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Colour Removal of Distillery Waste by *Saccharomyces* in Combination with Fungal Strains

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In distillery, sugars are fermented to alcohol through yeast. During fermentation all the sugars do not get utilized and some shown in the discharged liquid called spent wash. The yeast (*Saccharomyces cervisiae*) has the ability to utilize the non-reducing sugars involving enzymatic reactions. Fungi are recognized for their superior aptitudes to produce a large variety of extra cellular proteins, organic acids and other metabolites. That is why mixed culture of fungus and yeast was studied with concentrations of supplemented carbon source as 0.1, 0.05 and 0.02 %. The COD and colour removal observed in between 47.9-50.6 % and 60.8-53.1 %, respectively with yeast + *Rhizomucor pusillus,* whereas it was 47-69 % and 54.4-65.7 % yeast + *Rhizopus microsporus* with 0.1, 0.05 and 0.02 % glucose, respectively in 48 h. Most research or fungal capacities to purify polluted effluents has been performed on a laboratory scale, hence there is a need to extend such research to pilot scale and to apply it to industrial process.

Key Words: Mixed culture, Colour removal, Distillery effluent, Fungal culture.

INTRODUCTION

Molasses, procured from sugar industry, is a major material used in distilleries. In distillery, sugar is fermented to alcohol through yeast. The effluent called spent wash having dark brown owing to the presence of pigment called melanoidin. The spent wash generated during process has low pH, unpleasant smell and viscous in nature containing high-suspended solids and organic load (BOD 45000-60000 mg/L) alongwith organic acids and their salts, soluble proteins and carbohydrates. Fiechter and Seghezzi[1] has analyzed the two main extra cellular effectors for the regulation of glucose metabolism in yeast cells are oxygen and glucose itself. *S. cerevisiae* is excellent strain which can give effective results. Orlowki and Barford[2] and Tantirungkiji *et al.*[3] used different sugars and utilized *Saccharomyces* strain for degradation of colour. Castlla *et al.*[4] used fungal culture and found 87 % COD removal, whereas Nudel *et al.*[5] has used mixed culture and found effective reduction in colour. Ohmomo *et al.*[6,7] observed decolourization and COD reduction. Tantirungkiji *et al.*[8] used fungal strain for industrial effluent treatment. Coulibaly

et al.[9] mentioned that fungal biomasses are susceptible to engineering improvements and regeneration of their capabilities with regards to organic pollutants. Excessive nutrients and dyes fungi can remove them from waste waters leading to a decrease in their toxicities.

Other than this fungus have been attracting growing interest for the bio-treatment (removal and destruction) of waste water in gradients such as metals, inorganic nutrients and compounds[10].

EXPERIMENTAL

The waste was collected from M/s Chandigarh Distillers and Bottlers (P) Ltd., 25 Kms from Chandigarh at village Banur, India was selected for the collection of wastewater. The industry started its production in 1991 with licensing capacity of plant of 5000 KL/annum of rectified spirit, using molasses as raw material.

The samples were collected from the outlet of bio-methanation unit to achieve a reasonable uniformity in characteristics. The samples were collected thrice and analyzed for pH, BOD, COD, nitrogen (TKN), sugars, solids and phosphastes.

Strain: A pure culture of *S. cerevisiae* was obtained on the agar slants from the Biotech. Division of Thapar Corporate R&D Centre, Patiala. Pure fungal strains isolated from the tree bark namely *Rhizopus microsporus* var. *rhizopodiformis* (x) and *Rhizomucor pusillus* (W) were used in mixed culture. Culture maintenance, inoculum preparation and dilution *etc*. is same as reported by Rajor *et al.*[11].

Sample preparation: The pH of the diluted waste was adjusted to 5.0. The 100 mL waste was dispensed into 250 mL conical flask, plugged and sterilized. After sterilization and cooling added required extra carbon source and 2 mL of yeast cell and two tablets of fungal cells were inoculated into each flask. Incubation was done according to Rajor *et al.*[11]. The following parameters were done initially after 96 h study. Sugars were analyzed according to Sadasivam and Manickam[12].

Biomass: The final biomass (cell count) in the treated sample was determined turbiditymetrically[11]. The final biomass of fungus was determined gravimetrically. Colour measurement was done by spectrophotometrically described by CPPA[13].

