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BIOREMEDIATION AND DECOLOURIZATION OF DISTILLERY SPENT WASH BY *LACTOBACILLUS PLANTARUM* ABS0S11

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ABSTRACT

Most of the Indian distilleries utilize molasses as raw material for the production of alcohol. Alcohol production from molasses generates large volumes of high strength unwanted residual liquid called spent wash. The distillery spent wash pose a major threat to soil and aquatic ecosystem due to the presence of water soluble recalcitrant colouring compound called melanoidin. Therefore, adequate treatment is necessary before the discharge of distillery spent wash into the environment. The present study was undertaken for investigation of decolourization potential of *Lactobacillus plantarum* ABS0S11 isolated from soil. Different environmental and nutritional parameters were optimized for decolourization and degradation of distillery spent wash. *Lactobacillus plantarum* ABS0S11 showed maximum decolourization (41.3 %) in presence of glucose (1%, w/v) and peptone (0.3%, w/v) at pH 6.0 and temperature 35^0C under static condition. The ability of the bacterial strain to decolorize and degrade the spent wash was confirmed by High Performance Liquid Chromatography analysis. Further, phytotoxicity assay of distillery spent wash with *Phaseolus mungo* L. showed a reduction of toxicity in bacterial treated spent wash sample.

Keywords: *Lactobacillus plantarum*, decolourization, spent wash, static condition

INTRODUCTION

The distilleries are one of the major polluting industries in all over the world. Most of the Indian distilleries use molasses as a raw material for alcohol production. Distilleries generate large volumes of effluent waste water called spent wash. Spent wash is strongly acidic, dark brown coloured liquid with high BOD and COD. The dark brown colour of distillery spent wash is mainly due to the presence of high molecular weight recalcitrant organic compounds called melanoidin, which is formed by the "Maillard reaction" between amino and carbonyl groups of organic matters **[1]**.

The distillery spent wash is considered as the major source of soil and aquatic pollution creating a serious threat to the nearby aquatic and terrestrial habitats. The dark brown colour of spent wash adversely affects sun light penetration in water bodies which reduces photosynthetic activity and dissolved oxygen concentration causing harm to aquatic life. Its disposal on land reduces alkalinity of soil and inhibits seed germination **[2-5]**. It may also adversely affect the biogeochemical cycle of many constituents in natural water **[3]**. Hence, adequate treatment is necessary before disposal of distillery effluent into the environment **[4, 6]**.

Several physicochemical methods have been investigated for treatment of distillery spent wash but they are not eco-friendly and have lots of disadvantages **[6, 7]**. In recent years, more interest has been developed towards utilizing microbial activity for decolorization of spent wash.

Various authors have reported decolourization of distillery spent wash by using many fungal strains and some bacterial strains **[4, 6, 8, 9]**. Bacteria can adapt to different environmental conditions and show versatility in their biochemical requirements. Therefore, they have more potential for degradation of melanoidin compounds **[5, 10, 11]**. Looking towards this, the present study was undertaken to investigate decolourization potential of a bacterial strain *Lactobacillus plantarum* ABS0S11 isolated from soil. Different environmental and nutritional parameters were also optimized for maximizing decolourization and degradation of distillery spent wash. The ability of the bacterial isolate to decolorize and degrade the spent wash was confirmed by High Performance Liquid Chromatography analysis.

MATERIAL AND METHODS

Collection of distillery spent wash

The distillery spent wash was collected aseptically from the spent wash collection tank of Distillation Unit of Vishwasrao Naik Co-operative Sugar Factory, Chikhali, Dist- Sangli, Maharashtra, India. The collected spent wash sample was then transported to laboratory and stored at 4^0C for further study.

Medium to study decolourization

The spent wash medium containing (1.0%, w/v), glucose; (0.5% w/v), peptone; (0.2% w/v), K_2HPO_4; (0.1% w/v), KH_2PO_4; (0.05% w/v), $MgSO_4.7H_2O$; and (10 % v/v); distillery spent wash was used for decolourization study. The pH of the medium was adjusted to 6.0 using 0.1 N NaOH or 0.1 N HCl.

Inoculum preparation

A bacterial strain *Lactobacillus plantarum* ABS0S11 isolated from soil contaminated with distillery spent wash was used in this study. For preparation of the cell suspension, a loopful of 24 hour grown culture was inoculate into 50 ml spent wash broth and incubated at 35^0 for 24 hours in static condition. The suitable amount (0.5 %, v/v) of cell suspension was used for inoculation.

