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Microaerophilic Treatment of Biomethanated Distillery Spent Wash

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ABSTRACT
Distillery spent wash is the leading pollution issue however, it generates in large quantity due to demand of alcohol and its products. Thus, commercial and bio-friendly approaches are desired to treat spent wash. So, our aim was treatment of biomethanated distillery spent wash (BMDSW) having very high COD and BOD in microaerophilic down flow fixed film bioreactor. The study was operated under various OLR range from 2.3 to 23 kg COD/m³/day and 10 to 1 day HRTs. The microaerophilic reactor was able to remove 98.12±1.8 % of COD and 98.26 ± 0.34 % of BOD from BMDSW at 2 days HRT and 11.5 kg COD/m³/day of OLR. At this HRT, the reactor was also removed solids, phosphate, sulphate, reducing sugar and total carbohydrate concentration from wastewater. To understand biodegradation mechanism of microaerophilic process FTIR and HPTLC analysis was carried out. Furthermore, the treatment efficiency was evaluated using phytotoxicity study by the seeds of Sorghum bicolor. Thus, in a conclusion, the microaerophilic process took 2 days HRT for collective treatment of BMDSW. Therefore, in terms of time and cost sequential anaerobic-microaerophilic process was far better for treatment of BMDSW.
Key words: *Melanoidin, Microaerophilic, Phytotoxicity, FTIR, HPTLC*

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INTRODUCTION

Increment of distillery industries due to high demand and necessity of distillery products are continuously growing for molasses-based alcohol industries. Most of sugar industries extend distillery plant for manage molasses waste and took profit by production of alcohols as molasses utilized as raw material in alcohol production. Distillery industries produces huge amount of dark brown and highly acidic waste water known as distillery spent wash (DSW). History reported, distillation process of alcohol ranges between 5-12% by volume and in this process, it produces the huge amount of DSW 88-95% by volume [1]. For the every 1L alcohol production, 12 to 15L DSW generated through the process diluted molasses used with such as urea, phosphate and ammonium sulphate while adjusted pH by sulphuric acid. This supplement caused the major DSW pollution [2].

It is highly acidic in nature and has a diversity of recalcitrant colouring compounds such as melanoidins, metal sulphides and phenolics which are mainly responsible for the dark brown colour of DSW [3]. DSW was considered as a liquid waste generated with strong objectionable odour [4]. Complexity of DSW is due to COD and BOD ranging between 80000 to 100000 mg/L and 40000 to 50000 mg/L respectively and melanoidin [5]. The key pollutant of DSW is recalcitrant compound termed as melanoidin and other ones are caramel, phenolics and furfurals [6]. Reducing sugars and amino acids carried out from non-enzymatic reaction and yield melanoidins. That reaction is called Millard reaction [7]. Melanoidins are the major colorant compound formed after heating treatment, if it is present in high quantity it leads to environmental contamination. These are complex bio-polymer of amino-carbonyl compounds that can form stable complexes with metal cations [8].

The discharge of untreated DSW into the environment can be harmful and has high pollution potential like, eutrophication, global warming, toxicity, energy balance, water and land usage [9]. Many physicochemical and biological methods developed for melanoidin removal from that waste. Biological methods are trending now, as an anaerobic treatment was not able to completely remove the organic load from DSW [10]. One time treated anaerobically DSW can be termed as biomethanated distillery spent wash (BMDSW). Various aerobic, microaerophilic and anaerobic bioreactors are treading nowadays [11],

therefore the aim behind the study was using bacterial consortium to develop down flow microaerophilic fixed film bioreactor and treat BMDSW under different HRT condition.

MATERIAL AND METHODS

Waste water and soil sample collection

DSW collected from the Industrial area of Surat, Gujarat, India. It was treated in the biphasic anaerobic up flow fixed film bioreactor at our laboratory. The effluent from biphasic anaerobic up flow fixed film bioreactor was used as BMDSW. Liquor contaminated soil was collected in sterile bottle from different area of south and north Gujarat. The samples were stored at 4 ˚C temperature.

Physicochemical characterization of BMDSW

The BMDSW was physicochemical characterization for parameters according to standard methods stated in [12], which are specified in Table 1. All solutions were prepared in pure distilled water with AR grade chemicals.

Development of potent consortium

Bushnell Hass Medium (BHM) amended with 15% BMDSW was used for development and screening as well as optimized by physicochemical parameters for enrichment of consortium. Further it was isolated on solid media followed by identification.

Configuration and operation of down flow microaerophilic fixed film bioreactor

It is consisted of borosilicate glass column and 113 porous stones (size 2.0 ± 0.5 cm, total weight 999.46 g) collected from Sabarmati River bank, Gujarat, were used as a packing material in bioreactor for development of biofilm. The reactor was operated at room temperature (35 ± 2 °C) and inoculated with actively developed PN27 consortium for 30 days to adhered biofilm on stones. Specification of bioreactor was measured as outer diameter 4.5 cm, inner diameter 3.8 cm, hight 62 cm, total volume 1000 mL and working volume 550 mL. Schematic diagram is stated below as Fig. 1.

