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RESEARCH ARTICLE

DECOLOURIZATION AND BIODEGRADATION OF RAW SPENT WASH USING INDIGENOUS MICROBIAL ISOLATES

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ABSTRACT

The collection of indigenous bacterial cultures was performed to estimate its ability to degrade harmful pollutants present in molasses spent wash (MSW) and to understand its degradation potential through natural attenuation for which bacterial (*Bacillus subtilis*) and fungal strain (*Fusarium solani*) was utilized. From the following results 1.2% glucose/peptone concentrations were considered to be the optimized source for carbon and nitrogen for the organisms. Further, the degradation potential of the consortia in different concentrations of MSW along with its application on potted wheat plant was studied.

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INTRODUCTION

Industry an impact on water pollution

Industrial pollution has become a serious concern over degradation of the environment, in the whole world every year the total production of the distillery products is less than that of the production of distillery effluents. The effluents discharged from the industries that enter to the neighbouring streams has detrimental effects on the yield of crops and the land progressively becomes unsuitable for irrigation. Water is a crucial inorganic liquid that existed on planet earth before any form of life, it is an external medium of all aquatic forms. Water is known as a universal solvent and can function as a resource, condition and a habitat. The dumping of effluents which comprises of biodegradable and non-degradable components into the aquatic bodies affect the biogeochemical cycling in the aquatic ecosystem, may accelerate eutrophication and it reduces the productivity and availability of water for drinking purposes. It is imperative to acquire the power to modify or remediate ecological systems in various ways on an unprecedented scale. Sugarcane is one of the major cash crop of the farmers, the effluents of most of the sugarcane industries in India are discharged either directly into water bodies or adjoining crop areas. The toxic effluents shows biomagnification through food chain and their concentration become toxic in edible parts of crop plants. According to AIDA there are more than 300 distillery industries in India which release approximately 35 x 10^{16} KL of waste annually. To overcome this environment issue effluent treatment plants are installed in distilleries. Raw spent wash is subjected to biomethanation treatment to decrease the BOD and COD product obtained is known as biomethanated spent wash (BSW). The spent wash obtained is then concentrated subjected in an open field in form of a lagoon. The sludge is then subjected to evaporation prior to discharge. Treated distillery effluents consists of nutrients that have proved to be beneficial in increasing the crop yield.

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Overview on spent wash and its application

The sugarcane is a thick, tall, perennial grass that flourishes in tropical or subtropical regions. Primary sugar that is glucose is a product of photosynthesis that occurs in all green plants. Juices of sugarcane (*Saacharumofficinarum*) and sugar beet (*Beta vulgaris*) are two sugar crops that are main sources of commercial sucrose**.** India is a major producer of sugar in the world. Its production contributes to tremendous amount of economic development. The waste products like bagasse

(residue from sugarcane crushing) and molasses (final residue from sugar crystallization section) that is obtained from the sugarcane factory also prove to be economically viable. Bagasse is used for the production of paper, electricity and fuel. On the other hand Molasses is a cheap source utilized for the production of alcohol through fermentation. Pollution through spent wash is a major problem in India. There is a need to develop waste water treatment technologies for safer disposal of spent wash. It is estimated that about 15 litres of spent wash is discharged from every litre of ethanol produced.[1] Spent wash is serious waste water pollutant that is generated by distilleries. Spent wash is dark coloured, highly acidic due to the presence of organic acids, hydrophilic, viscous liquid waste with a strong unpleasant odour, high biochemical oxygen demand (BOD), chemical oxygen demand (COD) and consist of high biodegradable organic and inorganic nutrients. Thus, it could not be disposed of directly into the water bodies. It is resistant to bioremediation. Distillery spent wash is perceived as the most caramelized and recalcitrant wastes with high inorganic solid content and low pH.