Decolourization assay of the spent wash:

The decolourization assay was performed in triplicate by using spent wash medium. The bacterial strain was inoculated in the medium and incubated at 35^0C. After incubation, culture broth was centrifuged at 10,000 rpm for 10 min. The supernatant was taken and absorbance was measured at 475 nm using spectrophotometer. Uninoculated medium was used as control. The decolorization yield was expressed as the decrease in the absorbance at 475 nm against initial absorbance at the same wavelength **[4, 6, 12, 13]**.

Optimization of various parameters

Various parameters were optimized for maximizing decolourization. The effect of pH on decolourization of distillery spent wash was studied by varying the initial pH of medium from 5.0 to 9.0. Optimum temperature required for the decolourization was determined by varying the temperature from 30°C to 45°C.Time course of melanoidin decolorization was also studied. The effect of different carbon sources viz. glucose, sucrose, fructose, maltose, lactose and starch at 1 % (w/v) on distillery spent wash decolourization was evaluated. Optimum concentration of the best carbon source required for maximum decolorization was determined by taking the concentration of best carbon source in the range of 0.25 to 1.5 % (w/v). To study the effect of different organic and inorganic nitrogen sources viz. peptone, yeast extract, beef extract, ammonium chloride, ammonium sulphate and sodium nitrate were individually added into the medium at

0.5% (w/v). Optimum concentration of the nitrogen source required for maximum decolorization was determined by taking the concentration of best nitrogen source in the range of 0.1 to 0.6 % (w/v). Different concentrations of spent wash viz. 10, 15, 20, 25 and 30 % (v/v) supplemented in medium were inoculated with bacterial isolate and the decolourization yields were computed.

HPLC analysis of treated spent wash

HPLC analysis was carried out at Hitech lab and Consultancy, Sangli, India. Decolourization of spent wash was monitored by HPLC (Thermo Finnigan Surveyor HPLC System). 10 ml of samples were taken, and centrifuged, filtered through 0.45 µm membrane filter (Millipore). Filtered sample was analyzed using mobile phase consisting of acetonitrile and methanol (45:55) (HPLC grade) with 1ml glacial acid and 0.5 ml sodium acetate. The sample was eluted using C-18; reverse phase column of 5 µm SGE, 250 x 4.6mm SS. Resultant peak was analyzed with UV-detector 475 nm. The flow rate of the mobile phase was 1 ml/min **[7, 14, 15, 16]**.

Phytotoxicity assessment by seed germination test

The toxicity of untreated and bacterial treated distillery spent wash was evaluated with *Phaseolus mungo* L. seed germination using the petri dish method as described previously **[17-20]**. For the seed germination test, distillery spent wash was applied at different concentrations viz. 2, 4, 6, 8 and 10 % (v/v). The seeds were treated with 0.1% mercury chloride ($HgCl_2$) solution for 2 min before conducting the experiment to avoid any microbial contamination. Then, seeds were repeatedly washed with sterilized distilled water to remove the residual $HgCl_2$. Subsequently, ten seeds of *P. mungo* L. were placed in sterilized glass petri dishes lined with two Whatman no. 1 filter paper discs. These filter discs were then moistened with 10 ml of tap water for control and with the same volume of treated and untreated spent wash samples separately after which they are incubated at room temperature for a period of 3 consecutive days. Seed germination percentage was calculated by using methods described earlier **[19-21]**.

RESULTS AND DISCUSSION

Bacterial culture

The bacterial strain *Lactobacillus plantarum* ABS0S11 used in this study has ability to decolourize distillery spent wash.

Optimization of various parameters

The effect of pH on decolourization in the range of (5.0-9.0) showed the highest decolourization activity at pH 6.0. Any deviation from optimum pH reduced decolourization **(Figure 1)**.

Similar results were reported by several workers **[16, 22, 23]**. The decolorization activity might get reduced above and below of optimum pH due to inhibition of the enzyme production **[7]**.

The effect of temperature on decolourization in the range of 30^0C - 45^0C showed that the optimal temperature was 35^0C **(Figure 2)**. Further increase of temperature up to 45^0C showed reduction in decolourization ability of bacteria.

Several workers observed that the higher decolorization was obtained at a temperature range of 25-40°C **[16, 24]**. It was reported that decolorization ability of bacterial consortium is adversely affected by increasing temperature above 40^0 C **[6]**.