After biofilm development, inoculum was replaced by melanoidin linked BMDSW with HRT of 10 days. Further, the HRT was slowly changed from lower HRT (10 days with 2.3 kg COD/m^3/day OLR) to higher HRT (1 day with 23 kg COD/m^3/day OLR). Each HRT was operated triplicate for accuracy. The stability of the reactor was confirmed by constant COD and BOD removal of the effluent. The wastewater was fed downward direction into the reactor. The treated effluent was collected from bottom of the reactor and analysed.

Similar characterization parameters as BMDSW were employed for effluent of bioreactor to achieve best HRT for treatment.

Phytotoxicity study for effluent

The method was modified to find out toxicity effect of raw BMDSW and microbial treated effluent was studied on *Sorghum bicolor* seed germination and plant growth. Seed sterilized by 0.1% $HgCl_2$ for 2 min. Seeds were cultivated in fertile soil with equal volume of 5 mL/ seed irrigation of untreated BMDSW, treated BMDSW and tap water (control) for irrigation, Plant growth measurement include in Table 2 [13].

FTIR and HPTLC analysis

After treatment at best HRT to confirm degradation of melanoidins from BMDSW there was Fourier Transform Infrared Spectroscopy and High-Performance Thin-Layer Chromatography analysis performed. 5 mL treated and untreated samples were centrifuged at 11180 *g* for 15 minutes thrice. For FTIR, supernatant was collected as sample and the spectral resolution of 4000-400 cm^{-1} [14]. For HPTLC analysis, supernatant was used as a sample for sapplication on standard silica gel (TLC silica gel 60) plates. The mobile phase was butanol, acetic acid, water (2:4:1 v/v). The scanner (LINOMAT 50) at 254 nm for observation and scanning of silica gel plates (TLC) [15].

RESULTS AND DISCUSSION

Development of potent consortium

Development of bacterial consortium PN27 including six different culturable bacterial strains that were *Paracoccus pentotrophus*, *Pseudomonas balearica*, *Paracoccus sp.*, *Paracoccus pantotrophus*, *Paracoccus denitrificans* and ***Ochrobactrum ciceri***.

Influence of OLR on bioreactor operation

Influence of OLR on pH and redox potential

Any bioreactor majorly influenced by internal pH and redox potential to figure out its microbial health and processes. The influence of OLR on effluent pH values were ranged between 7.16 ± 0.5 to 8.3 ± 0.2 i.e., acceptable range of reactor operation. Feedstock had 8.66 ± 0.22 pH initially and the reactor outflows of treatments were indicated slightly alkaline pH (7.16 ± 1.3 to 8.3 ± 0.5). Maximum pH (8.3 ± 0.2) was attained at 11.5 kg COD/m^3/day of reactor operation and minimum pH (7.16 ± 0.5) was attained at 2.3 kg COD/m^3/day. Another linked parameter was measurement on value of redox potential which was

suggestive value for condition of internal environment e.g., anaerobicor microaerophilic in the bioreactor. The redox potential of BMDSW (influent) was -098 ± 0.3 mV and influence of OLR on redox potential values were in the range of -024 ± 0.5 to -60.6 ± 1.0 mV i.e., acceptable range of reactor operation for microaerophilic condition. From lower to higher HRT there was redox potential increased but get stable during 3, 2 and 1 HRT. Whereas in case of redox potential at 11.5 kg COD/m^3/day there was -45.5 ± 0.2 most stabled redox potential. Detailed Fig. 2 (a) represents all results of influence of OLR on pH and redox potential. Reported microaerophilic reactor study and they achieved 7.1–7.3 pH at 2.4 to 7.2 kg COD/m^3/day [16]. Our initial pH was fairly close to that study.

Influence of OLR on COD and BOD removal

COD and BOD removal efficiency are considered as the sole parameters of wastewater treatment system, under different organic loading rates (2.3 to 23 kg COD m^3/day) is shown in Fig. 2 (b). The results showed that the COD and BOD removal efficiencies were 98.12±1.8% and 98.26±0.3% respectively, during OLR of 11.5 kg COD m^3/day and 2 days HRT Removal of COD and BOD were 95.7 ± 1.2 and 94 ± 1.5 % at OLR of 5.75 kg COD/m^3/day which is comparatively higher COD and BOD removal. Present study can compare with [17], the results showed that, the COD and BOD removal efficiencies were 67 and 64%, respectively, during OLR of 1.0 kg COD m^3/day and 10 d HRT. Treatment of DSW under three stage reactors and achieved 93% reduction in COD level after three stage treatment [18].

Influence of OLR on phosphate and sulphate removal

The phosphate and sulphate concentration of BMDSW (influent) was 1,443 ± 20.6 mg/L and 21 ± 2.1 mg/L respectively. After treatment maximum phosphate concentration of effluent was 333 ± 12.1 mg/L at OLR of 2.9 kg COD/m^3/day and minimum phosphate concentration was 96.25 ± 4.6 at OLR of 2.3 kg COD/m^3/day. Minimum sulphate concentration observed at 2.3 kg COD/m^3/day and 10day HRT and maximum sulphate concentration was 5.85 ± 1.1 mg/L observed at 23 kg COD/m^3/day and 1day HRT. Both elements were detailed in Fig. 2 (c). The phosphate and sulphate concentration were consumed by microbes but after adaptation of condition it was quite steady and became little increased by each higher HRT. Condition created because the influence of with higher OLR there was created slight anaerobic condition, so biological phosphorus removal can be possible when the biomass is subjected alternatively to aerobic to anaerobic conditions. Under anaerobic conditions the microorganisms will first liberate phosphate and sulphate to the liquid phase. Phosphate as such is not harmful to the organisms till its concentration exceeds 3 mg/L [19]. In the study, phosphate content was about 4–5mg/L at 2 d HRT. Also, sulphate concentration of untreated as well as treated effluent was found in the range of 150–1000 mg/L which is much higher. Treatment of DSW by bioreactor, study tends to increase in compare to the phosphate concentration in the influent but there was no significant increase or decrease and no specific trend was observed [2]. In the study of anaerobic treatment of DSW by fixed film bioreactor, the sulphate reduction was not observed at higher HRTs and reactor having charcoal, sulphate concentration was 6500 mg/L at 20day HRT and at 8 days HRT more sulphate reduction was observed because of more acetate concentration in the reactor [20].

