STUDIES ON DECOLORIZATION OF MELANOIDIN PIGMENT DISTILLERY SPENT WASH BY MICRO-ORGANISMS

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ABSTRACT:

Spent wash generated by distilleries is a major polluting waste which has high COD, and dark color with strong objectionable odour. The present study includes the study of decolorization of distillery spent wash (DSW) by micro-organisms. Yeast, bacterial and fungal cultures were screened for decolorization and degradation of DSW. Organisms were isolated from soil samples near distillery. Maximum decolorization was achieved with bacterial isolate up to 61% and was identified as *Bacillus sp*. Spent wash dilution up to 10 % and external addition of 0.4 % glucose was needed for decolorization. Optimum conditions for decolorization were 28^{o}C, pH 7.0 and time 96 hrs. Yeast and bacteria found to be efficient as live culture. Dried and ground fungal biomass was used and achieved maximum decolorization of spent wash previously decolorized with yeast and bacteria species based on biosorption principle. Fungus used was identified to be *Aspergillus sp.* and found to be carried out decolourization of spent wash. Maximum COD reduction was achieved 81.25 % by using yeast isolate.

Key words: Decolorization, Spent wash, Yeast, Biosorption

1.0. INRODUCTION

Spent wash generated by distilleries is a major polluting waste which has high COD values. It is strongly acidic, dark brown colored, with strong objectionable odour. Dark brown color is due to presence of melanoidin pigments. These pigments are formed by non-enzymatic amino carbonyl reaction i.e.Maillard reaction (1). Disposal of distillery spent wash directly into water bodies is not safe. Its dark brown color causes less penetration of sunlight thus causes decrease in photosynthetic activity and dissolved O_2 concentration which in turn cause harm to aquatic life (2). Disposal on land may cause decrease in soil alkalinity and inhibition of seed germination and the retardation of vegetative growth. Thus spent wash needs to be treated before disposal into environment. Huge amount of such waste is generated by distilleries all over India. About 15 liters of spent wash is generated per liter of alcohol produced (3). Now a day, demand for ethanol is increasing, thus huge amount of spent wash is generated (4).

Physicochemical methods are not economical which remove colors, toxicants, suspended solids and COD. Biological treatment of waste water removes color and BOD .Microbiologically treated effluent may be less toxic and safe (1). Use of physical and chemical methods may generate significant amount of sludge that may lead to secondary pollution due to excessive chemical usage (5). Some drawbacks of these methods are formation of hazardous by products and intensive energy consumption (3).

Several researchers have investigated role of micro organisms in degradation of spent wash. Some bacteria were found to be efficient in degradation .Fungi also found to have capacities to remove dyes from industrial effluent (6). Dyes are removed by fungi by biosorption, biodegradation and enzymatic degradation and mineralization (7). Fungus *Aspergillus fumigatus* was used for treatment of spent wash and achieved 81% decolorization (2). Bacterial consortium was reported to decolorize effluent up to 67% within 24hrs and 51% COD reduction. Bacterial consortium includes *Pseudomonas aruginosa, Proteus mirabilis, Stenotrophomonas maltophila* (8).

Bacterial consortium including *Klebsiella oxytoca, Serratia marcescens and Citrobactor sp* carried out decolorization of 17.5, 9.5, 8.02 and 1.13% were achieved using sugarcane molasses waste water, vinandox source, beet molasses waste water respectively (3). Fungal consortium was used for primary treatment of spent wash using fluidized film aerobic system and algal biomass either free or in immobilized condition for secondary

treatment and achieved 75% decolorization (9). A mixed culture of *Cyanobacteria* achieved 63% decolorization and 72 % COD reduction after 20 days incubation at 35^0C.

2.0 MATERIALS AND METHODS

2.1. Enrichment and isolation of micro organisms:

Distillery spent wash was collected from Yashavantrao Mohite Krishna Sahakari Sakhar karkhana Rethare Budruk, Satara (M.S)India distillery unit and stoared at 4ºC. Characterization of effluent was done for color, pH, temperature, COD, BOD, phosphorus content, nitrogen content, carbon content and total solids according to standard methods (1)

For isolation of micro organisms for decolorization, soil sample suspended in sterile distilled water and serial dilutions were prepared that was spread inoculated on nutrient agar, sabourauds agar and GYE agar for isolation of bacteria, fungi and yeast respectively. Each medium were supplemented with 0.5% spent wash (10).