The effect of incubation period on decolourization showed that maximum decolourization was achieved in a 5 days incubation period. Further increase in incubation period did not increase decolourization **(Figure 3)**.

In previous studies, it was reported that 60% decolourization by *Issatchenkia orientalis* after 7 days of incubation **[25]**. It was also observed that *Citeromyces* sp. WR- 43-6 required 7 days of incubation for maximum decolourization **[11]**.

The effects of different carbon sources on decolourization activity showed that the high level of decolourization yield was obtained when glucose was used as a carbon source. Lowest decolourization yield was obtained when starch was used as a carbon source **(Figure 4)**.

There is presence of huge amount of sugar in spent wash but easily metabolizable carbon content of spent wash is almost negligible **[8]**. The addition of easily available carbon sources in medium increased decolourization efficiency. During initial growth phase, the organism utilizes readily available carbon sources present in the medium then it starts to degrade spent wash components for carbon source **[8]**.

The effect of different concentrations of glucose on decolourization showed that the optimum concentration of glucose for decolourization was 1 %. Further increase in glucose concentration did not increase decolourization **(Figure 5)**. There may be formation of excess gluconic acid in presence of high glucose concentration which may also inhibit bacterial growth **[26]**.

Similar observations have been reported by several workers **[10, 26-28]**. Lignolytic activity of laccase enzyme and oxidation activity of the peroxidase enzyme might be inhibited at high sugar concentration which may result in decolourrization reduction **[16, 24, 29-32]**.

The effect of nitrogen sources on decolourization activity showed that the highest decolourization yield was obtained using peptone **(Figure 6)**.

Several workers also reported that peptone was the most effective for colour removal **[11, 13, 16, 28, 33]**.

The effect of different concentrations of peptone on decolourization showed that the optimum concentration of peptone for decolourization was 0.3 %. Further increase in peptone concentration did not increase decolourization **(Figure 7)**. Surplus addition of nitrogen source may inhibit bacterial growth as well as decolourization process **[7, 26]**.

The effect of various concentrations of distillery spent wash in the range of 10% - 30% (v/v) showed the decolourization process was greatly influenced by concentration of distillery spent wash. Maximum decolourization was obtained at 10 % spent wash concentration. Decolourization yield decreased with increase in the concentration of distillery effluent **(Figure 8)**. Similar observations were also been reported in previous studies **[34]**.

HPLC analysis

The HPLC analysis of bacterial treated samples of spent wash has shown reduction in peak areas compared to control **(Figure 9)**. It indicates that decrease in colour intensity might be due to the ability of bacterial isolate to degrade colour containing components of the spent wash. The similar findings were reported by earlier workers **[12, 26, 35, 36]**.

Phytotoxicity assessment by seed germination test

The inhibitory effect on seed germination was not observed up to 2% (v/v) concentration of untreated spent wash. Further increase in spent wash concentration decreased percentage seed germination. The inhibitory effect might be due to presence of toxic organic compounds in spent wash **[19, 37]**. The higher percentage of seed germination was observed in bacterial treated spent wash samples **(Figure 10)**.

This might be due to degradation of organic compounds which caused adverse effect on seed germination **[19, 20, 36, 37]**.