Distilleries are responsible for the disposal of eco-toxic waste water spent wash. Ethanol production from sugarcane molasses generate a toxic effluent containing melanoidin. Melanoidin compound formed elevated temperature via the Maillard reaction between reducing sugars and amino acids (non-enzymatic amino-carbonyl reaction). The antioxidant nature of melanoidin compound is toxic for certain micro-organisms, including the ones that are responsible for waste water treatment process.[2] According to Pazoki (2008) "The main problem in treating distilleries spent wash is its color, which contains about 2% of the dark brown recalcitrant pigment known as melanoidin". The spent wash consist of caramel, melanoidin, and other toxic chemicals. Melanoidin is a recalcitrant pigment that is responsible to give brownish colour in the spent wash. [3]Melanoidin is a toxic compound that is responsible to harm aquatic life by reducing the photosynthetic activity and utilizing the dissolved oxygen in the water bodies it also induces toxicity in the soil, causing reduce in soil alkalinity and also in soil manganese availability eventually resulting in inhibition of seed germination and vegetation growth. It is common practice in India to use industrial effluent and sewage sludge on agricultural land this results in toxic metals to get accumulated in the soil these toxic ingredients have detrimental effects on plants and may result in health problems to man and animals. Molasses spent wash is highly coloured in nature which tends to block out the sun light from water bodies thus reducing the oxygenation activity of the water along with the ability of the plants to perform photosynthesis. Due to its presence of excess of nutrients it results in eutrophication subsequently leading to contamination of water sources. The dark brown colour of spent was is mainly due to the melanoidin pigment. Spent wash consists of carcinogenic and mutagenic compounds such as dioxins, phenols, lignins etc. Molasses consists high amount of sucrose which can be used as a carbon source in fermentation process and can be used as a livestock feed. The land application of spent wash offers benefits of water pollution control and agricultural production due to its rich in organic nutrients. Use of spent wash for agricultural purposes such as fertilizer and has subsequently lead to water pollution control. Use of spent wash for agricultural purposes has led to increase in salt content. It can be applied directly to land as irrigated water thereby helps restoring and maintaining the soil fertility, improving micro flora and maintaining physio-chemical properties in soil. Due to limitation of knowledge on utilization of spent wash as a fertilizer, overuse of spent wash and overuse of spent wash by farmers has led to salinity, infertility of soil and immature growth of crops.

Field Description

Bhima Shakari Sakhar Karkhana Limited (BSSKL), Patas, Pune. The plant was incorporated in December 1976 by late Mr. Madhukarro Gangajirao Shitole as a co-operative society to undertake manufacturing of sugar related production. The crushing capacity conducted in the sugar season 1979-80 was about 12,500 tonnes of cane crushed per day, which subsequently enhanced tremendously to about 5000 TCD on March 31st, 2014.[4] BSSKL commissioned a 45 kilo-liters per day (KLPD) distillery unit and subsequently installed a bagasse co-generation unit with an installed capacity of 19.5 mega-watts (MW) in November 2012.

MATERIALS AND METHODS

Physio-chemical Analysis

Distillery spent wash consists of rich organic nutrients such as nitrogen, phosphorus, potassium, calcium and sulphur. Micro nutrients such as iron, zinc, copper, manganese, boron and molybdenum is present in sufficient amounts. It this therefore, imperative to analyse the physio-chemical characteristics of spent wash prior subjecting it to treatment and use as fertilizer to avoid crop damage.

Isolation of Micro-organisms and optimization of glucose and peptone concentration

Step 1: Pure culture of Fungus (*Fusarium solani*) and Bacteria (*Bacillus subtilis*) strain no. 168W23 was inoculated on Basal medium agar plate containing 10% molasses.

Step 2: Growth is transferred in broth with in different concentration of glucose and peptone (1%, 1.2% and 1.4%) in 250ml conical flask containing 10% molasses spent wash. The flasks were incubated at 30°C for 7 days in laboratory shaker at 160 rpm. After the incubation was complete chemical oxygen demand and Absorbance was taken at 475 nm with

1Banu, J. Rajesh, S. Kaliappan, M. Rajkumar, Dieter Beck, and others. 2006. "Treatment of Spent Wash in Anaerobic Mesophilic Suspended Growth Reactor (AMSGR)." Journal of Environmental Biology 27 (1): 111–17.

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[4]BhimaSahakariSakharKarkhana Limited, Care Ratings Professional Risk Opinion, http://www.careratings.com/upload/CompanyFiles/PR/BHIMA%20SAHAKARI%20SAKHAR%20KARKHANA%20LIMITED-08-07-2014.pdf

spectroscopy to monitor the rate of decolourization of spent wash sample. This procedure continuously carried out for 7 to 21 days. Isolation was obtained from pure culture of fungi on basal medium with 10% molasses spent wash. Isolation of microbial strains from molasses spent wash was done by streak plate technique.

Consortia Study

Step 1: Pure culture of Fungus (*Fusarium solani*) and Bacteria (*Bacillus subtilis*) strain no. 168W23 was inoculated on Basal medium agar plate.