Influence of OLR on total nitrogen and carbohydrate removal

Primary treatment that was anaerobic-digestion but few amounts of sugar in the form of total carbohydrate and total nitrogen still remain in effluent. Initially total nitrogen and carbohydrate concentration of BMDWS was 2128 ± 400 mg/L and 4,000 ± 100 mg/L. The nitrogen concentration of effluent was ranged between 1083.4 ± 220 to 1550.4 ± 200 mg/L at OLR of 2.3–23 kg COD/m^3/day. Maximum nitrogen concentration was observed at OLR of 1550.4 ± 200 and 23 kg COD/m^3/day and minimum concentration 1083.4 ± 220 was observed at 2.3 kg COD/m^3/day. The results indicated that nitrogen concentration considerably increasing of organic loading rate due to increase the growth of microbial population and their metabolic activity like synthesis of enzymes for degradation of organic matter, production of amino acids and another by-product. Treatment of DSW by bioreactor, total nitrogen in reactor with coconut coir nitrogen was decreased with each change in HRT and at 6 days HRT it was 1140 mg/L due to the denitrification process in anaerobic conditions [21]. The total carbohydrate concentration varies between 120 ± 15.5 to 640 ± 40.8 mg/L at organic loading rate of 2.3–23 kg COD m^3/day. Maximum removal total carbohydrates at 3.8 kg COD m^3/day, which drop down total carbohydrate up to 120 ± 12.2 mg/L. It was gradually increased initially but, at higher HRT it was constantly decreased. It was due to consumption of carbohydrate by internal flora Fig. 2 (d).

Influence of OLR on solids removal

The effect of the OLR on the concentration of different types of solids in the process effluents is shown in Fig. 2 (e). The concentration of TS and TVS of PMDSW (influent) was 29 ± 4.3 and 7 ± 2.1 mg/L respectively. The concentration of TS and TVS of effluent of microaerophilic reactor was 8 ± 0.6 to 9 ± 0.3 mg/L and 8 ± 0.1 to 5 ± 0.5 mg/L, respectively during an OLR of 2.3 kg COD m^3/L and HRT of 10 days. Study was observed at the increased OLR of 23 kg COD m^3/day, having 2 ± 0.3 mg/L of TS and TVS

concentration respectively. The concentration of TDS and TSS of BMDSW (influent) was 24 ± 2.3 and 6 ± 0.5 mg/L respectively. The organic loading rate between 5.75 ± 2.1 to 23± 5.3 and 2.9 to 4.6 kg COD m^3/L, TDS was constantly between 2 ± 0.1 and 4 ± 0.5 mg/L concentration observed respectively. The highest concentration of TSS was 2 ± 0.3 mg/L observed at OLR of 2.3 kg COD /m^3/L which was completely removed up to OLR of 23 kg COD /m^3/L Fig. 2 (f). The relation between OLR and solids removal have been reported with the decrease in HRT from 15 to 5 day suspended solids concentration increased [22]. Another reported work on fixed film reactor at 6.2 kg COD/m^3/day, TS of the effluent were 435000 mg/L and 42000 mg/L respectively, but as the loading rate increased solid removal decreased [23].

Influence of OLR on decolorization

One of the primitive parameters was carried out to find out with all other impurities of BMDSW, the chromophore group was also to be remove necessarily because the BMDSW contain dark brown pigment due to melanoidin content. So, the breakdown of chromophore group was clearly observed and the results are stated in Fig. 2 (g). The lower HRT gives maximum decolorization that was 99.99 ± 0.01% and eventually when HRT increased and OLR also get increased the decolorization was decreased. At OLR of 23 kg COD/m^3/day treatment can remove 94.34 ± 1.3 % with steady state, between OLR 11.5 to 23 kg COD/m^3/day the decolorization noted quite stable. Decrement in decolorization must be due to extreme amount of OLR and internal flora cannot adapt easily with inhibitory effects of pollutant and flora could utilized it slowly. The reported study shown steady condition of reactor was detected till OLR of 5.0 kg COD/m^3/day and 2 d HRT had 80% color removal elsewhere, the color removal was decreased by 40% when OLR increased [16]. Summarized research work with 98% color removal by microaerophilic treatment of textile dyes with bioreactor impactfully done [19]. Thus, after anaerobic treatment microaerophilic condition was positively impacted on BMDSW in case of decolorization as well as other impurities reduction which stated in Fig. 2 (a to g) below.

