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MICROBIAL DECOLOURIZATION OF DISTILLERY SPENT WASH BY *PSEUDOMONAS STUTZERI* ABS009

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ABSTRACT

Cane molasses-based distilleries are major contributors to water pollution due to their discharge of dark brown coloured waste water having high values of chemical oxygen demand (COD) and biological oxygen demand (BOD). Improper disposal contaminates aquatic and terrestrial ecosystems, necessitating effective pretreatment methods. In this study, a bacterial strain *Pseudomonas stutzeri* ABS009 was used to decolourize distillery spent wash. The decolourization process was evaluated under varying environmental conditions and with different nutritional parameters. Maximum decolourization (38.90%) was achieved at 35°C after 6 days of incubation under static conditions with 1.25 % glucose (w/v) and 0.3% peptone (w/v) at pH 6.0. A seed germination test using *Phaseolus mungo* L. showed a notable decrease in the toxicity of the spent wash that had been treated with bacterial strain. These findings contribute to the development of an eco-friendly, cost-effective, and socially acceptable method for treating distillery spent wash.

INTRODUCTION

Distilleries are a significant source of global pollution, particularly in India, where most use molasses to produce alcohol. This process generates large quantities of wastewater known as spent wash, which is acidic, and contains high levels of chemical oxygen demand (COD) and biological oxygen demand (BOD). The dark brown colour of spent wash, caused by melanoidin compounds formed through the Maillard reaction, poses serious environmental threats, especially to aquatic ecosystems, by reducing sunlight penetration and lowering dissolved oxygen levels. When disposed of on land, it harms soil quality, affecting seed germination and soil alkalinity (Mohana et al., 2007; Chandra et al., 2018). Therefore, spent wash must be properly treated before being disposed away.

Traditional methods for treating spent wash, such as physicochemical approaches, are costly, inefficient, and environmentally unfriendly (Pant & Adholeya, 2007). The biological methods have also proven less effective because of the toxicity of melanoidins (Chandra et al., 2008). However, recent attention has focused on microbial decolourization of spent wash, with bacterial strains showing promise due to their adaptability and biochemical capabilities (Pant & Adholeya, 2007). Studies have reported the success of certain bacteria in degrading melanoidins and reducing the harmful effects of spent wash (Chandra et al., 2018, Shinde, 2022). Employing bacteria is seen as a more sustainable and effective approach for spent wash treatment, and efforts continue to optimize environmental and nutritional conditions to maximize its decolourization and degradation potential. This study investigates the decolourization potential of a bacterial strain, *Pseudomonas stutzeri* ABS009, under optimized environmental and nutritional parameters.

MATERIALS AND METHODS

Collection of distillery spent wash

The spent wash was aseptically collected from the spent wash tank of the distillery associated with Vishwasrao Naik Co-operative Sugar Factory, Chikhali, Dist- Sangli, Maharashtra, India. The collected samples were brought to the laboratory and kept at 4˚C till subsequent analysis.

Medium used for decolourization studies

The medium consisted of distillery spent wash (10%, v/v), glucose (1.0%), peptone (0.5%), K_2HPO_4 (0.2%), KH_2PO_4 (0.1 %,), and $MgSO_4 \cdot 7H_2O$ (0.05%). The medium's pH was adjusted to 6.0 (Shinde, 2022).

Preparation of inoculum

In this study, a bacterial strain, *Pseudomonas stutzeri* ABS009, obtained from contaminated soil, was used (Shinde & Nakade, 2021a). A loopful of 24-hour-old culture of the bacterial strain was inoculated in the spent wash medium and incubated at 35˚C under static conditions for 24 hours. The resulting cell suspension (0.5%) was used as inoculum.

Decolourization assay

The decolourization experiment was conducted in triplicate. The bacterial strain was grown in spent wash broth at 35˚C, followed by centrifugation at 10,000 rpm for 10 minutes. Absorbance of the supernatant was recorded at 475 nm, with an uninoculated medium as the control. By comparing the initial and final

absorbance at 475 nm, the decolourization yield was estimated (Bhargava et al., 2009; Shinde & Nakade, 2021a).