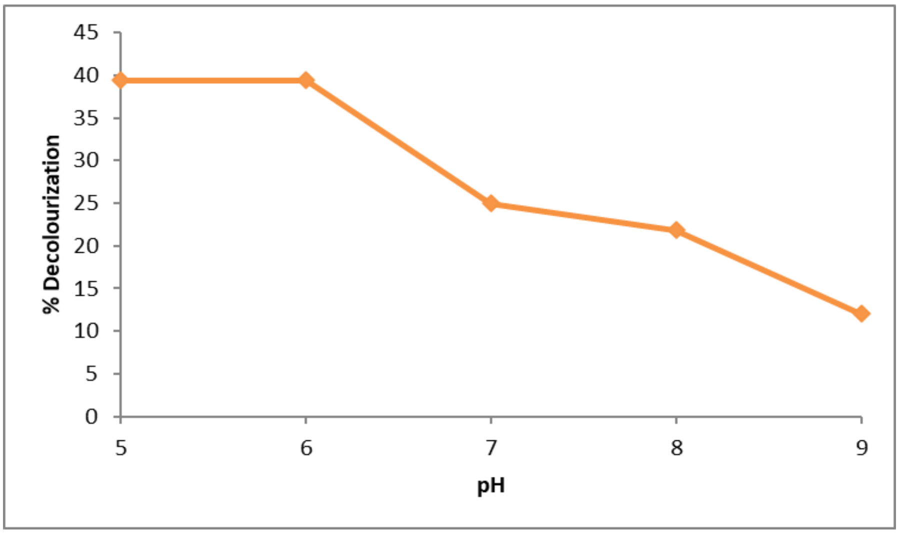

Figure 1: Effect of different pH on decolourization

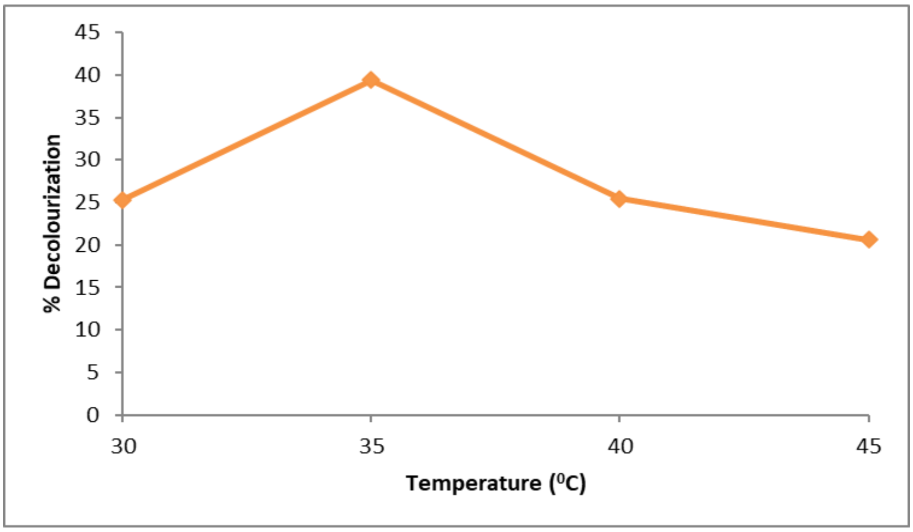

Figure 2: Effect of different temperatures on decolourization

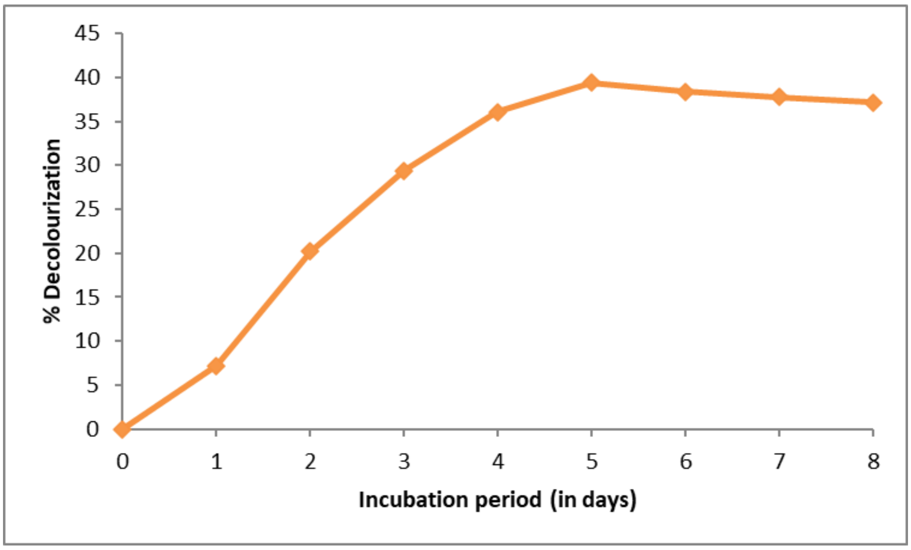

Figure 3: Effect of different incubation periods on decolourization

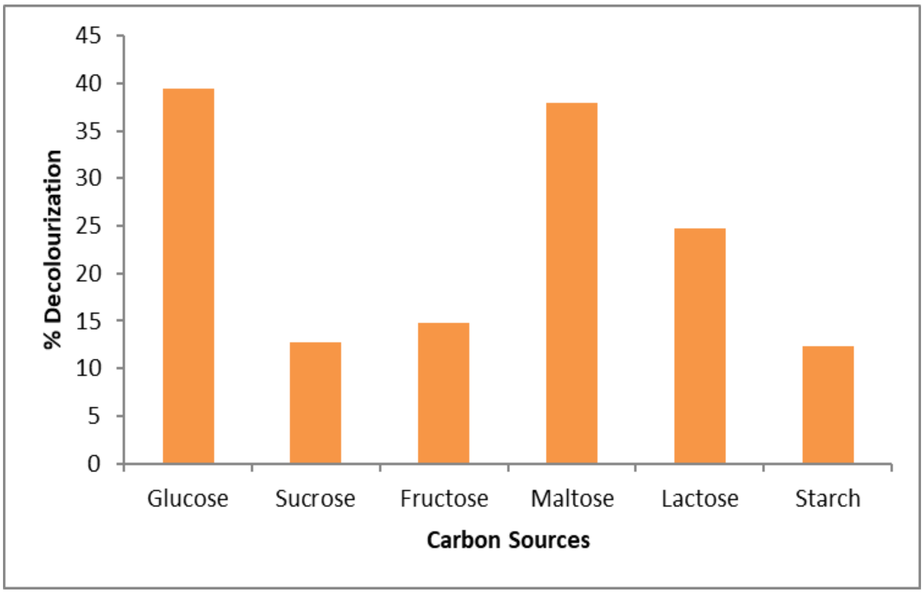

Figure 4: Effect of different carbon sources on decolourization

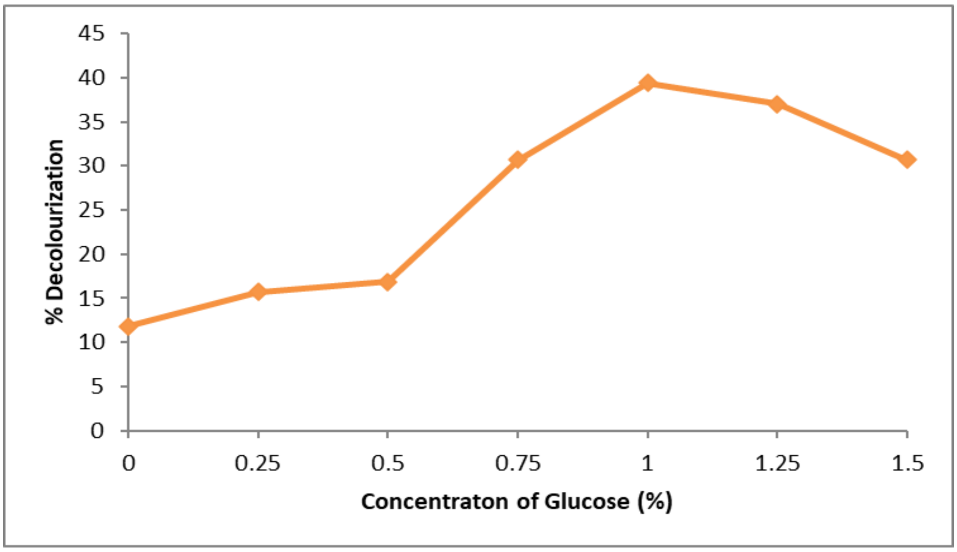