2.2. Decolorization studies:

A loopfull of pure culture of each isolate from spent wash agar containing melanoid pigment was transferred to minimal medium containing 10 % spent wash. The spent wash modified medium consist of glucose 0.4%, K2HPO4 0.02%, Mgso4 0.0009% (11). To study effect of concentration of spent wash on percent decolorization, various concentrations of spent wash as 5%, 10% and 20% were supplemented in medium.

2.3. Use of combine cultures:

For studying ability of decolorization using combine cultures, minimal medium containing 10% spent wash was prepared and inoculated with various combinations of culture. Fungal mat was allowed to dry and ground in mortar and pestle. The biomass was weighed. One gm, 5gm and 10gm quantities were inoculated separately in 100 ml 1:10 diluted raw spent wash and incubated on rotatory shaker for 10 min then kept steady for 30 min for the mass to settle. Supernatant from these flasks were centrifuged at 5000 rpm for 15 min and percent decolorization was calculated. Uninoculated 1:10 diluted spent wash was used as control.

3.0 RESULTS AND DISCUSSION:

Distillery spent wash was collected from Yashavantrao Mohite Krishna Sahakari Sakhar karkhana Rethare Budruk, Satara (M.S), India distillery unit and stoared at 4ºC.

Characterization of effluent was done for color, pH, temperature, COD, BOD, phosphorus content, nitrogen content, carbon content and total solids according to standard methods (1). Spent wash generated by distilleries is pollution intensive waste water. Because of its high acidity, high COD, BOD values, it is need to be treated (10,11). Soil sample was collected from disposal site of distillery for screening of isolates for decolorization of spent wash.

Table 1: Characterization of DSW Yashavantrao Mohite Krishna Sahakari Sakhar karkhana Rethare Budruk, Satara(M.S)India

Sr. No.	Parameters	Value
1	Color	Dark brown
2	Temperature	85^{o}C
3	pH	4.03
4	COD	1, 28, 000 mg/lit.
5	Carbon Content	3.6%
6	Organic matter content	7.2%
7	Total solids	1,57,870 mg/ lit
8	Total Suspended solids	3070 mg/lit
9	Total dissolved solids	1,54,800 mg/lit
10	Phosphorous content	1420 mg/ lit
11	Nitrogen content	0.141%
12	BOD	19,592 mg/lit

3.1.Decolorization studies:

A loopful of pure culture of each isolate from spent wash agar was transferred to minimal medium containing 10 % spent wash. The spent wash modified medium consist of glucose 0.4%, K2HPO4 0.02%, Mgso4 0.0009%. pH of medium for bacterial isolate was

adjusted to 7.2 and for yeast and fungal isolate; it was adjusted to 5.4 and incubated on rotatory shaker. Samples were withdrawn after 72, 96 and 120 hrs and decolorization activity was determined by measuring decrease in color intensity as absorbance at 475 nm on uv-visible spectrophotometer. Percent decolorization was calculated by using formula:

% Decolorization = (Initial Abs_{475} – final Abs_{475})/Initial $Abs_{475 \times 100}$ ……..(1)

The time at which maximum decolorization was achieved was selected for further studies.

Table 2: indicates that maximum decolorization was achieved in 96 hrs of incubation. Out of all isolates, *B.subtilis* and *S.pombe* shows maximum. decolourisation.

Isolates	**% decolorization in**		
	72 hrs	**96 hrs**	**120hrs**
S.cerevisiae	21.25	30.76	16.50
Citromyces	15.22	18.14	12.50
Candida tropicalis	2.05	6.79	4.00
S.pombe	31.18	38.70	22.15
Candida krusei	3.51	6.79	5.22
B.subtilis	41.25	53.84	35.5
P.putida	33.33	46.23	17.75
Aspergillus niger	26.75	26.23	21.00