Optimization of environmental and nutritional parameters

The effects of various physical factors on decolourization were assessed. The medium's pH was modified within the range of 5.0 to 9.0, and the decolourization yield was recorded. To evaluate the influence of temperature, inoculated media were incubated at 30°C to 45°C. The effect of the incubation period was determined by sampling at 24-hour intervals over 8 days (Shinde and Nakade, 2021b; 2021c).

The impact of various carbon sources including glucose, sucrose, fructose, maltose, lactose, and starch) was evaluated, and the optimal concentration of the best carbon source (0.25–1.5%) was determined. Similarly, diverse nitrogen sources such as peptone, yeast extract, beef extract, ammonium chloride, ammonium sulfate, and sodium nitrate) were tested at 0.5%, and the optimal concentration of the best nitrogen source (0.1–0.6%) was established. The influence of spent wash concentrations (10–30%, v/v) on decolourization was also assessed (Shinde and Nakade, 2021b; 2021c).

A set of optimized physical and nutritional parameters was selected, and the decolourization potential of the bacterial strain was studied.

Evaluation of phytotoxicity by using seed germination test

The toxicity of bacterially treated and untreated spent wash was assessed using a seed germination test with *Phaseolus mungo* L. seeds. After being surface sterilized and thoroughly rinsed with sterile distilled water, the seeds were placed in sterilized Petri plates containing Whatman No. 1 filter paper. Each plate contained 10 seeds that had been moistened with tap water (control) or untreated or treated distillery spent wash. Seed germination percentages were recorded after three days incubation at room temperature (Shinde and Nakade, 2021b; 2021c).

RESULTS AND DISCUSSION

The bacterial strain *Pseudomonas stutzeri* ABS009 demonstrated the capability of spent wash decolourization.

Optimization of environmental and nutritional parameters for enhancing decolourization

To maximize the efficiency of spent wash decolourization, various environmental and nutritional parameters were systematically optimized.

Effect of pH

Maximum decolourization efficiency was observed at pH 6.0, while deviations from this optimal pH resulted in reduced activity (Fig. 1). These findings align with previous studies, which reported that suboptimal pH levels inhibit enzyme production critical for decolourization (Nikam et al., 2014; Sridevi and Mullai, 2014).

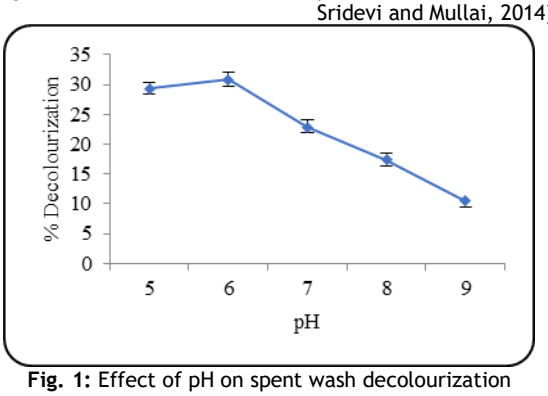

Fig. 1: Effect of pH on spent wash decolourization

Effect of temperature

The bacterial isolate exhibited peak decolourization at 35°C, with activity declining at temperatures exceeding this optimal point (Fig. 2). Previous studies corroborate these findings, suggesting optimal decolourization occurs within the temperature range of 30°C to 37°C (Nikam et al., 2014; Chandra et al., 2018).