Step 2: Growth was transferred in broth with optimized 1.2% of glucose and peptone (based on results optimized carbon and nitrogen source) in 250ml conical flask containing different molasses spent wash concentrations between 10%-40%.

Step 3: After the incubation was complete chemical oxygen demand and Absorbance was taken at 475 nm with spectroscopy to monitor the rate of decolourization of spent wash sample. This procedure continuously carried out for 7days.

Study the effect of treated distillery treated spent wash on soil properties and crop yield

Treated molasses spent wash with different concentrations between 10%-40% were applied to potted plants containing wheat seeds. 50 wheat seeds were used for each pot. For the first three days only water was applied following treated spent wash (10-40%). Soil samples were analysed in the laboratory by following standard procedures (Page *et al.,* 1982)

RESULTS AND DISCUSSION

Distilleries from spent wash when disposed into water bodies eventually leads to water pollution. The purpose of this study is to understand the quality of effluent generated from the distillery and its dilution prior to discharge.

Table 1. Concentration of glucose and peptone in flasks

1%	1.2%	1.4%
2.5gms of glucose and peptone	3gms of glucose and peptone	3.5gms of glucose and peptone

Physical Parameters

Table 4. Physical Parameters

Serial number	Parameters	Unit	Result
1.	Colour	-	Dark Brown
2.	Odour	-	Unpleasant
3.	Total Solids	mg/l	156460
4.	Total Suspended Solids	mg/l	69960
5.	Total Dissolved Solids	mg/l	86500
6.	Electric Conductivity	mS/cm	53.4
7.	Turbidity	NTU	9100
8.	Total Hardness ($CaCO_3$)	mg/l	22000

Source: (own draft)

Chemical Parameters

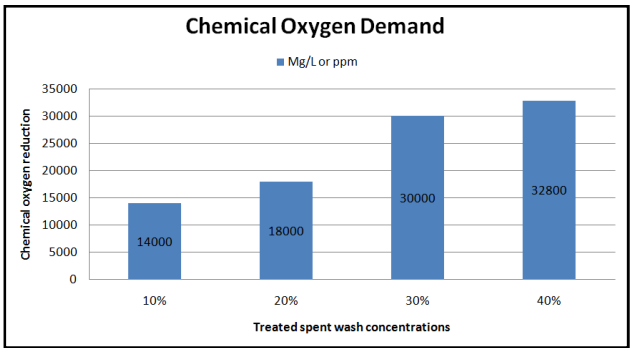
S.No.	Parameters	Unit	Result
1.	pH		3.91
2.	Chemical Oxygen Demand (COD)	mg/l	142800
3.	Alkalinity ($CaCO_3$)	mg/l	2742
4.	Total Nitrogen (N)	mg/l	3493.06
5.	Total Phosphate (P)	mg/l	256
6.	Total Potassium (K)	mg/l	3875
7.	Oxygen Demand (OD)	mg/l	nil
8.	Biochemical Oxygen Demand (BOD)	mg/l	29300

Optimization of glucose and peptone concentrations

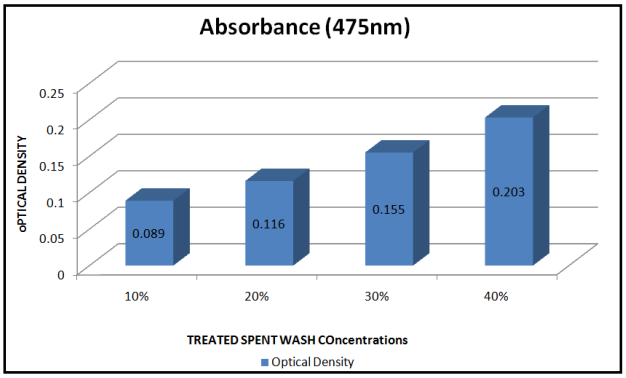
Several bacteria and fungi have been proven effective in degrading molasses spent wash but it is important to improve the performance of the system and increase the product yield without increasing the cost hence, Based on results 1.2% concentrations of glucose and peptone were most effective for both Fungus (*Fusarium solani*) and Bacteria (*Bacillus subtilis).*

Data Obtained (Consortia Study)

Pure culture of Fungus (*Fusarium solani*) and Bacteria (*Bacillus subtilis*) strain no. 168W23 was inoculated on Basal medium agar plate. Growth was transferred in broth with optimized 1.2% of glucose and peptone in 250ml conical flask containing different molasses spent wash concentrations between 10%-40%. After the incubation was complete chemical oxygen demand and Absorbance was taken at 475 nm with spectroscopy to monitor the rate of decolourization of spent wash sample. This procedure continuously carried out for 7days.