RESULTS AND DISCUSSION

There are limited technologies available to tackle the problem of colour removal even after primary and secondary treatment. Due to promulgations of Environmental laws, health considerations and aesthetics it has become mandatory to reduce the values to the Minimal National Standards (MINAS) before discharging it in water body.

To achieve colour removal from the distillery waste an attempt has been made, herein, by using biological tool (yeast and fungus), which has the capacity to degrade sugars and colour concomitantly. Table-1 shows the comparative removal efficiency, COD, colour, total sugar and reducing sugars by *Saccharomyces* fungal strains with different glucose concentrations.

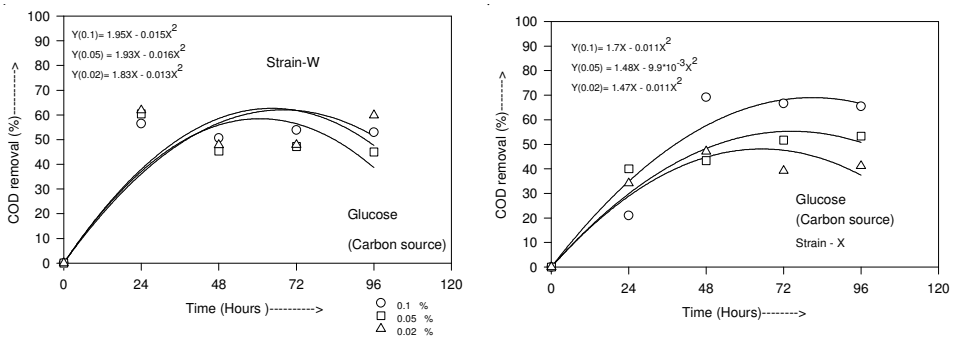
TABLE-1
ANALYSIS OF PARAMETERS (MIXED CULTURE AND SUBSTRATE: GLUCOSE)
W = *S. cerevisial* + *Rhizomucor pusillus*
X = *S. cerevisial* + *Rhizomucor microsporus* Var. *rhizopodiformis*

Time (h) / conc. (%)	W 0.1	W 0.05	W 0.02		X 0.1	X 0.05	X 0.02
0	2600	2000	1880		2999	2222	1889
24	1132(56.50)	792 (60.4)	717(61.9)	COD	2370(20.97)	1333(40.01)	1245(34.1)
48	1283(50.65)	1094(45.3)	981(47.8)		926(69.12)	1259(45.14)	1000(47.1)
72	1200(53.80)	1056(47.2)	980(47.9)		1000(66.65)	1074(51.66)	1148(39.3)
96	1224(52.90)	1100(45.0)	745(59.8)		1037(65.42)	1037(53.30)	1111(41.2)
0	3447	3447	3447		3447	3447	3447
24	1341(61.10)	1667(51.6)	2083(39.6)	Colour	1602(46.50)	1803(47.69)	2037(40.9)
48	1617(53.10)	1985(42.2)	2098(60.9)		1182(65.70)	1379(59.90)	1572(54.4)
72	2106(38.90)	2227(35.4)	2144(37.8)		1431(58.50)	1500(56.48)	1629(52.7)
96	2109(38.80)	2359(31.6)	2474(28.2)		1398(59.40)	1527(55.70)	1560(54.7)
0	1067	896	499		1538	1022	559
24	480(55.01)	589(34.3)	469(6.01)		734(52.30)	650(36.10)	380(32.01)
48	462(56.70)	555(38.1)	429(14.2)	TS	300(80.50)	383(62.50)	337(39.71)
72	559(47.60)	522(41.7)	424(15.3)		371(75.90)	376(63.01)	359(35.78)
96	472(55.8)	520(41.96)	410(17.83)		404(73.73)	404(60.59)	356(36.31)
0	724	495	269		877	517	300
24	175(75.83)	132(73.3)	119(55.76)		314(64.16)	300(41.97)	150(50.00)
48	118(83.70)	124(74.95)	110(59.12)	RS	136(84.49)	146(71.96)	140(53.30)
72	101(86.05)	115(76.77)	106(60.59)		128(85.40)	134(74.08)	129(57.00)
96	111(84.44)	130(73.74)	129(52)		147(83.40)	137(73.50)	153(49.00)
Final pH	4.0	4.3	4.6		3.9	4.0	4.4

Values in paranthesis indicates per cent decrease over initial value.