Phytotoxicity study

The release of DSW effluent into environment deteriorates the quality of surface water. This water can be used for agricultural purpose in India. Use of such untreated DSW has a direct impact on fertility of soil. Thus, it was needed to assess toxicity of DSW before and after degradation. The study analyzed the toxicity of BMDSW (untreated) and its degradation products (2 days HRT treated effluent) with *Sorghum bicolor*. Experiment was gained 86.66 % germination, all parameters are encoded in Table 2 below, implanted for utilization of BMDSW for examined phytotoxicity [13]. Study was included seed germination of green gram (*P. mungo*) and *Triticum aestivum*, it was resulted that *P. mungo* was less affected than *T. aestivum*. Reported study on phytotoxicity of textile wastewaster on *P. mungo*, the plumule and radical length of *P. mungo* were 25.8 ± 0.44 and 8.6 ± 0.19 cm respectively as a control with 100% germination and the germination rate of *P. mungo* seeds were inhibited (85%) and plumule and radical length were decreased drastically when treated with simulated wastewater. In contrast, plumule and radical length of *P. mungo* was found to be 24.7 ± 0.31 and 8.3 ± 0.15 cm respectively, with 100% germination [17].

FTIR and HPTLC analysis

The change occurred in spectra, area and the functional group of compounds present in BMDSW studied through FTIR and HPTLC spectral analysis by associating treated samples with the untreated samples. The Fig. 3showing obtained peaks of untreated and treated samples, respectively.

The FTIR analysis of untreated sample between 4000-400 cm^{-1} resulted the peaks at 690.06, 834.82, 1038.99, 1107.99, 1319.20, 1627.07, 1922.49, 2339.92, 2625.93, and 3244.09 cm^{-1} that represents C−I stretching in aromatic, S=O stretching sulfonic acid, S=O stretching as well as C-Cl stretching, C-O stretching of secondary alcohol, C-H bend in alkyne, represents N=N stretching and C=C in alkynes, C=N in acetylene and C=C in acetylene, O-H stretching and C-H in acetylene respectively. Same range was utilized for analysis of the treated sample and achieved peaks at 545.02, 624.24, 991.92, 1114.29, 1368, 1635.35, 2142.79 and 3349.93 cm^{-1} which represents aliphatic C-I stretching, C-H deformation in benzene as well as C-Cl stretching in ring, out of plane C=C−H bends, O-H deformation, C-N stretching, N=N stretching in azo compounds, C=C in acetylene, and O-H stretching respectively with changes in % transmission. Most important as per reported data melanoidin can detect between 1600-400 $cm^{-1}$and results of study was indicate the very clear results that the peaks were gradually decreased and disappeared very well, as well as metabolite compounds were indicating another new peak in treated sample which proved that degradation was carried out there by consortium PN27 under microaerophilic state. Fig. 3 (a) and (b)presents the spectra of untreated and treated sample**.** The present study can compare with reported research work by [14] and [24], in their treatment study, the degradation of melanoidins was proved by receiving smaller and disappearance of peaks with lesser % transmission analysed by FTIR.

The degradation of PMDSW was confirmed by high performance thin layer chromatography. Resulted spots with different R*f*-values observed as compared to untreated BMDSW in UV-light. The BMDSW was

observed in UV light at 254 nm. Mentioned results of R*f* values and % area of treated sample was lower than untreated sample which can clearly indicate that the degradation of BMDSW was successfully carried out by developed bioreactor. The table 3presenting obtained peaks of untreated and treated samples, respectively. The peak was gradually decrease very well as well as another peak in treated sample designate new metabolite compound must be present there indicating degradation was carried out there by consortium PN27 in microaerophilic condition. Previously recorded study of melanoidin pigments adapted molecular alteration and breakdown, hence proved that melanoidin can be removed with aid of microorganisms [15].

Microaerophilic down flow fixed film reactor has proven to be successful in treatment of BMDSW, it was characterized before and after treatment and study concluded that, the treatment process was able to reduce COD (98.12±1.8 %) and BOD (98.26±0.34 %) from BMDSW within 2 days HRT. The good germination rate resulted of phytotoxicity study and the biodegradation of melanoidins confirmed by FTIR and HPTLC analysis successfully. Thus, in terms of time and cost sequential anaerobic-microaerophilic process approach was far better for treatment of spent wash.

Table 1 Physicochemical characterization of biomethanated distillery spent wash

Parameters	Results
pH	8.66 ± 0.22
Redox potential (mV)	-098 ± 0.3
TS (mg/L)	29 ± 4.3
TVS (mg/L)	7 ± 2.1
TDS (mg/L)	24 ± 2.9
TSS (mg/L)	6 ± 1.2
Phosphate (mg/L)	1,443 ± 20.6
Sulphate (mg/L)	21 ± 2.1
Reducing Sugar (mg/L)	3,000 ± 100
Total Carbohydrate (mg/L)	4,000 ± 100
Nitrogen (mg/L)	2,128 ± 400
BOD (mg /L)	13,800 ± 2000
COD (mg/L)	23,000 ± 3000

