehydic group), Ca–C, O-H stretching respectively shown in Fig.6 (b). Some peaks disappeared and formed in treated sample clearly indicate that metabolites were formed and also get broken. In FTIR analysis, untreated sample noted for three peaks of 1922.49, 2339.92 and 2625.63 which were completely disappeared in treated sample and formation of different new peaks of 3218.2, 3251.30, 3444.4 and 1358.80 indicated that biodegradation occurred in treated sample.

DISCUSSION

Developed consortium identified with six culturable isolates were also reported by many authors for treatment of distillery spent wash (Mahla and Bhatt, 2020. Statistical approach was used in the present study. Mohana *et al*., (2008) utilized RSM statistical approach for optimization of media for dye degradation by bacterial consortia DMC. They reported dye concentration, yeast extract and inoculum size as significant parameters. Dye concentration has linear effect on the response with maximum decolorization achieved at low dye concentrations. This decrease in decolorization with increase in initial dye concentration is attributed to the toxicity of the dyes to the growing microbial cells at higher dye concentrations (Ali *et al*., 2010). The induction of MnP for decolorization and degradation of melanoidin has been also reported

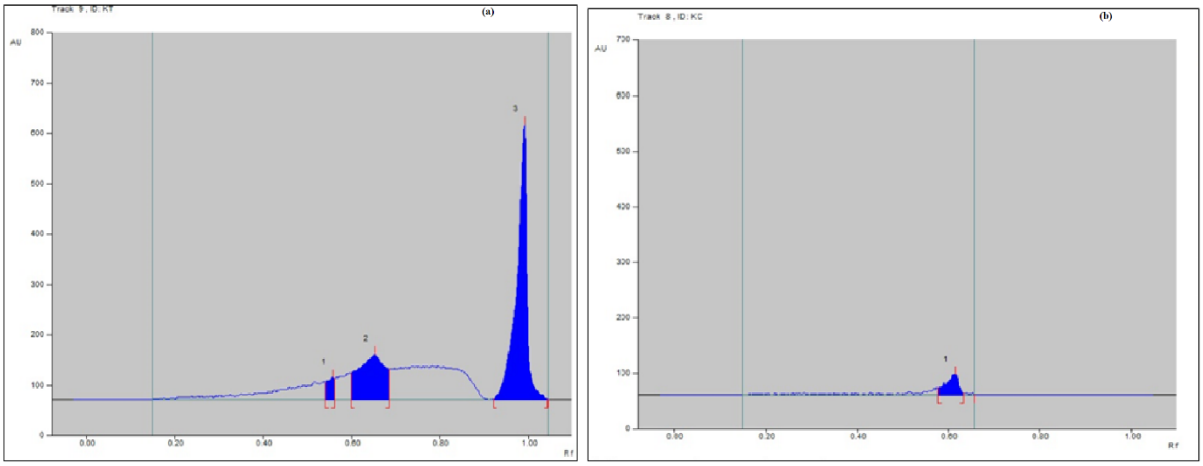

Fig. 5. HPTLC analysis of untreated and treated PMDSW, (a): Untreated and (b) Treated

Table 4. Phytotoxicity test

Parameters	Control	Treated	Untreated
Germination (%)	100	**95**	25
No. of leaves	2	2	2
Color of leaves	Dark green	Green	Faded green
Shoot length (cm)	20.02 ± 2.3	16.21 ± 1.5	10.10 ± 0.6
Pre-weight shoot (g)	1.92 ± 0.6	1.16 ± 0.4	0.28 ± 0.04
Dry-weight shoot (g)	0.24 ± 0.03	0.08 ± 0.003	0.02 ± 0.02
Root length (cm)	15.67 ± 3.5	8.28 ± 1.8	2.24 ± 0.9
Pre-weight root (g)	0.9 ± 0.05	0.48 ± 0.03	0.08 ± 0.002
Dry-weight root (g)	0.16 ± 0.02	0.04 ± 0.001	0.01 ± 0.01