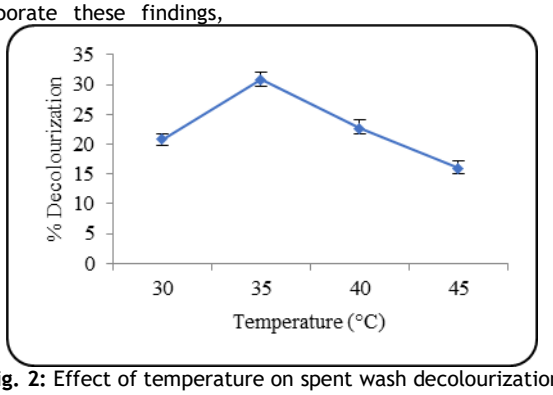

Fig. 2: Effect of temperature on spent wash decolourization

Effect of incubation period

The decolourization efficiency increased with the extension of the incubation period, reaching a maximum at 144 hours. Beyond this period, no significant improvement in decolourization yield was observed (Fig. 3). Studies by Bharagava and Chandra (2010) demonstrated similar trends, where 70% degradation of colourants was achieved after 144 hours of incubation. Comparable results were obtained by Ruhi et al. (2017) and Singh and Singh (2020), with bacterial consortia achieving peak efficiency within 96 to 168 hours.

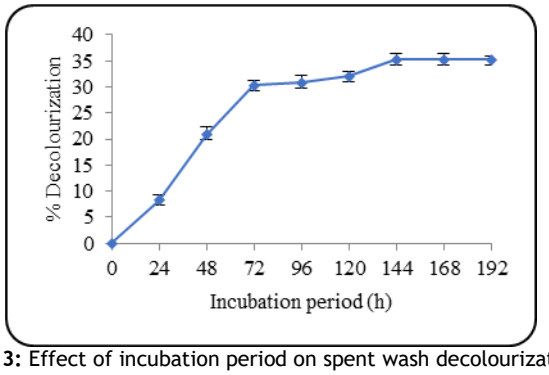

Fig. 3: Effect of incubation period on spent wash decolourization

Effect of carbon sources

Decolourization was enhanced by supplementing the medium with easily metabolizable carbon sources. Among various sources tested, glucose yielded the highest decolourization (Fig. 4). Although the spent wash inherently contains sugars, their limited bioavailability necessitates supplementation with readily metabolizable carbon. During the initial growth phase, the bacteria utilize these additional carbon sources before transitioning to metabolize spent wash components (Kumar et al., 1997, Shinde, 2022).

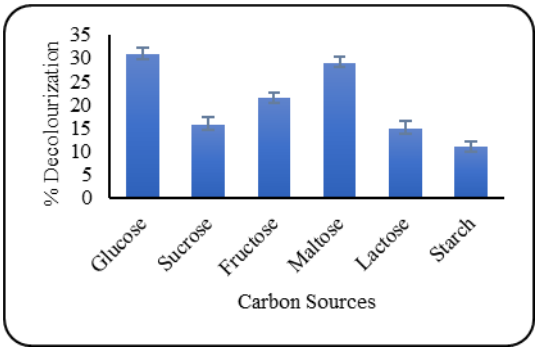

Fig. 4: Effect of different carbon sources on spent wash decolourization

Effect of glucose concentration

The optimal glucose concentration for maximum decolourization was determined to be 1.25%. Higher concentrations did not significantly enhance the decolourization yield (Fig. 5), consistent with findings reported by Mohana et al. (2007), and Chandra et al. (2018).

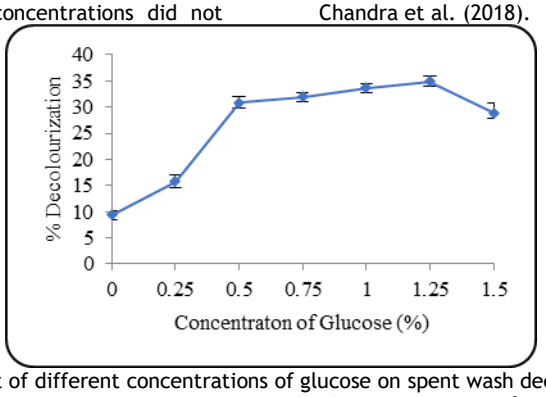

Fig. 5: Effect of different concentrations of glucose on spent wash decolourization

Effect of nitrogen sources

Among the nitrogen sources evaluated, peptone was the most effective for maximizing decolourization (Fig. 6). Previous research has similarly identified peptone as the preferred nitrogen source for enhancing decolourization efficiency (Bharagava and Chandra, 2010; Chandra et al., 2018).