Table 2 Values of phytotoxicity study

Parameters	Control	Test	Untreated
Germination (%)	100	86.66	10
Shoot Length (cm/seed)	15 ± 0.21	13 ± 2.20	4.5 ± 0.32
Root Length (cm/seed)	11 ± 0.42	9 ± 1.02	6 ± 0.13
Wet Shoot Length (g/seed)	0.119 ± 0.02	0.89 ± 0.31	0.003 ± 0.001
Wet Root Length (g/seed)	0.642 ± 2.4	0.412 ± 0.2	0.019 ± 0.005
Dry Shoot Length (g/seed)	0.116 ± 0.033	0.086 ± 0.04	0.001 ± 0.0001
Dry Root Length (g/seed)	0.103 ± 0.024	0.081 ± 0.03	0.004 ± 0.002
Number of leaves	2 Dark green and bigger	2 Dark green and bigger	2 Green and smaller

Table 3 Peak values of HPTLC analysis

Track	Peak	Start Position (R*f*)	Start Height (AU)	Max Position (R*f*)	Max Height (AU)	Max %	End Position (R*f*)	End Height (AU)	Area %
	1	-0.02	4.0	0.00	474.2	73.38	0.07	36.4	62.89
	2	0.51	10.3	0.55	97.8	15.14	0.59	75.6	30.73
Untreated	3	0.61	67.8	0.61	74.2	11.48	0.64	8.9	6.38
	1	-0.02	0.0	-0.02	17.9	4.66	-0.01	12.3	2.36
	2	-0.01	2.4	0.01	187.9	48.82	0.05	26.4	48.38
	3	0.25	2.9	0.26	15.6	4.06	0.27	2.7	1.60
Treated	4	0.44	11.3	0.47	55.1	14.33	0.58	47.8	20.50

Fig. 1. Schematic representation of down flow microaerophilic fixed film bioreactor(A) untreated wastewater; (B) microaerophilic fixed film reactor; (C) packing material; (D) rubber bungs; (E) treated effluent

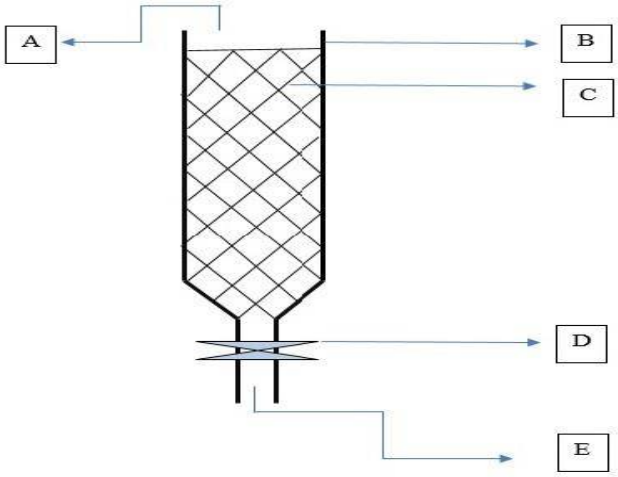
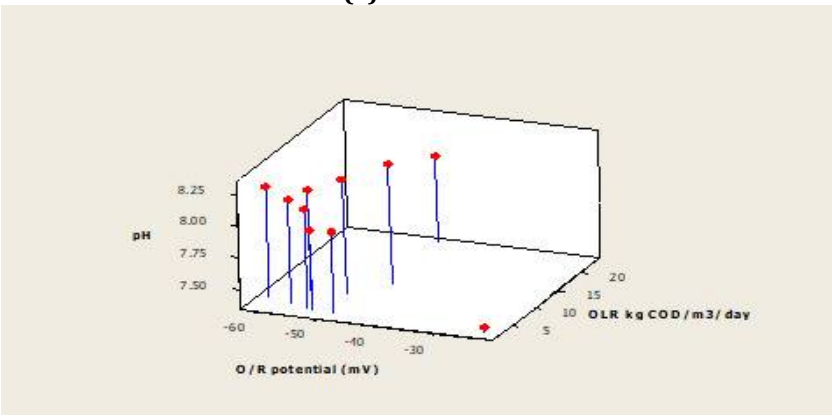


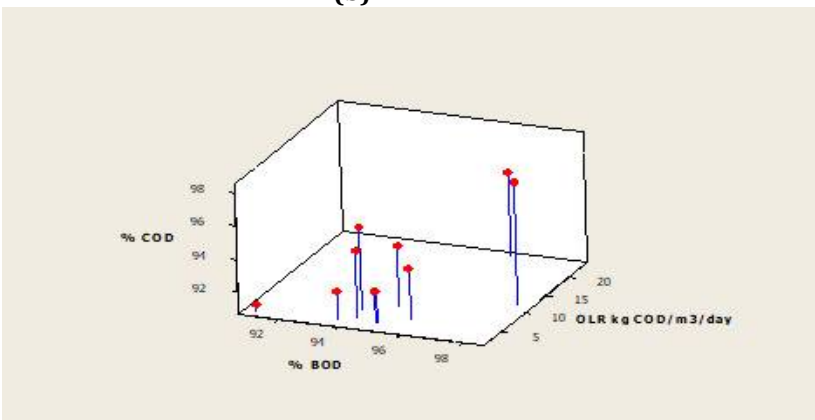
Fig. 2. Influence of OLR on bioreactor operation