To get satisfactory level of colour removal the yeast cells were used in combinations with two fungal strains namely *Rhizomucor pusillus* (W) and *Rhizopus microsporous* var. *rhizopodiformis* (X) 2 mL of liquid culture of yeast and two tablets of fungus (preweighed) were inoculated. The distinct advantage of fungus was that the yeast cells harvesting were entrapped in fungal mycelia so the problem of yeast cells from the treated sample was eliminated. Due to unknown symbiotic relationship between yeast and fungal strains high level of colour, COD, TS, RS reduction consuming smaller amounts of sugar was achieved (Table-1).

COD: The COD reduction efficiencies for *Rhizomucor pusillus* (Fig. 1a) were 56.5, 60.4 and 61.9 % corresponding to glucose concentration of 0.1, 0.05 and 0.02 at the end of 24 h and then removal decreased slightly. It may be probably due to resuspension of enzymes in the system. The removal with yeast and *Rhizopus microsporous* var. rhizopodiformis (Fig. 1b) was maximum 69.12 with 0.1 % of glucose at the end of 48 h, but in case of 0.05 % the maximum removal occurs at the end of 96 h and in 0.02 % the concentration the maximum COD removal was at the end of 48 h and decreased beyond 48 h. Some of the concertrations alone of glucose was used with *Saccharomyces*[11] and observed mixed cultures found better degradation.



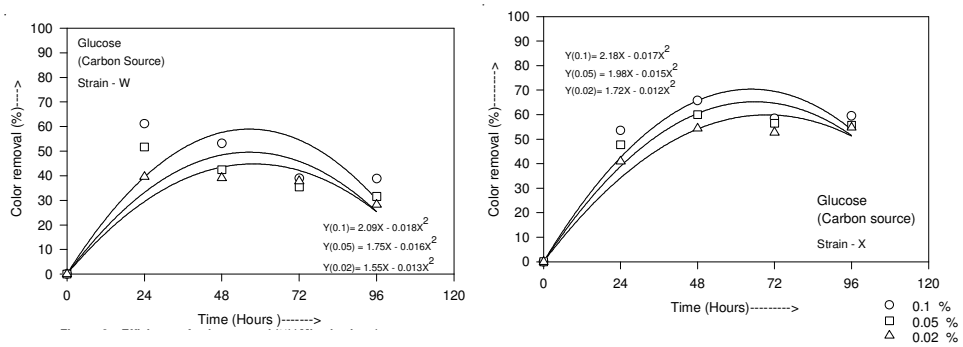
(a) (b)

Fig. 1. Efficiency of COD removal (%) (mixed culture)

Nudel *et al.*[5] has used mixed culture for biomass production and COD reduction and found 30 to 65 % COD reduction in batch process with *Candida utilis* and *Aspergillus niger* in two step continuous culture it was obtained 89 %. Castlla *et al.*[4] studied mixed culture of *Candida utilis* with *Aspergillus niger* and achieved 87 % COD reduction. Coulibaly *et al.*[14,15] used *Aspergillus niger* in laboratory and found 72 % COD reduction in domestic wastewater, while Garcia *et al.*[16] have used four fungus and found good removal of COD by *Geotrichum candidum* with olive mill wastewater.

Colour: The degradation and the mineralization of some recalcitrant dyes organ chlorinated compounds are effective by certain fungi. However fungal aptitudes for raw wastewaters remain dependent on salt concentration, culture conditions and especially on the amendment of carbon and nutrient sources.