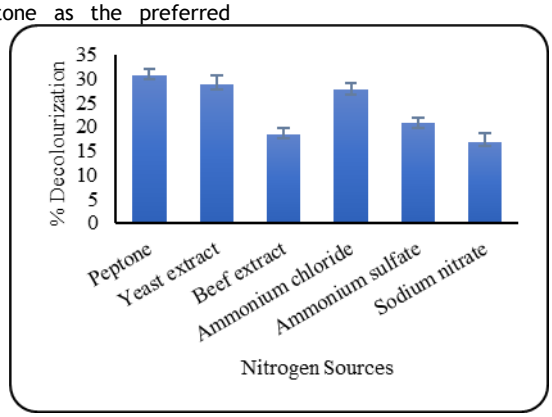

Fig. 6: Effect of different nitrogen sources on spent wash decolourization

Effect of peptone concentration

The optimal peptone concentration was determined to be 0.3%, beyond which no significant increase in decolourization yield was observed (Fig. 7). Excess nitrogen in the medium may inhibit bacterial activity and limit decolourization efficiency, as previously reported by Ravikumar et al. (2011).

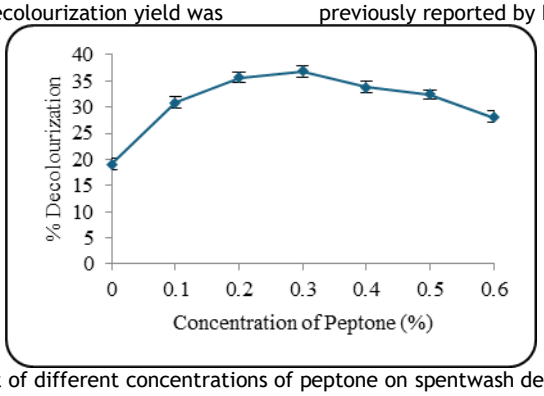

Fig. 7: Effect of different concentrations of peptone on spentwash decolourization

Effect of spent wash concentration

Decolourization efficiency was influenced by the spent wash concentration, with maximum yield observed at 10% (v/v). Higher concentrations adversely impacted the efficiency (Fig. 8), likely due to increased toxicity. These results are in agreement with earlier studies (Rani and Saharan, 2009).

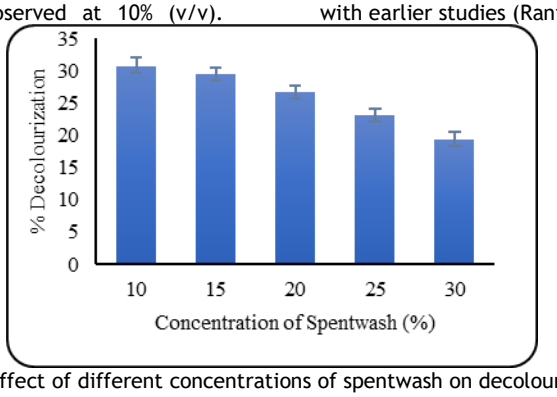

Fig. 8: Effect of different concentrations of spentwash on decolourization

Evaluation of phytotoxicity by using seed germination test

The existence of hazardous chemicals in the untreated sample dramatically reduced seed germination (Chandra and Kumar, 2017). In contrast, bacterial treatment of the spent wash improved seed germination to 80%, attributed to the breakdown of organic toxins (Yadav and Chandra, 2013; Chandra and Kumar, 2017).

CONCLUSION

Pseudomonas stutzeri ABS009 achieved 38.90% decolourization after 6 days of incubation at 35°C under static conditions in a medium with 1.25% (w/v) glucose and 0.3% (w/v) peptone at pH 6.0. Furthermore, the reduction in toxicity was validated by enhanced seed germination in the treated samples. These findings underscore the potential of the bacterial strain for efficient decolourization of distillery spent wash while mitigating its environmental and phytotoxic impacts.

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