Figure 5: Effect of different concentrations of glucose on decolourization

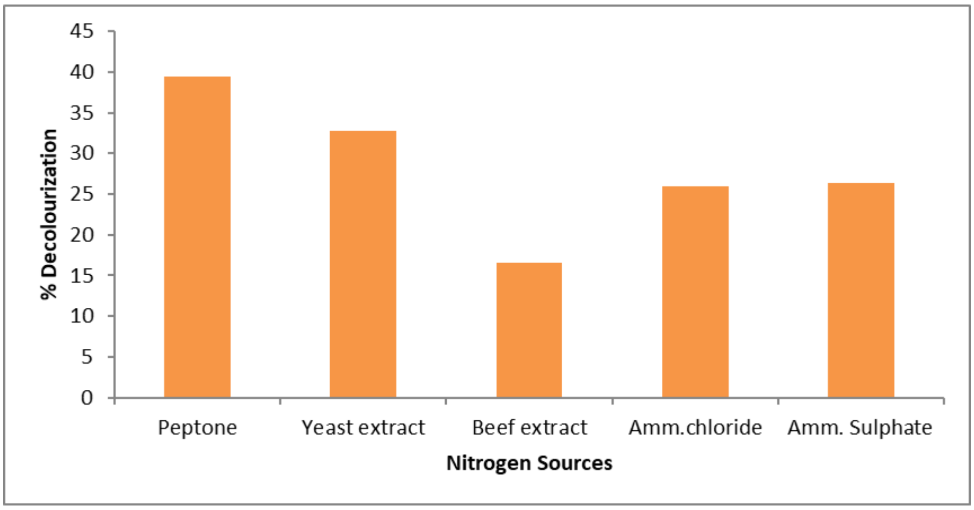

Figure 6: Effect of different nitrogen sources on decolourization

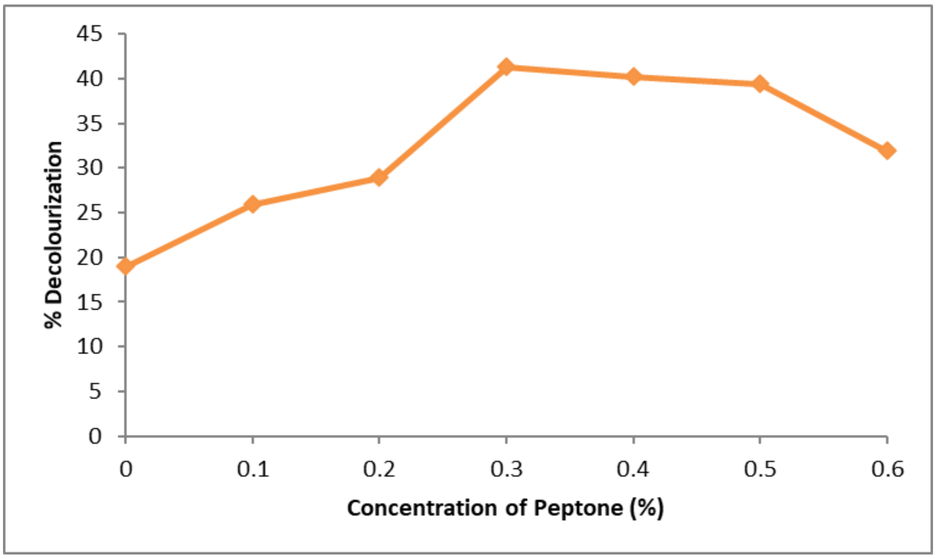

Figure 7: Effect of different concentrations of peptone on decolourization

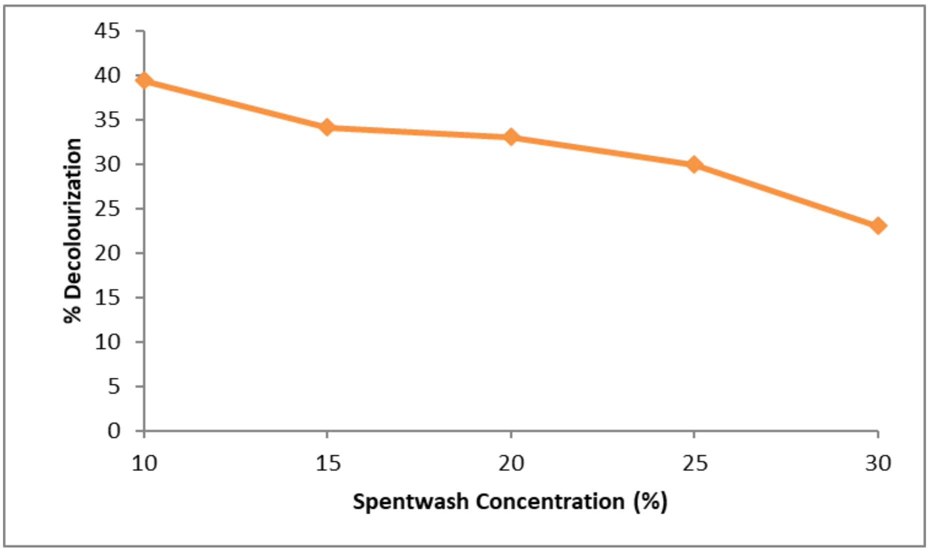

Figure 8: Effect of different concentrations of spent wash on decolourization