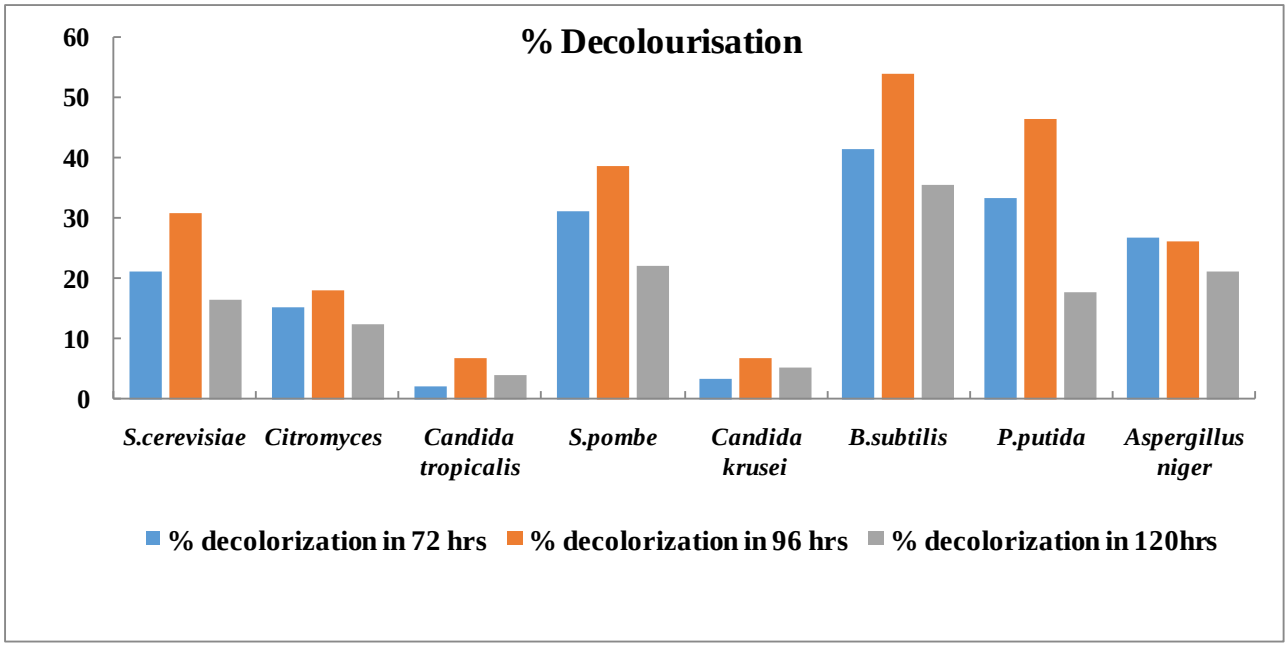


Fig. 1. Spent wash decolorization (%) at 72 h, 96 h and 120 h, using various isolates

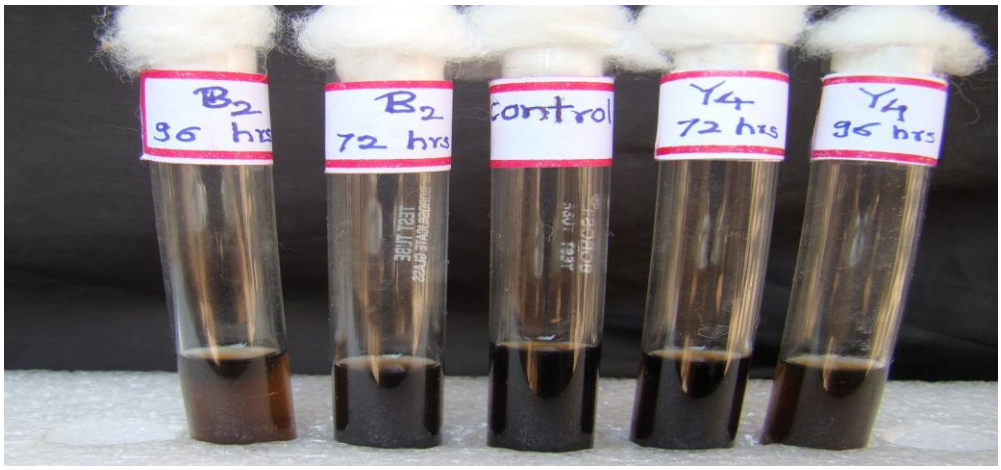


Fig. 2. Spent wash decolorization (%) at different time interval

For further studies, effect of concentration of spent wash on percent decolorization was studied. Various concentrations of spent wash as 5%, 10% and 20% were supplemented in medium and inoculated with *S.cerevisiae, S.pombe, B.subtilis* and *Pseudomonas putida* and fungus *Aspergillus niger* and incubated on rotatory shaker and decolorization was studied. Uninoculated medium supplemented with 5%, 10% and 20% spent wash was used as control (12).