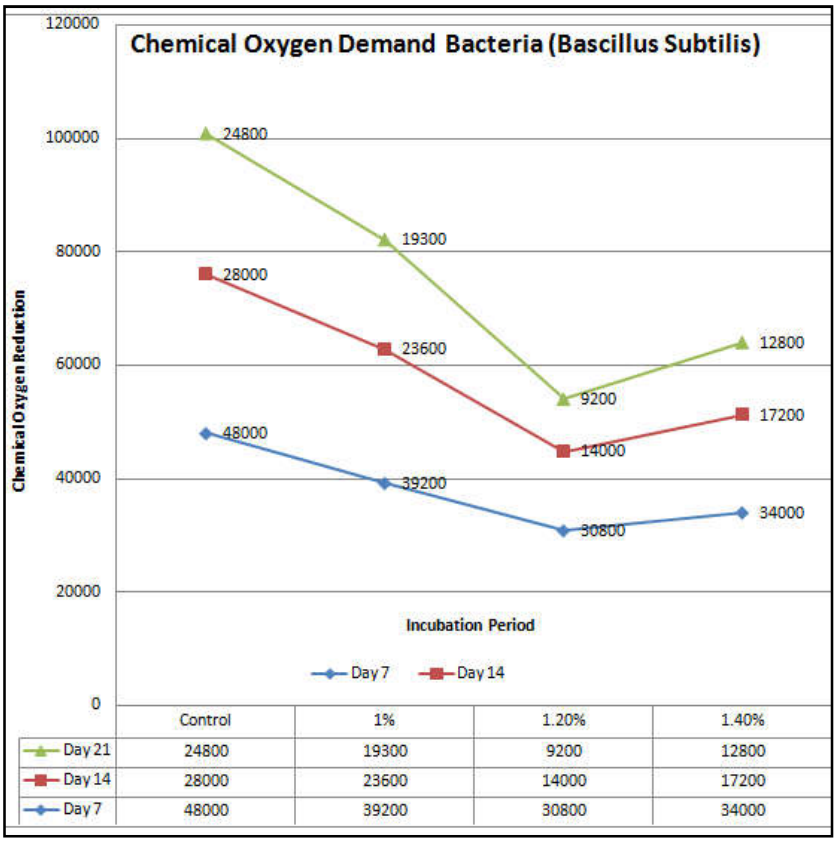


Graph 1. Determination of chemical oxygen demand for Bacteria (*Bacillus subtilis*) & Fungi (*Fusarium solani*)