Raghukumar and Rivonkar[17] isolated white rot fungus from a marine sample and found good decolourization. The colour of *Rhizomucor pusillus* (Fig. 2a) is always less than *Rhizopus microsporous* (Fig. 2b) in each concentration. It was 66, 60 and 54 % at the end of 48 h corresponding to the concentration of 0.1, 0.05 and 0.02 % in *Rhizopus* while in *Rhizomucor pusillus* was 61, 52 and 40 %, respectively for same substrate concentrations at the end of 24 h. It was found that after maximum removal of colour, again the colour reappears in the system. The fungus removes the colour by absorbance phenomenon, so it may be said that after saturation resuspension of colour occurs. The influence of co-substrate detoxification and decolourization rates has also been observed with *Rhizomucor pusillus*, *Phanerochaete chrysosporium*, *Trametes versicolor, Rhizopus oryzae* and *Ceriporiopsis subvermispora* by Van Driessel and Christov[18]; Nagarathnamma and Bajpai[19]; Nagarathnamma *et al.*[20], Tantirungkiji *et al.*[8] have used white rot fungi and found good biodegradability. Their enzyme producing activity makes them effective decolourizers.

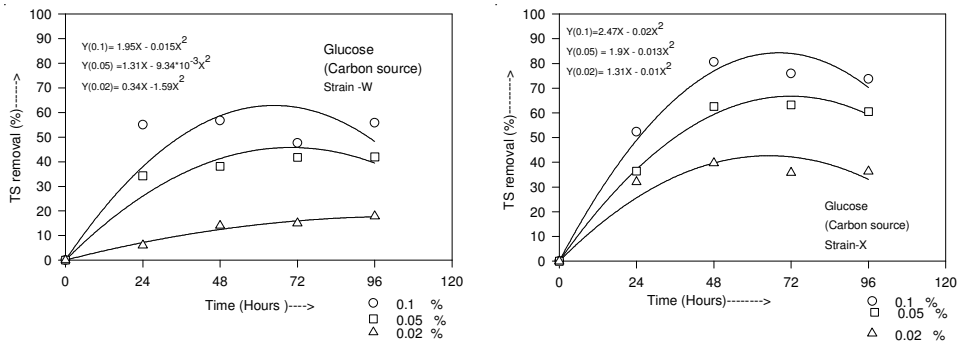


(a) (b)

Fig. 2. Efficiency of colour removal (%) (mixed culture)

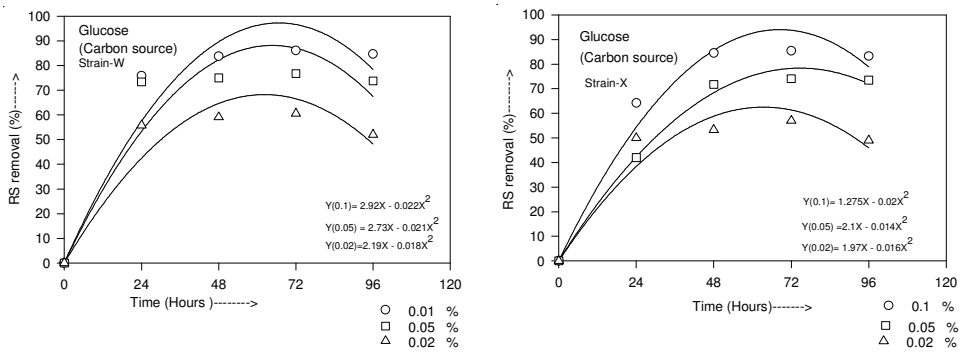
FitzGibbon *et al.*[21] studied behaviour of four fungi *Geotrichum candidum, Corilous, versicolour, Phanerochaete chrysosporium* and *Myceliasterilia* in distillery waste and found maximum 63.3 % decolourization whereas Miranda *et al.*[22]; Ohmomo *et al.*[7] used only *Aspergillus niger* and *Aspergillus fumigatus* and found good 69 and 70 % colour reduction by the fungus from molasses wastewater. Ohmomo *et al.*[7] observed 70 % removal of melanoicin with thermophillic strain *Aspergillus fumigatus*. Assas *et al.*[23]used *Geotrichum candidum* for decolourization of olive mill waste water and found 70 % of colour removal. Thus fungi have shown to removed colour by bisorption[24], biodegradation[25] and enzymatic mineralization[26,27]. Low molecular weight co factors that serves as redox mediators in addition to the enzymes themselves influence fungal removal rate of colours. Kim and Makoto[28] and Nakajim Kambe *et al.*[29] achieved 87 % decolourization of molasses after 12 d of time with *G. candidum* whereas Miyata *et al.*[30] and Fujita *et al.*[31] used *Corilous hirsutus* for decolourization of melanoidin in the presence of nitrogen and carbon source. Strong and Burgess[32] found that white rot fungi have been shown to exihibit unique biodegradable capabilities primarily due to their production of extracellular and broad substrate range enzymes that are capable of mineralization lignin and recalcitrant biopolymer.