(a) Influence of OLR on pH and redox potential **(b)** Influence of OLR on COD and BOD removal **(c)** Influence of OLR on phosphate and sulphate removal **(d)**Influence of OLR on total nitrogen and carbohydrate removal **(e)** Influence of OLR on Total solids and Total Volatile Solids removal **(f)** Influence of OLR on Total dissolve solids and Total suspended solids removal**(g)** Influence of OLR on decolorization

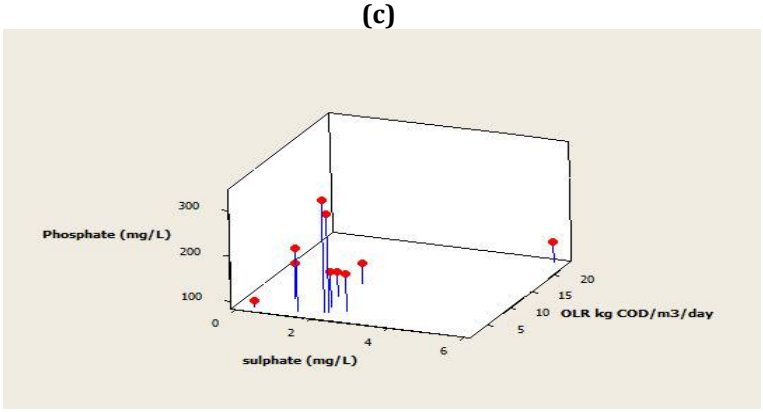
(a)



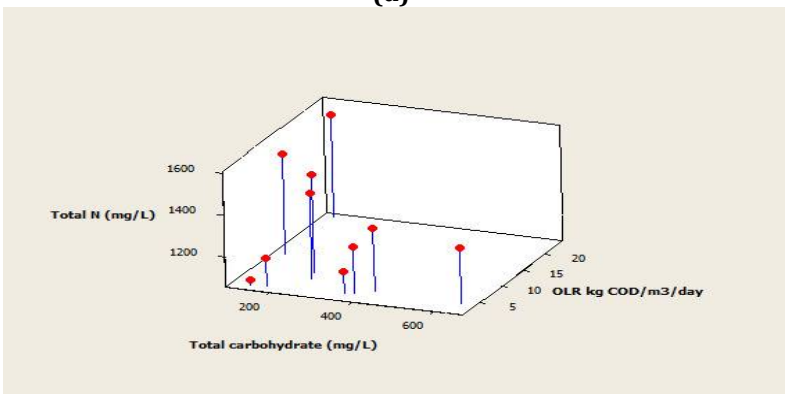
(b)



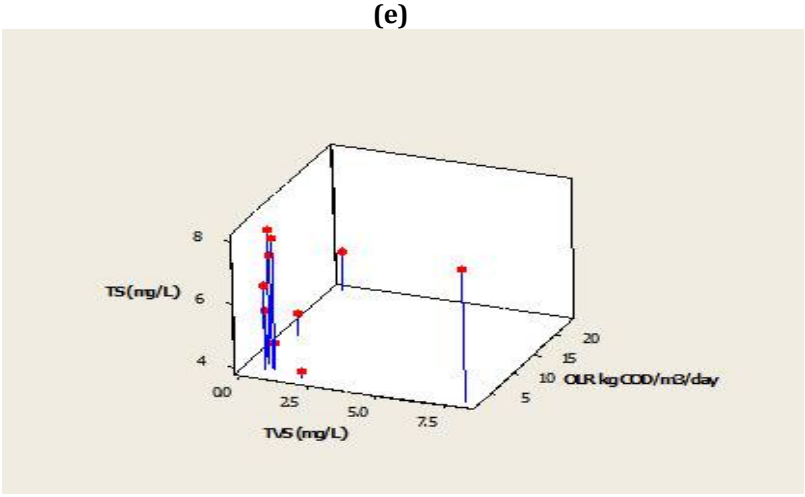
(c)


Phosphate (mg/L)
300
200
100
0
2
4
6
sulphate (mg/L)
5
10
15
20
OLR kg COD/m3/day

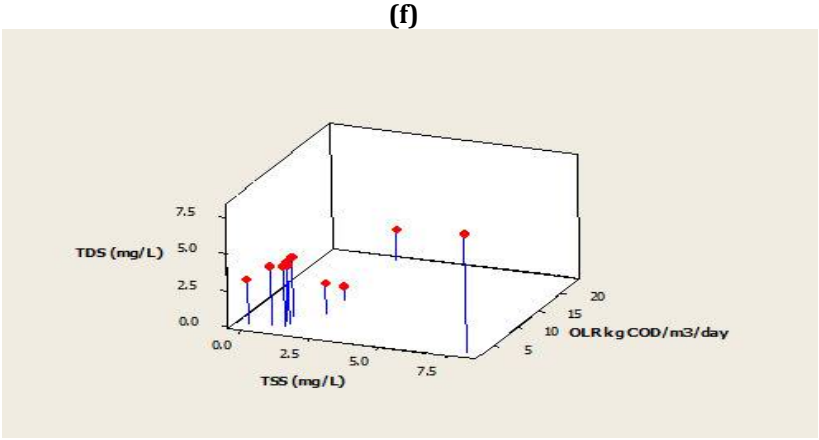
(d)


Total N (mg/L)
1600
1400
1200
200
400
600
Total carbohydrate (mg/L)
5
10
15
20
OLR kg COD/m3/day

(e)