Table 3: Indicate percent decolorization effect of different concentration of spent wash

Isolates	% decolourisation when spent wash concentration was		
	5%	10%	20%
Saccharomyces cerevisiae	21.51	30.76	11.00
Saccharomyces pombae	15.73	38.70	12.50
Bacillus subtilis	26.62	53.84	20.51
Pseudomonas putida	20.12	46.23	15.00
Aspergillus niger	25.62	46.23	16.25

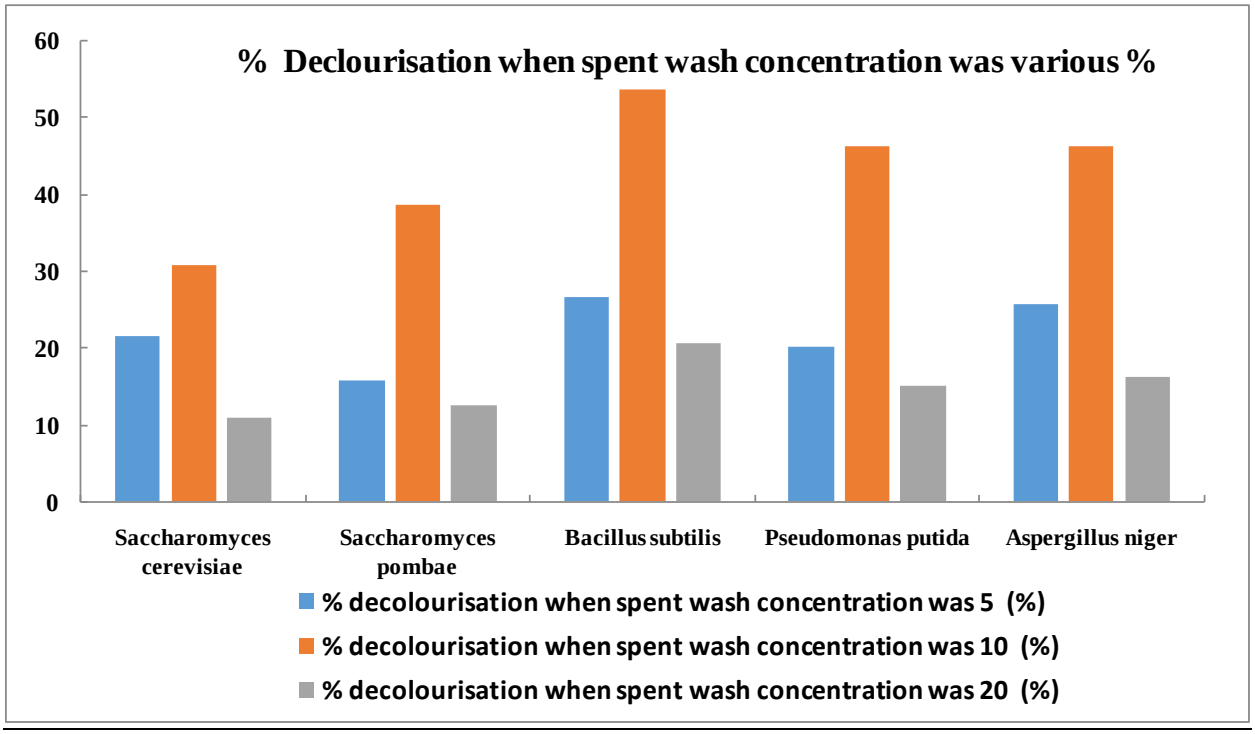


Fig. 3 Indicate percent decolorization effect of different concentration of spent wash

Maximum percent decolourisation was achieved when 10 % spent wash was supplied in medium. Thus, the concentration of spent wash selected for further study was 10%. *Aspergillus niger* was not found very efficient in color removal but as fungi in such cases are known to work more by adopting physical adsorption principle rather than degradative

removal. It was decided to test its ability from that angle (13). Ability of decolorization using combine cultures, minimal medium containing 10% spent wash was prepared and inoculated with various combinations of culture. Use of live fungus *Aspergillus niger* spores in combination with *S.pombae* and *B.subtilis, P.putida* isolated were not found to be satisfactory thus, another method was used (14).