Sugars (TS and RS): The total sugar (TS) removal efficiencies for *Rhizomucor pusillus* (Fig. 3a) and *Rhizopus microsporous* var. *rhizopodiformis* (Fig. 3b) were found 56.8, 38 and 14 % and 80.5, 62.5 % and 39.7 % in 48, 72, 96 h with 0.1, 0.05 and 0.02 %, respectively whereas reducing sugar (RS) removal efficiencies for *Rhizomucor* (Fig. 4a) and *Rhizopus* (Fig. 4b) were 86, 77 and 60.59 % and 86, 74 and 57 % corresponding to 0.01, 0.05 and 0.02 substrate concentration after the end of 72 h. Beyond this efficiency was slightly less. It may be due to resuspension of enzymes during desorption mechanism and may be more growth rate occurring in the richer concentration.



(a) (b)

Fig. 3. Efficiency of total sugar removal (%) (mixed culture)

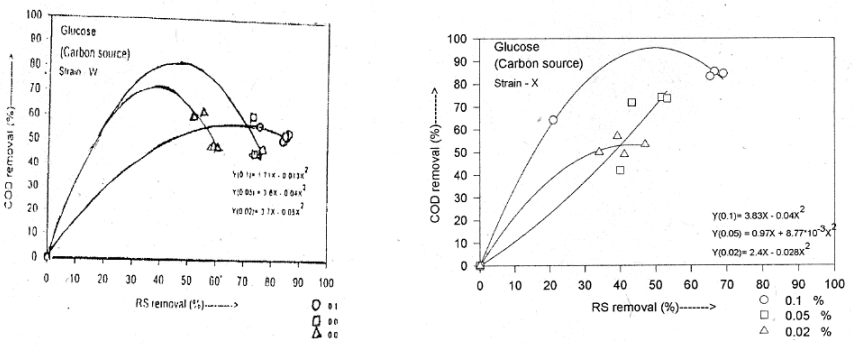


(a) (b)

Fig. 4. Efficiency of reducing sugar removal (%) (mixed culture)

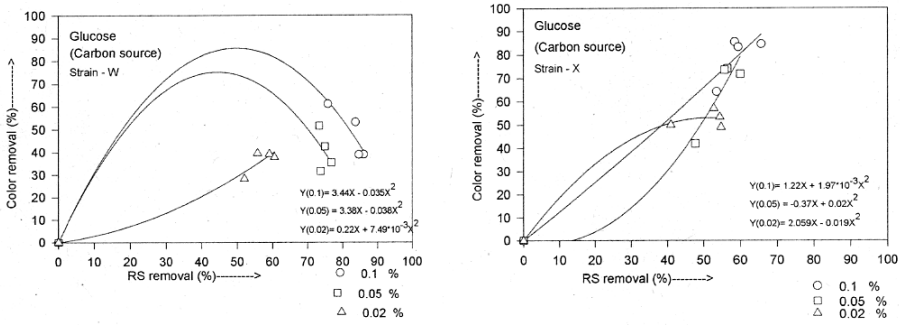
Functional relationship: The COD removal efficiency corresponding to reducing sugar removal for *Rhizomucor pusillus* (Fig. 5a) and for *Rhizopus microsporous* (Fig. 5b) shows that at every instant the COD removal was lesser as compared to RS removal, which may be due to presence of non-biodegradable substances in the system. The colour removal efficiencies of *Rhizomucor pusillus* (Fig. 6a) and of *Rhizopus microsporous* (Fig. 6b) shows that the rate of reducing sugar removal was more than colour removal. Whenever the reducing sugar removal rate decreased the colour removal was less. It may be due to the activity of culture which were faster in the early stage where more nutrients were available. The efficiency decrease with decrease in nutrients.