TS (mg/L)
8
6
4
0.0
2.5
5.0
7.5
TVS (mg/L)
5
10
15
20
OLR kg COD/m3/day

(f)


TDS (mg/L)
7.5
5.0
2.5
0.0
0.0
2.5
5.0
7.5
TSS (mg/L)
5
10
15
20
OLR kg COD/m3/day

(g)

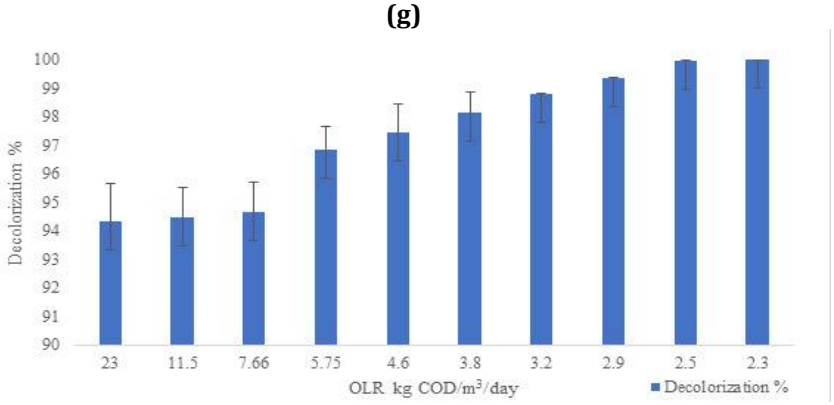
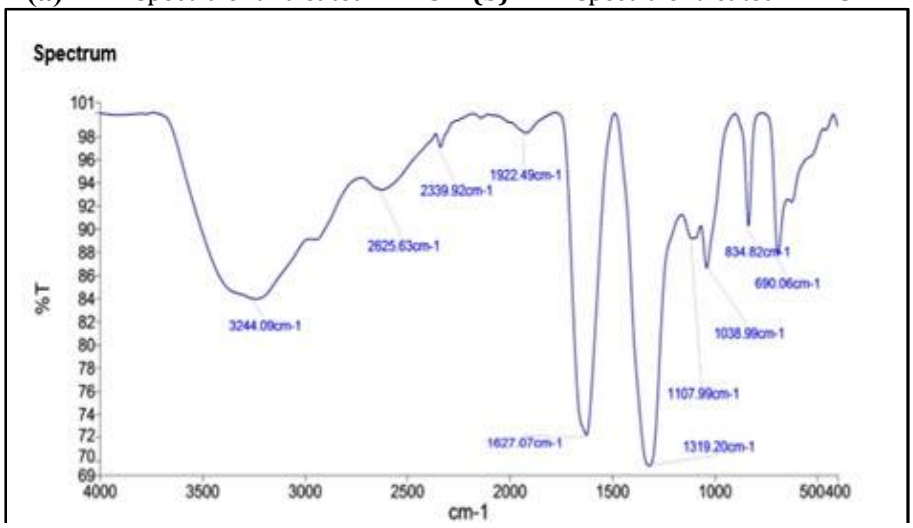
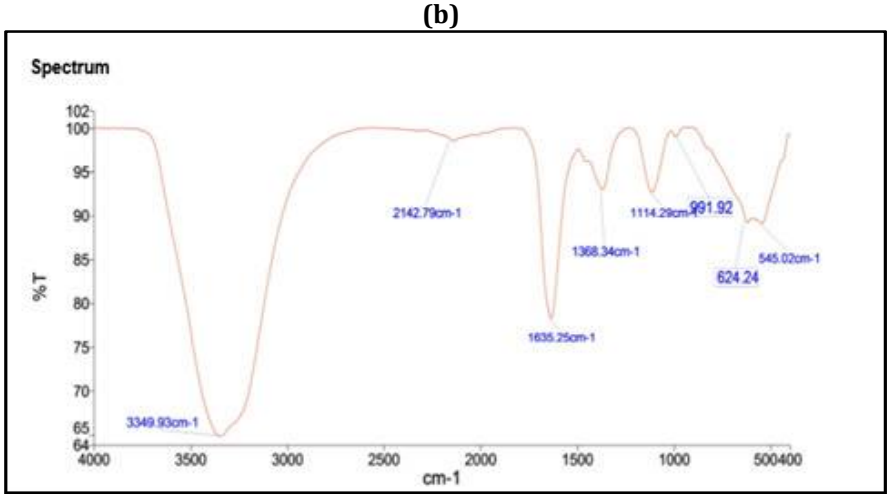


Fig. 3. FTIR and HPTLC analysis

(a) FTIR spectra of untreated BMDSW **(b)** FTIR spectra of treated BMDSW



(b)



CONCLUSION

Treatment of distillery spent wash was aimed and experimented because it is hazardous to surrounding environment including living entities. Microaerophilic down flow fixed film reactor has proven to be successful in treatment of BMDSW. The biomethanated distillery spent wash was characterized before and after treatment and study concluded that, microaerophilic bioreactor took 30 days for successful biofilm development. The treatment process was able to reduce COD (98.12±1.8 %) and BOD (98.26±0.34 %) from BMDSW within 2 days HRT. The positive results of phytotoxicity study in both of the cases proved the efficiency of treatment as well as the biodegradation of melanoidins confirmed by FTIR and

HPTLC analysis successfully. Thus, in terms of time and cost sequential anaerobic-microaerophilic process approach was far better for treatment of spent wash.