Table 4: Combination of isolates % decolorization

Combination of isolates	Decolorization (%)
S.pombae +B.subtilis	43.01
S.pombae + P.putida	36.55
B.subtilis + P.putida	50.23

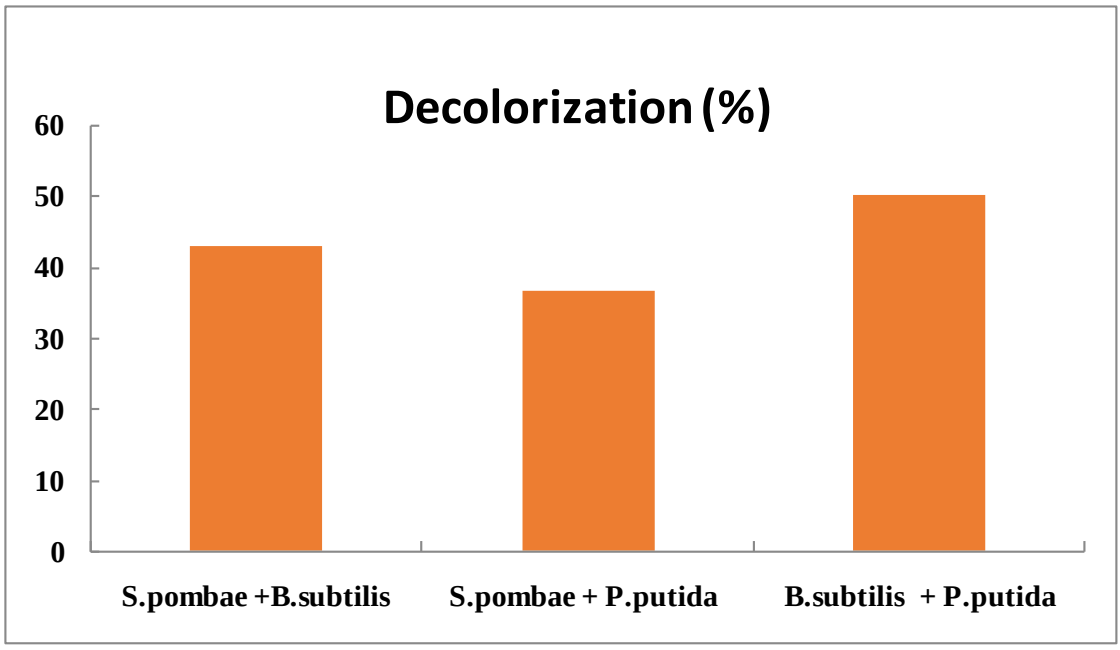


Fig. 4. Combination of isolates % decolorization

Isolates showed better decolorization were selected for further studies. It was reported that nowadays *Aspergillus niger* have shown capacities to remove dyes from industrial effluents by biosorption principle (9). Biosorption is the passive uptake of toxicants by dead/inactive biological materials or by materials derived from biological sources (14). Fungal mat was allowed to dry and ground in mortar and pestle. The biomass was weighed. One gm, 5gm and 10gm quantities were inoculated separately in 100 ml 1:10 diluted raw

spent wash. The flask were kept on rotatory shaker for 10 min then kept steady for 30 min for the mass to settle. Supernatant from these flask were centrifuged at 5000 rpm for 15 min and percent decolorization was calculated.Uninoculated 1:10 diluted spent wash was used as control (15). Out of three flasks inoculated with 1 gm, 5 gm, 10 gm biomass, medium in flask showing maximum decolorization was noted.

Table 5: Different quantity of fungal biomass % decolorization

Quantity of fungal biomass	**Decolorization (%)**
1g	26.82
5g	39.75
10g	30.12

The table indicates that maximum decolourisation was achieved after addition of 5g of dried ground *A.niger* biomass. Thus, 5g quantity of dried fungal biomass was decided to use for further study. Use of dried fungal mass for decolorization of spent wash previously treated with bacteria and yeast (16).

The amount of biomass that showed maximum decolorization activity in previous experiment was noted. Same amount of biomass was added in flasks containing spent wash previously treated with bacteria and that of yeast. Flasks were incubated on rotatory shaker for 10 min then kept steady for 30 min. Supernatant from these flasks was withdrawn and centrifuged. Supernatant was used to measure decrease in Abs 475 nm and percent decolorization was determined (17).

Table 6: Table decolourisation (%) of spent wash previously treated using yeast and bacterial cultures.