The RS/TS ratio corresponding to time for *Rhizomucor pusillus* (Fig. 7a) and *Rhizopus microsporous* (Fig. 7b) shows that the ratio was decreasing in both the cases. It is 0.68 to 0.24, 0.55 to 0.25 and 0.54 to 0.31 and 0.57 to 0.36, 0.51 to 0.34 and 0.54 to 0.43 with substrate concentration of 0.1, 0.05 and 0.02. It was observed that non-reducing sugars are being converted to reducing sugars and simultaneously used by the cell. Regarding the relationship between COD and reducing sugar removal, it shows that at any particular time the reducing sugars removal was more



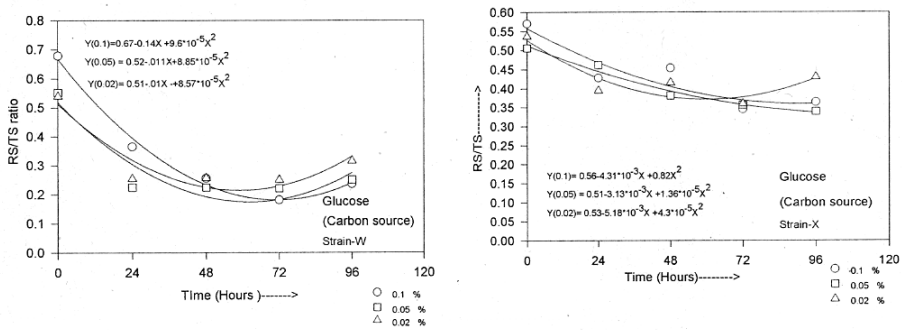
(a) (b)

Fig. 5. COD *vs.* reducing sugar removal (%) (mixed culture)



(a) (b)

Fig. 6. Colour *vs.* reducing sugar removal (%) (mixed culture)



(a) (b)

Fig. 7. RS/TS removal *vs.* Time (mixed culture)

than COD removal. It indicates the *S. cerevisiae* and fungal strains have utilized the reducing sugars for growth as well as for new all production.

So it may be concluded that due to low rate, decrease in *Rhizopus microsporous* the conversion of non-reducing sugar to reducing sugar was more but in case of *Rhizomucor pusillus*, the rate of decrease was more due to greater utilization rate than conversion of non-reducing sugar to reducing sugar.

The final pH values ranges 3.9 to 4.6 (initial pH 5.5) shows that there was production of acids. The rate of acid production was directly proportional to activity of culture. Ohmomo *et al.*[6] reported that treatment of molasses waste water with decolourization enzyme reduced the colour and at the different same time same useful organic acids were produced.

Conclusion

On the basis of work done following conclusions can be drawn: (**1**) Distillery spent wash is one of the worst pollutants both in magnitude and strength. It is about 200-300 times more in pollution load as compared to domestic sewage. (**2**) A dilution of 1:10 and three concentrations of carbon source (glucose) 0.1, 0.05 and 0.02 % were selected for the detailed time study. Repeated inoculations tend to improve the performance with lower concentrations. (**3**) Mixed cultures of fungus and yeast were used with different concentration of glucose. Distillery waste contains degradable sugars (reducing and non-reducing) and fungus can utilize them but not all. Extra carbon source supplements growth and release of extra cellular enzymes those convert non-reducing sugars to reducing sugars. Thus, it appears that colour removal in initial phases is due to surface adsorption mechanisms supplemented by degradation because of extra cellular enzymes in the later stages. Whereas COD removal is purely a metabolic activity. So more work required for concerning enzymatic degradations and reactions. (**4**) To gain confidence with the results these investigations are performed on synthetic colour. Consequently work on pilot and the development of treatment plants are to be encouraged.

Significance: The study conducted revealed that the *Rhizopus* and *Rhizomucor* are capable of degrading the pigment melanoidin. The exact methodology would be established following continuous reactor studies: Melanoidin is a non-enzymatic produced compound with high molecular weight. This compound cannot be transported through membranes and needs enzymatic breakdown to simpler molecules. This is achieved by extra cellular enzymes produced by these fungal strains, when an extra carbon source is added. So an optimization of the culture media in carbon sources or nutrients and mediators molecules is very important to obtain a good output of pollutants degradations. Table-1 shows the comparative removal efficiency of *Saccharomyces* with strains W and X with different glucose concentrations.

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