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CONFLICT OF INTEREST
Authors are declaring mutual participation for whole work.

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Not applicable.

REFERENCES

1. Wagh, M. P. and Nemade, P. D. (2015). Treatment of distillery spent wash by using chemical coagulation (CC) and Electro - coagulation [EC]. *Am. J. Environ. Prot.,***3**(5), 159–163.
2. Fito, J., Tefera, N., Kloos, H. and Van Hulle, S. W. H. (2018). Anaerobic treatment of blended sugar industry and ethanol distillery wastewater through biphasic high rate reactor. *J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng*. 1-10.
3. Thiyagu, R and Sivarajan P. (2019). Isolation and characterization of novel bacterial strain present in a lab scale hybrid UASB reactor treating distillery spent wash. *Environ. Technol. (United Kingdom)*. **40**(25), 3351-3357.
4. Prajapati, A. K. and Chaudhari, P. K. (2015). Physicochemical treatment of distillery wastewater—a review. *Chem. Eng. Commun*., **202**, 1098–1117.
5. Chowdhary, P., Raj, A and Bharagava, R. N. (2018). Environmental pollution and health hazards from distillery wastewater and treatment approaches to combat the environmental threats: A review. *Chemosphere*. **194**, 229–246.
6. Fito, J., Tefera, N. and VanHulle, S. W. H. (2019). Sugarcane biorefineries wastewater: bioremediation technologies for environmental sustainability. *Chem. Biol. Technol. Agric.*, **6**, 1–13.
7. Ravikumar, R. and Karthik, V.(2016). Effective utilization and conversion of spent distillery liquid to valuable products using an intensified technology of two-stage biological sequestration. *Chem. Biochem. Eng. Q*. **29**, 599–608.
8. Santal, A. R., Singh, N. P. and Saharan, B. S. (2016). A novel application of Paracoccus pantotrophus for the decolorization of melanoidins from distillery effluent under static conditions. *J. Environ. Manage*., **169**, 78–83.
9. Krishnamoorthy, S., Premalatha, M. and Vijayasekaran, M.(2017). Characterization of distillery wastewater – An approach to retrofit existing effluent treatment plant operation with phycoremediation. *J. Clean. Prod*., **148**, 735–750.
10. Mohite, D. D. and Salimath, S. S. (2019). Performance optimization of anaerobic continuous stirred-tank reactor operating on distillery spent wash. *J. Environ. Eng. (United States)*., **145**, 1–5.
11. 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.
12. Eathon, A. D., Clesceri, L. S. and Greenberg, A. E. (2012). APHA Standard Method for the Examination of water and wastewater, 22nd edition. American Public Health Association, Washington, DC, USA.
13. Chandra, R. and Kumar, V. (2017). Detection of Bacillus and Stenotrophomonas species growing in an organic acid and endocrine-disrupting chemical-rich environment of distillery spent wash and its phytotoxicity. *Environ. Monit. Assess*., 189(1):1-9.
14. Rameshkumar, N., Dhanapaul, S., Krishnan, M. and Kayalvizhi, N. (2017). Bioremediation and Sustainable Technologies for Cleaner Environment. *Springer,* 329–345.
15. 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. Soil. Pollut.*, **224**(11), 1-10.
16. Balapure, K., Bhatt, N. and Madamwar, D. (2015). Mineralization of reactive azo dyes present in simulated textile waste water using down flow microaerophilic fixed film bioreactor. *Bioresour. Technol*. **175**, 1–7.
17. 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.
18. Kaushik, G., Gopal, M. and Thakur, I. S. (2010). Evaluation of performance and community dynamics of microorganisms during treatment of distillery spent wash in a three stage bioreactor. *Bioresour. Technol*., **101**, 4296–4305.
19. Shah, B., Patel, A. and Madamwar, D. (2016). Kinetic modeling and community dynamics of microaerophilic treatment of textile dyes containing effluent by consortium VIE6. *Environ. Process*., **3**(2), 397–411.
20. Kazemi, N., Tavakoli, O., Seif, S. and Nahangi, M. (2015). High-strength distillery wastewater treatment using catalytic sub- and supercritical water. *J. Supercrit. Fluids*., **97**, 74–80.
21. Litaor, M. I., Meir-Dinar, N., Castro, B., Azaizeh, H., Rytwo, G., Levi, N., Levi, M. and MarChaim, U. (2015). Treatment of winery wastewater with aerated cells mobile system. *Environ. Nanotechnology Monit. Manag*., **4**,

17–26.
22. Moosvi, S., Kher, X. and Madamwar, D. (2007). Isolation, characterization and decolorization of textile dyes by a mixed bacterial consortium JW-2. *Dye Pigment*., **74**, 723–729.
23. Tiwari, S., Gaur, R. and Singh, R. (2012). Decolorization of a recalcitrant organic compound (Melanoidin) by a novel thermotolerant yeast , Candida tropicalis RG-9.*BMC biotechnology*,**12**(1):1-8.
24. Bharagava, R. N. and Chandra, R. (2010). Biodegradation of the major color containing compounds in distillery wastewater by an aerobic bacterial culture and characterization of their metabolites. *Biodegradation*. **21**, 703–711.

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