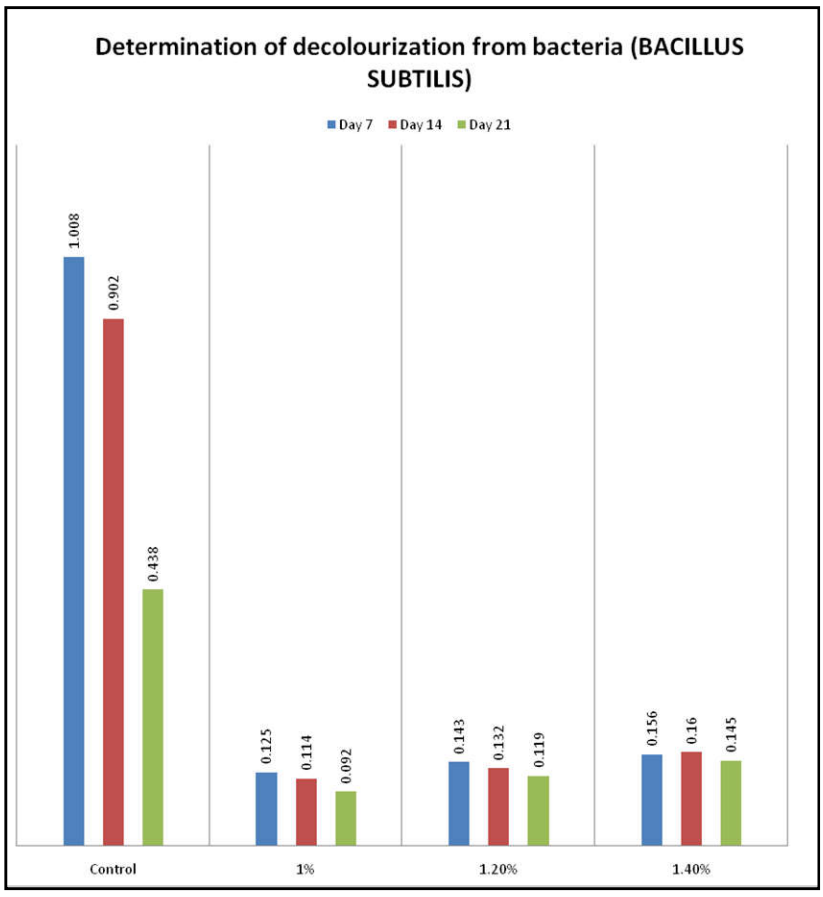


Graph 2. Bacteria (*Bacillus subtilis*) and Fungi (*Fusarium solani*) Absorbance at 475nm

Analyses of biodegradation and decolourization of molasses spent using Bacteria (Bacillus *subtilis*)

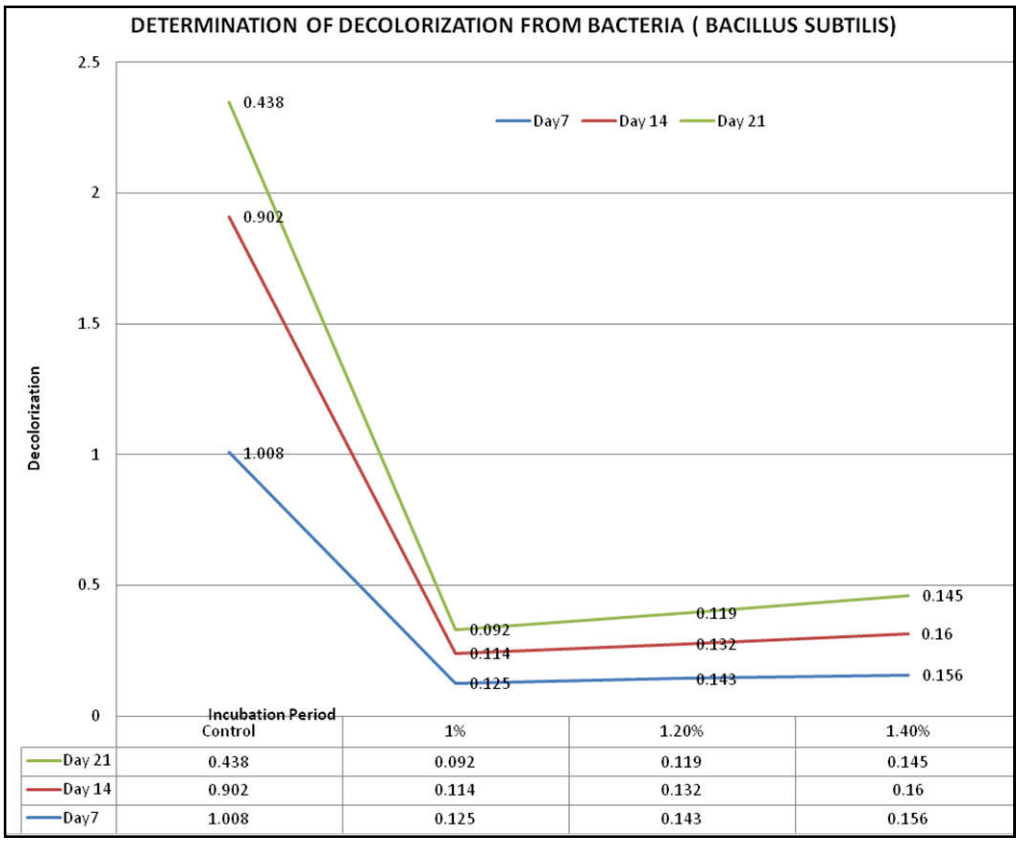


Graph 3. Determination of chemical oxygen demand Bacteria (*Bacillus subtilis*, day 7-21)