Initial decolorizing isolate	**Total decolourisation after addition of fungal biomass(%)**
S. cerevisiae	59.46
Bacillus subtilits	61.43
Pseudomonas putida	60.37

S. pombae+ Bacillus subtilits	48.01
S. pombae + Pseudomonas putida	40.55
Bacillus subtilits + Pseudomonas putida	51.46

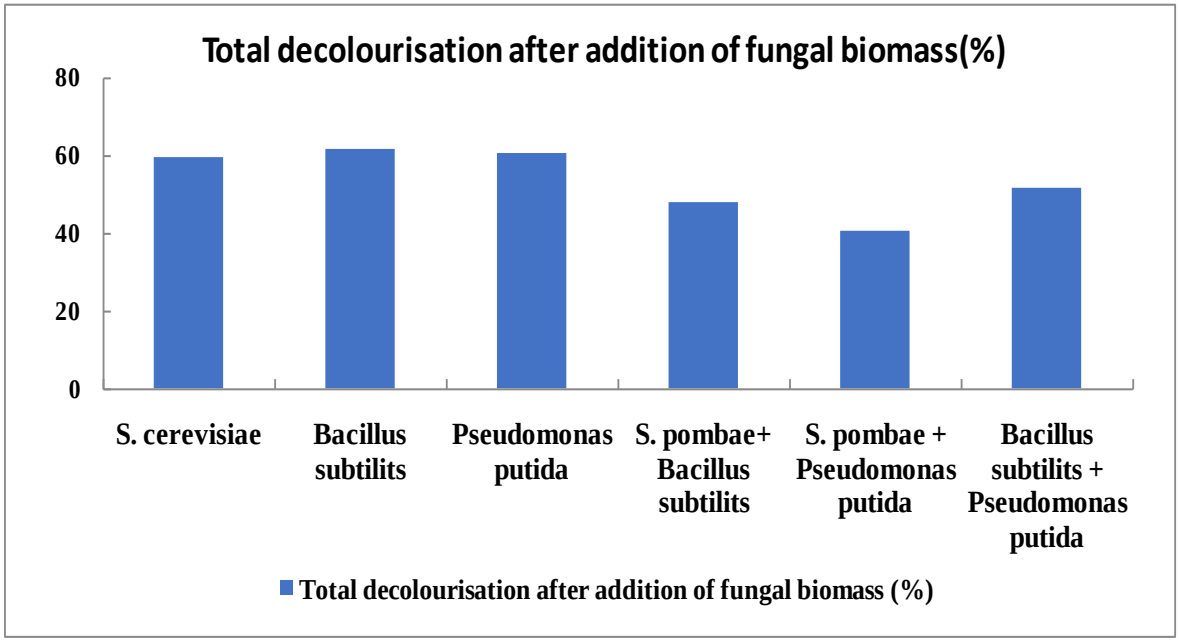
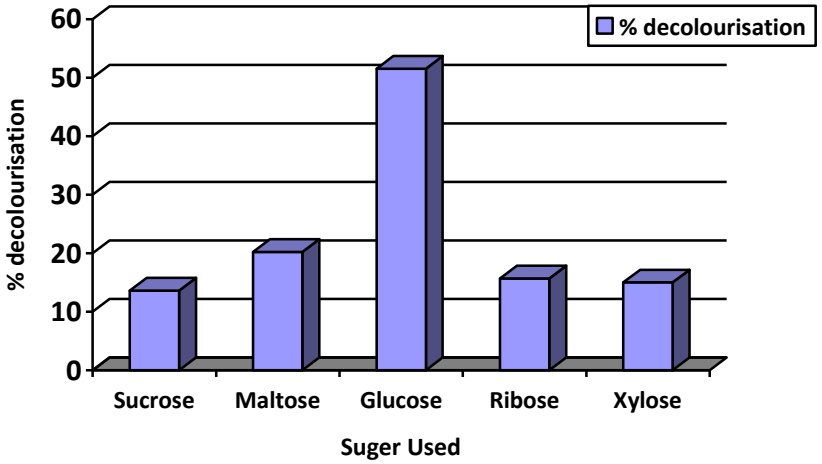


Fig. 5. Table decolourisation (%) of spent wash previously treated using yeast and bacterial cultures.