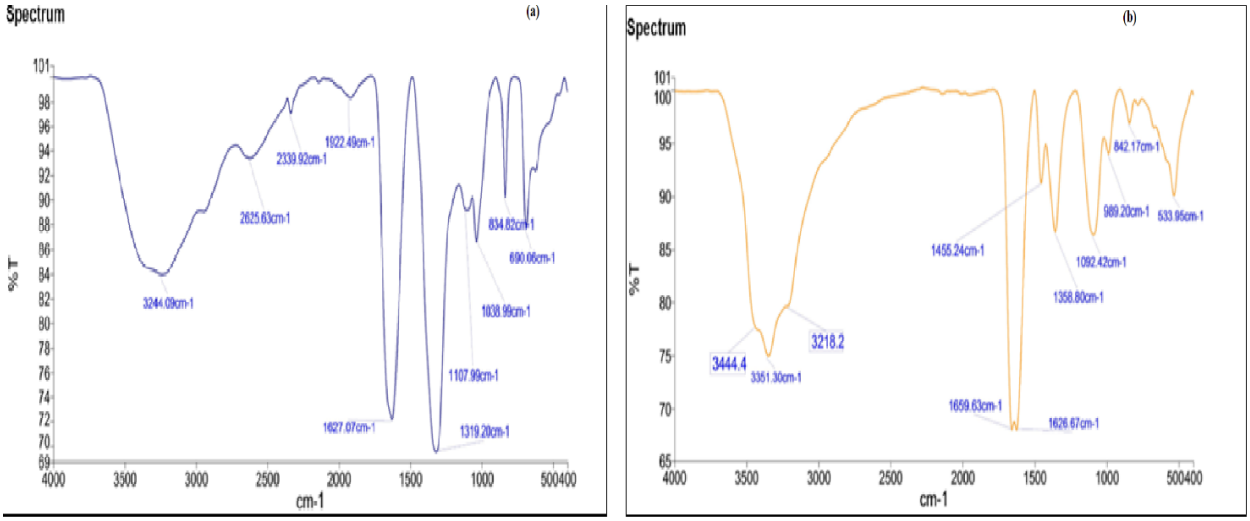

Fig. 6. FTIR analysis of untreated and treated PMDSW, (a): Untreated and (b) Treated

by various researches for bacteria (Pantand Adholeya, 2007). MnP activity 5.3 U/ml/h also has been reported for decolorization of distillery spent wash or melanoidin by Bhargava *et al*., (2009). The presented data also suggested that the extensive enzyme gave higher activity compared laccase activity to breakdown of melanoidin linked with spent wash. It ensures that the whole process was not physico-chemical but bacterial enzyme was responsible. MnP enzyme caused complete degradation of melanoidin linked contaminated PMDSW. Chavan *et al*., (2013) reported the maximum laccase activity 0.9±0.01 U /ml/h after 8 days. It had verified and also investigated that ligninolytic enzymes play a major role in remediation of colored effluents for utilization of complex recalcitrant compounds suchas melanoidin (Santal and Singh, 2013). The phytotoxicity results represent that untreated PMDSW was toxic and hazardous to plant and land ecosystem that depend on plants and treated sample show the germination as well as better plant growth. Results specified that treated sample was not toxic compared to untreated due to the capacity of consortium PN27 treatment could mineralize of PMDSW and its impurities in nontoxic residual metabolites, which could be easily utilized by *Vigna raadiata* as a nutrient material for the germination. Yadav and Chandra, (2019) detected the effect of distillery spent wash on soil (10, 20, 40, 60, 80 and 100 %) and found the favourable results. Chandra and Kumar, (2017) reported phytotoxicity study with *Phaseolus mungo* L. for spent wash treatment confirmation and got positive results. It was further confirmed by analytical methods that treatment was taken placed there and toxicants were degraded. Naik *et al*., (2010) reported FTIR for synthetic caramel and melanoidin degradation and they also achieved confirmation of degradation by peak disappeared and new peak formation. Zhao *et al*., (2018) experimented for Millard reaction and its products like melanoidin formation. Balapure *et al*., (2016) utilized FTIR for confirmation of high strength waste water treatment by bacterial consortium. Our study also represented degradation confirmation by HPTLC and FTIR and got positive results as test samples have disappeared, newly formed and less in percent transmission peaks than control samples. So, Present investigation successfully showed less utilization of nutrients as well as degradation and decolorization of PMDSW containing melanoidin and reuse of treated spent wash in the agriculture sector.

ACKNOWLEDGEMENT

The authors thank the GBRC, Gandhinagar for molecular study and MNIT, Jaipur, Rajasthan for analytical assistance.

REFERENCES

Acharya, B. K., Pathak, H., Mohana, S., Shouche, Y., Singh, V. and Madamwar, D. 2011. Kinetics modelling and microbial community assessment of anaerobic biphasic fixed film bioreactor treating distillery spent wash. *Water Research*. (45): 4248-4259.

Ali, H. 2010. Biodegradation of synthetic dye: a review. *Water Air Soil Pollut*. 213 : 251-273.