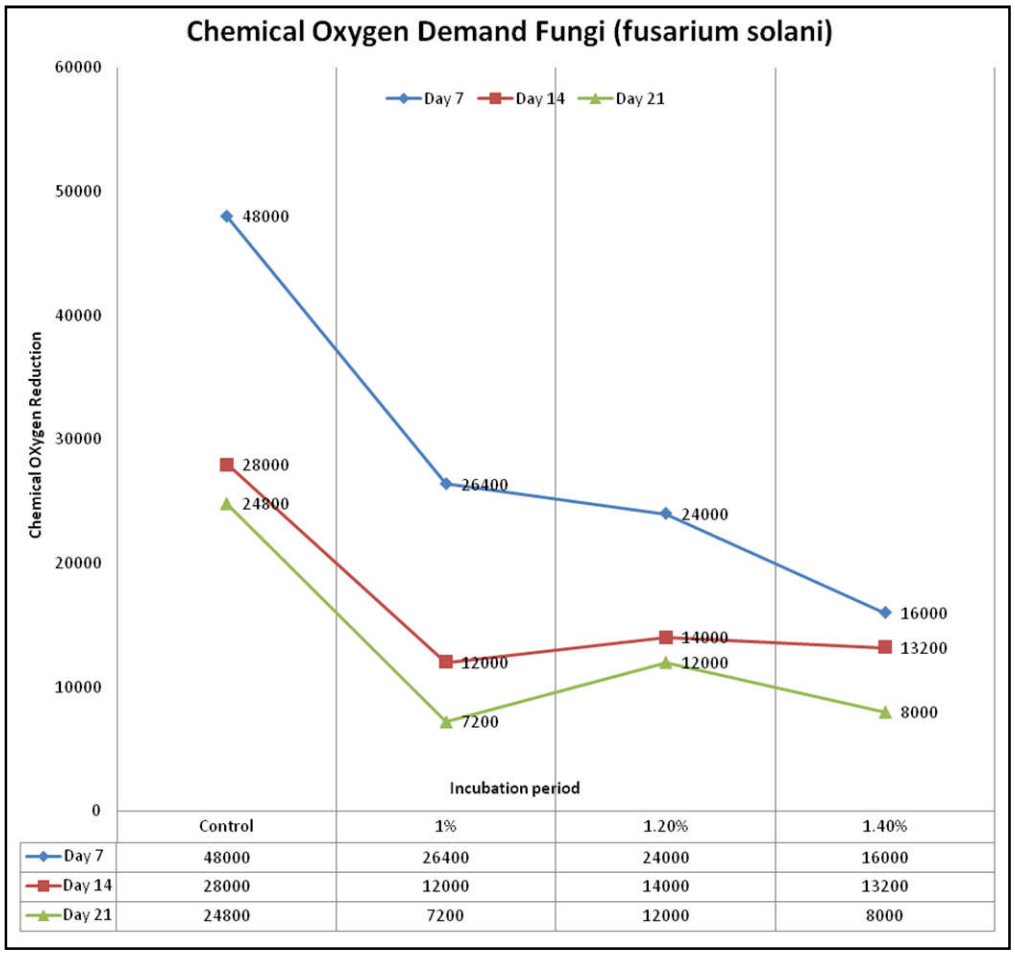
Graph 4. Bacteria (*Bacillus subtilis*) absorbance at 475nm (Day 7-21)

Analyses of biodegradation and decolourization of molasses spent using Fungus (Fusarium *solani*)