Maximum decolourisation was achieved after addition of dried fungal mass in spent wash that was treated by, *B.subtilis* better decolourisation was achieved after treatment of fungus to the spent wash that was previously treated with *P.putida* and *S.cerevisiae.* Further study of effect of various factors on decolourisation of distillery spent wash was studied and found that

3.1.1. a. Carbon sources:

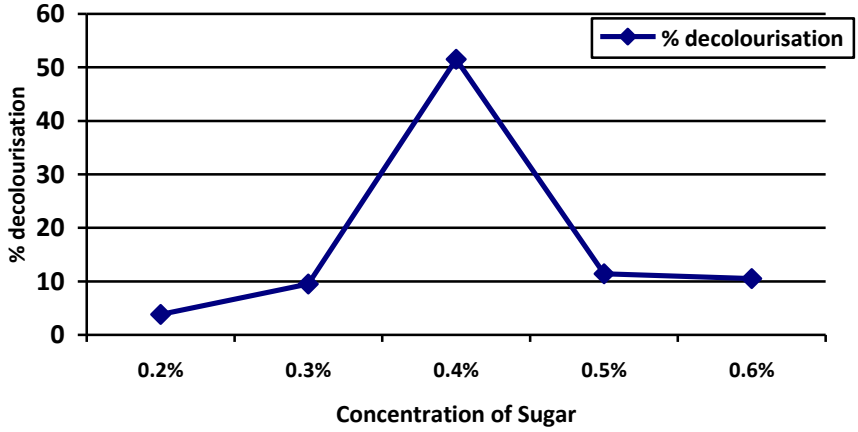
Effect of 'C' sources in decolorization activity was studied by supplying five carbon sources in medium. Out of five, best results were obtained with glucose. Results were represented using graph 2.



Graph 2. Effect of carbon sources in decolorization activity

3.1.2. b. Concentration of sugar:

Different concentration of glucose were supplemented in medium and checked for its contribution to decolorization .Optimum concentration was found to be 0.4% of the medium. Results of decolorization at different glucose concentrations were represented in graph 3.



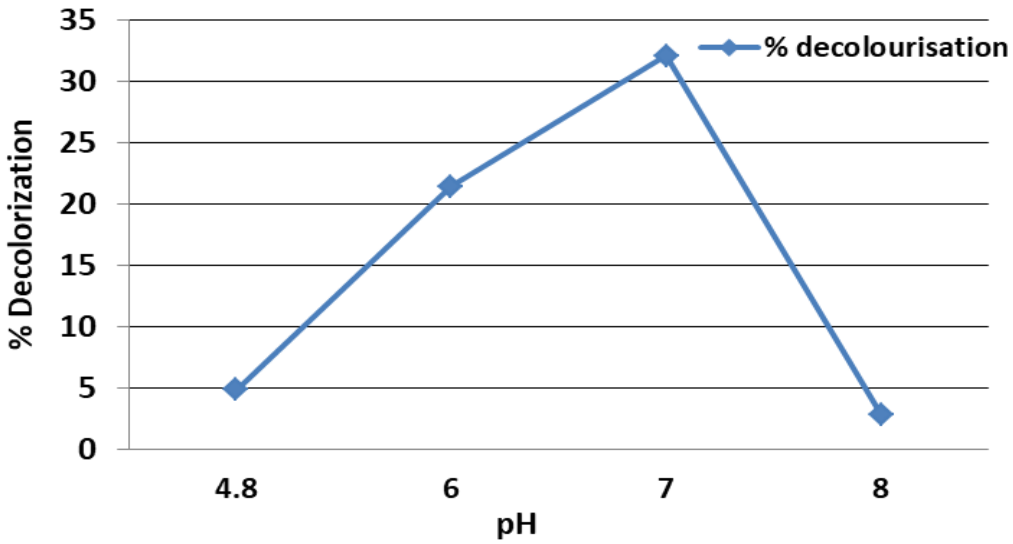
Graph 3. Different glucose concentration effect on decolorization

3.1.3. c. Effect of pH:

Decolorization at different pH values is represented in table 7. Maximum decolorization was achieved at pH 7.0 with fairly good decolorization at pH 6.0. in graph 4.

Table 7: Decolorization at different pH values

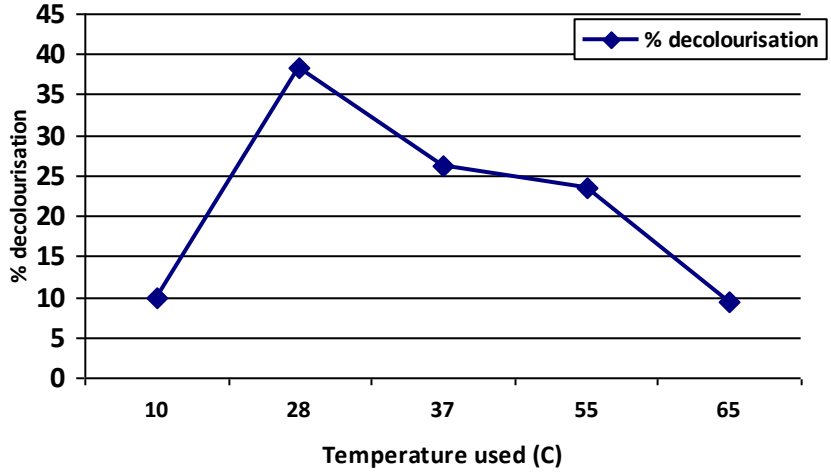
pH used	% decolourisation
4.8	4.83
6	21.42
7	32.07
8	2.81