APHA. 2012. Standard Methods for the Examination of water and wastewater. APHA, U.S.A.

Balapure, K., Jain, K., Bhatt, N. and Madamwar, D. 2016. Exploring bioremediation strategies to enhance the mineralization of textile industrial wastewater through sequential anaerobic-*microaerophilic process. Int. Biodeterior. Biodegrad*. (106) : 97–105.

Bharagava R. N., Chandra, R. and Rai, V. 2009. Isolation and characterization of aerobic bacteria capable of the degradation of synthetic and natural melanoidins from distillery waste water. *World Journal of Microbiol Biotechnology*. (25) : 737–744.

Boopathy, M. A. and Senthilkumar, S. N. S. 2014. Media optimization for the decolorization of distillery spent wash by biological treatment using pseudomonas fluorescence. *International Journal of Environmental Engineering Technology*. 4(1) : 8-15.

Chandra, R. and Kumar, V. 2017. Detection of *Bacillus* and *Streptophomonas* species growing in an organic acid and endocrine-disrupting chemical-rich environment of distillery spent wash and its phytotoxicity. *Environment Monit Assess*. (189): 19-26.

Chaudhari, A. B., Dandi, N. D., Vandnere, N. C., Patil, U. K. and Chincholkar, S. B. 2012. Bioethanol critical appraisal. In Satyanarayana, T. Johri B. N. and Prakash, A. *Microorganisms in Sustainable Agriculture and Biotechnology.* (1) : 793-924.

Chavan, M. N., Dandi. N. D., Kulkarni, M. V. and Chaudhari, A. B. 2013. Biotreatment of melanoidin-containing distillery Spent wash effluent by free and immobilized *Aspergillus oryzae* MTCC 7691. *Water, Air and Soil Pollution*. (224) : 1755.

Khandekar, Y. S. and Shinkar, N. P. 2020. Distillery Spent Wash Biological Treatment Techniques: A Review. *International Journal of Innovative Research in Advanced Engineering* (IJIRAE), (7): 252-528.

Mahla, P. and Bhatt, N. 2020. Microbial Strategies for the Decolorization and Degradation of Distillery Spent Wash Containing Melanoidins. *Bioremediation Technology: Hazardous Waste Management.* 37-61.

Mohana, S., Shrivastava, S., Divecha, J. and Madamwar, D. 2008. Response surface methodology for optimization of medium for decolorization of textile dye direct black 22 by a novel bacterial consortium. *Bioresour. Technol.* 99 : 562.

Naik, S. N., Goud, V. V., Rout, P. K. and Dalai, A. K. 2010. Production of first- and second-generation biofuels: a comprehensive review. *Renewable and Sustainable Energy Reviews*. 14(2): 578-597.

Pant, D. and Adholeya, A. 2007. A Biological approach for treatment of distillery wastewater: a review. *Bioresource Technology.* (98): 2321-2334.

Patel, S. and Jamaluddin. 2018. Treatment of Distillery Waste Water: A Review. *International Journal of Theoretical & Applied Sciences*. 10 (1): 117-139.

Santal, A.R. and Singh, N.P. 2013. Biodegradation of Melanoidin from Distillery Effluent/: Role of Microbes and Their Potential Enzymes. Biodegrad. *Hazard. Spec. Prod*. 71–104.

Santal, A.R., Singh, N. P. and Saharan, B. S. 2016. A novel application of *Paraccous pantotrophs* for the decolorization of melanoidins from distillery effluent under static condition. *Journal of Environment Management*. (169) : 78-83.

Singh, K.G., Lapsiya, K.L., Gophane, R.R. and Ranade, D.R. 2016. Optimization for butanol production using Plackett-Burman Design coupled with Central Composite Design by *Clostridium beijerenckii* strain CHTa isolated from distillery waste manure. 7: 1063–1068.

Yadav, S. and Chandra, R. 2012. Biodegradation of organic compounds of molasses melanoidin (MM) from biomethanated distillery spent wash (BMDS) during the decolorization by a potential bacterial consortium. *International Biodeteroationand Biodegradation*. (23) : 609–620.

Yadav, S. and Chandra, R. 2019. Environmental health hazards of post-methanated distillery effluent and its biodegradation and decolorization. *Environmental Biotechnology*. (4) : 73-101.

Zhao, X., Huang, X., Yao J., Zhou, Y. and Jia, R. 2018. Fungal growth and manganese peroxidase production in a deep tray solid-state bioreactor, and in vitro decolorization of poly R-478 by MnP. *Journal of Microbial Biotechnology*. 25(6) : 803–813.