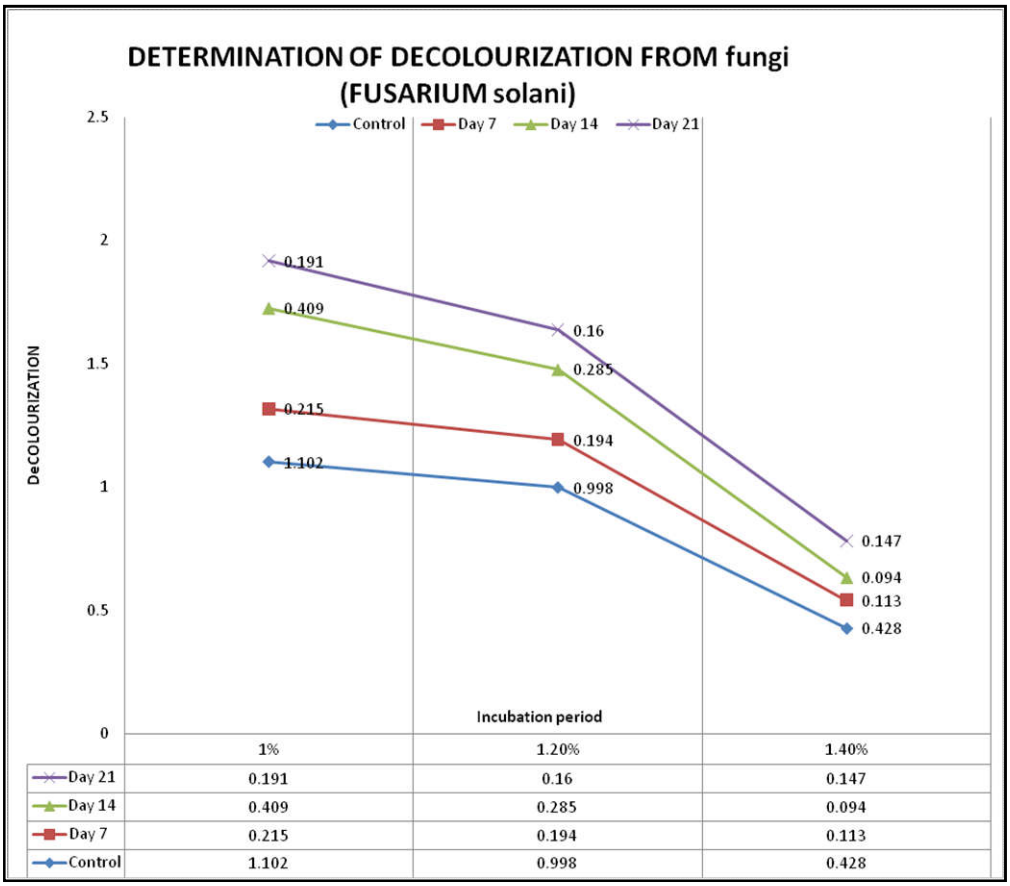
	Control	1%	1.20%	1.40%
Day 21	0.438	0.092	0.119	0.145
Day 14	0.902	0.114	0.132	0.16
Day7	1.008	0.125	0.143	0.156

Graph 5. Bacteria (*Bacillus subtilis*) absorbance at 475nm (Day 7-21)



	Control	1%	1.20%	1.40%
Day 7	48000	26400	24000	16000
Day 14	28000	12000	14000	13200
Day 21	24800	7200	12000	8000

Graph 6. Determination of chemical oxygen demand Fungi (*Fusarium solani*day 7-21)



	1%	1.20%	1.40%
Day 21	0.191	0.16	0.147
Day 14	0.409	0.285	0.094
Day 7	0.215	0.194	0.113
Control	1.102	0.998	0.428

Graph 7. Fungi (*Fusariumsolani*) absorbance at 475nm (Day 7-21)

Characteristics of experimental soil

Soil samples were analysed in the laboratory by following standard procedures (Page *et al.,* 1982)

Characteristics of experimental soil

Soil samples were analysed in the laboratory by following standard procedures (Page *et al.,* 1982).

Table 6: Characteristics of experimental soil

Parameters	Values
pH (1:2 soln)	7.8
Electric Conductivity	530 µS
Organic Carbon	1.44%
Available Nitrogen	397 mg/L
Available Phosphorous	202 mg/L
Available Potassium	108/L

Source: (own draft)

Effect on treated spent wash application on soil pH and electric conductivity Soil pH

Table 7. Effect on treated spent wash application on soil pH

Spent Wash Concentrations	Initial	After Harvest
10%	7.15-8.06	7.01-7.70
20%	7.45-8.25	8.02-8.12
30%	8.37-8.67	8.24-8.57
40%	7.13-8.26	7.10-8.04

Source: (own draft)

Soil Electric Conductivity

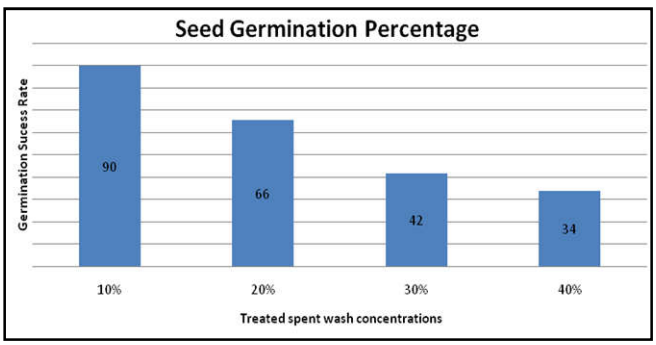
Table 8. Effect on treated spent wash application on soil electric conductivity

Spent Wash Concentrations	Initial	After Harvest
10%	0.05-0.31	0.12-1.22
20%	0.09-0.25	0.18-0.51
30%	0.18-1.12	0.26-1.15
40%	0.06-0.28	0.15-0.65