Graph 4. Different pH effect on decolorization

3.1.4. d. Effect of temperature:

Maximum decolorization was achieved at 28^{o} C. Decolorization was achieved even at 55^{o}C but then declines drastically. This indicates that the culture could not withstand high temperature. Decolorization at different temperature values is represented in graph 5.



Graph 5. Different temperature effect on decolorization

This indicates that the culture cannot withstand high temperature. pH optimization studies lead us to conclude that neutral pH was favorable for decolorization as compared to acidic and alkaline pH. The present results matches the results reported (12).

3.1.5.COD determination of distillery spent wash:

COD of spent wash treated yeast and a bacterial culture was determined in terms of mg/litre by using dichromate reflux method.

Table 8. COD Values before and after treatment.

COD (mg/ lit.) Before treatment	**After Treatment**	
	Treatment with *B.subtilis*	Treatment with *S.pombe*
1,28,000	1,12,000	24,000

Table 9: Different isolate with Percent COD reduction

Isolate	Percent COD reduction
S.pombe	81.25
B.subtilis	12.5

Maximum COD reduction was achieved after treatment with *S.pombe* culture. It was found to be 81.25% Thus, *S.pombe* culture was more efficient in COD reduction

Table 10: Characterization of DSW Yashavantrao Mohite Krishna Sahakari Sakhar karkhana Rethare Budruk, Satara(M.S)India after treatment with mixed cultures

Sr. No.	Parameters	Value
1	Color	light brown
2	Temperature	85^{o}C
3	pH	6.5
4	COD	24, 000 mg/lit.
5	Carbon Content	3.6%
6	Organic matter content	7.2%
7	Total solids	1,57,870 mg/ lit
8	Total Suspended solids	3070 mg/lit
9	Total dissolved solids	1,54,800 mg/lit
10	Phosphorous content	1420 mg/ lit
11	Nitrogen content	0.141%
12	BOD	19,592 mg/lit

CONCLUSION

The two bacterial strains of genus *Bacillus* and two yeasts were found to be most efficient as live cultures for decolrization. The fungal isolate was not found to be very efficient in this. *Aspergillus niger* isolate was found to be very efficient in decolorization by physical adsorption (biosorption) principle. It was found to be even more effective when applied on waste partially decolorized previously by yeast and bacteria were also found to be fairly efficient. The best concentration of waste was found 10% and decolorization time was 96 hrs. COD reducing efficiency 0f selected isolates were also tested in which yeast Y_4 was found to be most effective reducing COD by 81.25%**.** By treating the spent wash with mixed culture not only melanoidin pigment is degraded but there is drastic reduction in BOD ,total solids, total dissolved solids, organic matter, carbon content, pH. By using mixed culture one can treat DSW and reduce undesirable characteristics of DSW and this is one of the effective method for distillery industries.

AUTHOR CONTRIBUTIONS

Each of the writers has made significant donation to the consensus and data, acquirement of information, examination and the understanding of the information; participated in drafting the creation of article and changing it fundamentally for significant and scholarly substance; All of the writers were consented to submit it to the ongoing diary; every one of the writers gave the last approval of the variant of the structure to be distributed; and consented to be at risk for the every one of the parts of the current work. The creators are all qualified to be a creator according to the global panel of clinical diary editors (ICMJE) necessities and rules.

FUNDING

The authors are thankful to the Rayat Institute of Research and Development, Satara for providing prestigious Dr. B.M.Sawant fellowship as a financial support for the research project and providing laboratory facilities.

CONFLICTS OF INTEREST

All of the authors report no monetary or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study doesn't involve the experimentation on any animal creature or human subjects.

PUBLISHER'S NOTE

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DATA AVAILABILITY

All the data gained during the study are presented in this manuscript. Any further added inquiries for additional for further more information are available up on request from the corresponding author

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