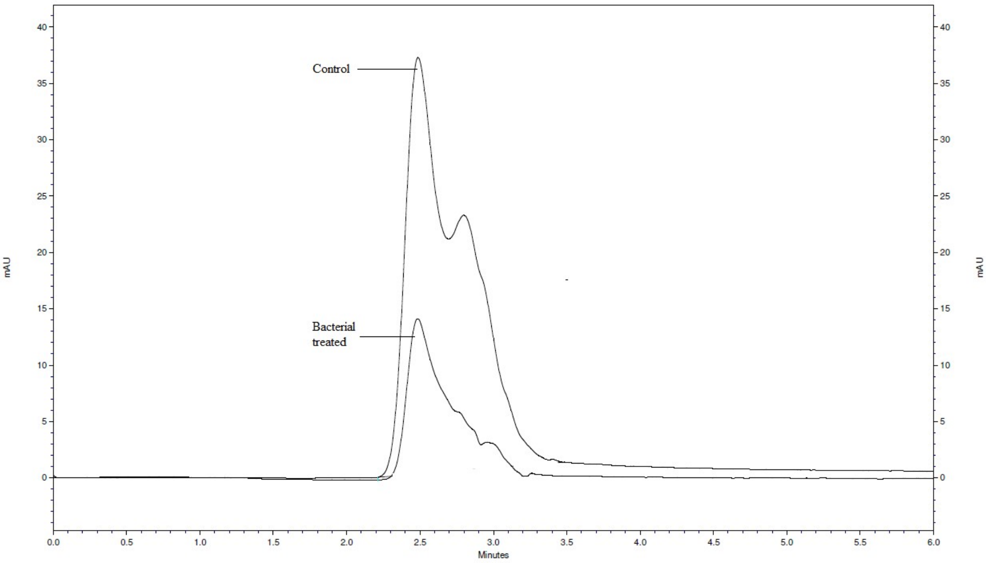

Figure 9: Comparative HPLC chromatogram of spent wash before and after bacterial decolourization

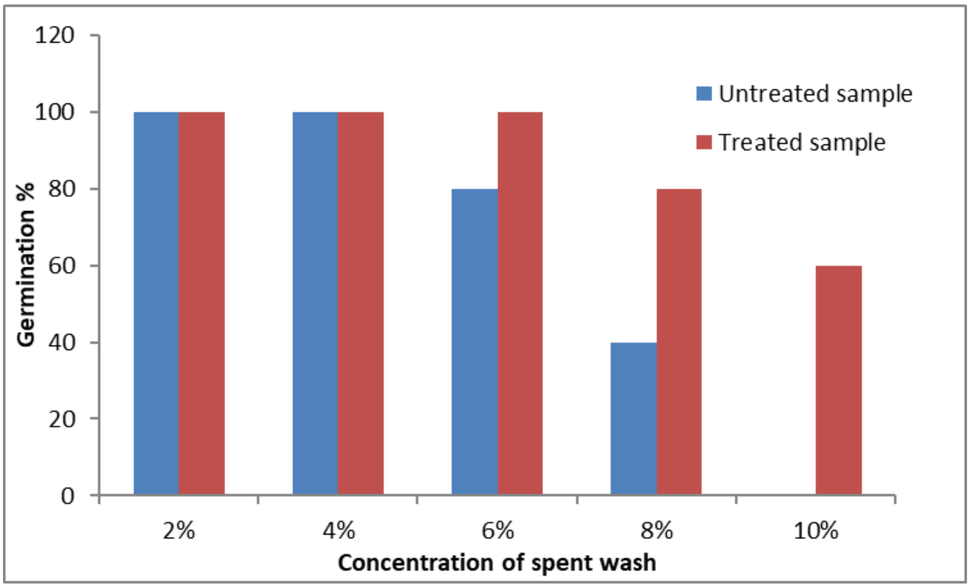

Figure 10: Effects of different concentrations of spent wash on germination of *Phaseolus mungo* L.

CONCLUSION

The bacterial strain *Lactobacillus plantarum* ABS0S11 has ability to decolourize distillery spent wash. It showed maximum decolourization (41.3 %) in presence of glucose (1%, w/v) and peptone (0.3%, w/v) at pH 6.0 and temperature 35^0C under static condition. The HPLC analysis of bacterial treated sample has shown smaller peaks in compared to control, confirming biodegradation of colour containing compounds of spent wash by the bacterial isolate. Seed germination test confirmed toxicity reduction of distillery spent wash after bacterial treatment.

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