Source: (own draft)

Seed Germination Percentage

50 Wheat seeds were sowed in five pots containing different treated spent wash concentrations (10-40%)



Graph 8. Seed germination success rate in percentage

DISCUSSION

Disposal of waste could pose a serious health hazard if not handled and treated appropriately. Distillery spent wash is perceived as one of the most caramelized and recalcitrant waste with organic content and low pH. Pazoki, 2008 stated that "The main problem in treating distilleries spent wash is its colour which contains about 2% of the dark brown recalcitrant pigment known as melanoidin". Melanoidin compound is recalcitrant pigment responsible for dark brownish colour in the spent wash it reduces the photosynthetic activity and utilizes the dissolved oxygen subsequently resulting in eutrophication. The purpose of collection of indigenous bacterial cultures is to screen its ability to degrade harmful pollutants present in spent wash and to understand its degradation potential. It is necessary to add external carbon source for the growth of the microorganisms along with maintaining the factors affecting its growth such as temperature, pH, moisture content and redox potential. This approach is proved to be cost effective, eco-friendly for the remediation of waste water. Physico-chemical methods for treatment of spent wash are not sufficient for decolorisation. [5]Biological methods are necessary to reduce the biochemical and chemical oxygen demand of the spent wash but regardless of the treatment substantial amount of organic pollutants are still left untreated, which requires secondary treatment.[6] Microbial treatment effluent may be less toxic and safe device for effluent management.[7] In the present research work use of indigenous bacterial and fungal strain for decolourisation and COD reduction of the spent wash was performed. Spent wash sample collected from distillery unit of Madhukar Co-operative Sugar Factory at Faizpur, Dis. Jalgon (MMSK) the key physicochemical parameters were analysed. Soil sample was collected from the disposal site of the distillery unit of MMSK with the intention of isolating microorganisms having a melanoidin degrading ability. The least oxygen demand required to oxidise organic matter at 1.2% glucose/peptone concentration for *Bacillus subtilis*. As far as decolourization is concerned 1%-1.2% showed maximum reduction (graph number: 1 and 2). Effective chemical oxygen demand reduction was observed in 1% glucose/peptone concentration and decolourization is1.2% glucose/peptone concentration for *Fusariumsolani*. From the following results 1.2% glucose/peptone concentrations was considered to be the optimized source for carbon and nitrogen for the organisms. It is difficult to treat melanoidins via biological process because melanoidin consist of antioxidant properties that proves to be toxic to aquaticmacro and certain microorganisms. Biological decolourization using indigenous organisms such as Enterobacter sp, Corynebacterium sp, Micrococcus sp and Corynebacterium sp have reported to have total dissolved solid and chemical oxygen demand reduction in sugar wash. Highest COD reduction and TDS reduction was observed when a consortia of all the three organisms were undertaken which is about 32% TDS reduction and 35% COD reduction. [8] The following study then consists of consortia of both Fungus (*Fusarium solani*) and Bacteria (*Bacillus subtilis*). Growth was transferred in broth with optimized 1.2% of glucose and peptone containing different molasses spent wash concentrations between 10%-40%. Highest decolorization and chemical oxygen demand reduction was observed in 10% treated spent wash concentration. Untreated molasses spent wash results in inhibition of seed germination and vegetation growth. It reduces the soil alkalinity and manganese availability. The State Pollution Control Board permits the distilleries for controlled land application of treated spent wash having pH of 7.5-8.5 to fallow lands during non-rainy season (November to May). Effect of treated distillery spent wash on soil properties and crop yields were studied. At the initial stage the soils were neutral to alkaline in reaction, with initial pH values ranging from 7.13 to 8.67.The treated spent wash ranging from 10-40% decreased the soil pH slightly for at harvest it ranged from 7.01 to 8.57. Since the pH of the treated spent wash was also in the same range there was no adverse effect on the soil reaction. Highest germination success was observed in 10% treated spent wash concentration. Conductivity in liquid signifies the total dissolved solids, it also signifies the effect of diverse ions on physiology of plants and animals as well as corrosion rates. Soil solution salt concentration as measured in terms of electrical conductivity (EC) increased by two to three fold due to spent wash application. The high content of dissolved salts in the spent wash contributed to the addition of salts in soil. However, the soil remained non saline since electric conductivity < 2.0 dS/m, indicating that the quantity of spent wash applied to the soils was appropriate with no adverse effects on soil.

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