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# Application of *Citeromyces siamensis* for Molasses Wastewater Decolourization

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***Abstract*** **– Molasses, a brownish black viscous liquid, is a byproduct of the cane sugar process. It finds applications in various industries such as fertilizer production, animal husbandry, alcohol production, monosodium glutamate production, and the production of acetic acid. In Thailand, it is primarily used for alcohol production and as animal feed. However, molasses wastewater is characterized by high levels of biochemical and chemical oxygen demand, as well as suspended solids. Untreated release of this wastewater into the environment can lead to numerous environmental issues. Additionally, the presence of melanoidins in molasses wastewater makes its removal challenging, resulting in a dark colour of the treated water. Discharge of this dark-coloured water into natural water sources can lead to an abnormal discolouration of the water. This study aims to investigate the optimal conditions for utilizing *Citeromyces siamensis* microorganisms in the biological treatment of molasses wastewater. The experiment was conducted to determine the conditions that facilitate the reduction of colour in molasses wastewater. The findings indicate that the addition of 1 % glucose and 1 % peptone was more effective in decolourizing molasses wastewater using *Citeromyces siamensis* compared to other nutrient additions. While the addition of additives may incur additional costs, it remains a more economical option compared to the use of chemicals for wastewater colour removal. Furthermore, the use of the additives is considered safe for living organisms.**

***Keywords – Citeromyces siamensis*; decolourization; molasses; wastewater.**

## 1. INTRODUCTION

Molasses is one of the raw materials used in alcohol production. However, the wastewater generated during the alcohol production process using molasses contains various substances that contribute to environmental pollution, including its characteristic dark brown to black colour. This dark colour in the wastewater is primarily attributed to the presence of melanoidin, a complex compound formed when sugars and amino acids react at high temperatures through a browning or Maillard reaction. The presence of melanoidin makes the wastewater even more contaminated and darker when released into water sources. The high coloured components in rivers, lakes, and lagoons can reduce sunlight penetration. The reduction of sunlight will be effect on photosynthetic activity and the levels of dissolved oxygen in water. These have negative impacts to aquatic life. Addressing this issue requires employing different methods, one of which involves the use of microorganisms to remove the colour. Nonetheless, treating the wastewater proves challenging due to the inherent difficulty in biodegrading melanoidin.

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Microorganisms that have been studied include bacteria, fungi and yeast. Miranda *et al.* studied the colour removal of molasses wastewater using *Aspergillus niger*, as in [1]. It was found that 69 % of the residue colour was removed, and 75 % COD (Chemical Oxygen Demand) was reduced. Benito *et al.* studied the colour removal of molasses wastewater by using *Trametes versicolour,* as in [2]. It was found that 82 % of the residue colour was removed and 77 % COD was reduced. Jimenez *et al.* studied the method of wastewater treatment by using aerobic and anaerobic system that uses 4 strains of fungus, as in [3]. Sirianuntapiboon *et al.* selected 205 strains of yeast from Thailand fruit samples and found some strain had the highest colour removal capacity of 68.91 % at 30 °C, as in [4]. Sirianuntapiboon *et al.* studied 170 species of acetogenic bacteria and found that species which able to remove maximum colour of 76.4 ± 3.2 % at 30 °C, as in [5]. Raghukuma *et al.* studied the melanoidin removal by whitewash fungi, *Flavadon flavus,* as in [6]. By 10 % cell immobilization with polyurethane foam, *Flavadon flavus* was able to remove 60–70 % of colour. In a study conducted by *Chandra et al*, a consortium of aerobic bacteria was investigated. The consortium consisted of *Klebsiella pneumoniae* (KU321273), *Salmonella enteric* (KU726954), *Enterobacter aerogenes* (KU726955), and *Enterobacter cloacae* (KU726957), as in [7]. The researchers found that this bacterial consortium exhibited optimal decolourization of molasses-melanoidins, achieving a remarkable 81 % removal rate. This was accomplished through co-metabolism in the presence of glucose (1.0 %) and peptone (0.2 %) as carbon and nitrogen sources, respectively.

The objective of this study was to identify the optimal culture media conditions for evaluating the effectiveness of *Saccharomyces cerevisiae*, a well-known yeast, in decolourizing molasses in wastewater. The researchers aimed to compare the decolourization capabilities of Saccharomyces cerevisiae with those of *Citeromyces siamensis* and *Issatchenkia orientalis*, potentially leading to the discovery of a more efficient microbial treatment for water pollution elimination. The only variables investigated in the experiment were the carbon and nitrogen sources.

For carbon sources, sucrose, lactose, and glucose are chosen for comparing the activity of microorganisms. These sugars are widely available and easily obtainable, making them convenient. Sucrose, lactose, and glucose have distinct chemical structures, which can influence the activity of microorganisms. The chosen nitrogen sources in this study were urea, peptone, and yeast extract. They are all rich sources of nitrogen, making them suitable options for providing ample nitrogen supply in various applications. This comparison provides valuable insights into the ability of microorganisms to utilize various substrates and adapt to different environmental conditions. This study aims to develop a practical and easily implementable method for controlling water pollution.

## 2. METHODS

The aim of this study was to investigate the impact of carbon and nitrogen sources on the reduction of colour in molasses wastewater. The researchers aimed to compare the effects of various carbon and nitrogen sources on the efficiency of colour reduction. By conducting this comparison, they aimed to identify the optimal combination of carbon and nitrogen sources that would promote the most effective decolourization and elimination of pollutants in the molasses-containing wastewater.

### *2.1. Appropriate carbon sources*

Inoculate yeasts (*Citeromyces siamensis* TISTR5778, *Issatchenkia orientalis* TISTR5690 and *Saccharomyces cerevisiae* TISTR5003) to 30 mL of MYGP broth (Malt Extract 3.0 g $L^{-1}$, Yeast Extract 3.0 g $L^{-1}$, Glucose 10.0 g $L^{-1}$, Peptone 5.0 g $L^{-1}$ pH 6 at 30 °C) which has 10 % of synthetic molasses wastewater, as in [8]. Incubate at 30 °C for 24 hours. Then measure the absorbance with a spectrophotometer at wavelength 660 nm, as in [9]–[10]. The desired value is 0.5. Add 5 % of yeast solution (by volume) to 30 mL of synthetic molasses wastewater which has 1, 2 and 3 % of sucrose, lactose and glucose. Incubate at room temperature for 1–7 days in 200-rpm shaker, as in [10].

At the end of each period, pick sample. Analyse by centrifugation at 5000 rpm for 20 minutes. Then get supernatant. Diluted 1 mL of supernatant with 0.1 M acetate buffer. The absorbances are measured by spectrophotometer at 475 nm, as in [11].

Yeast was inoculated into 30 mm of MYGP broth at 30 °C, which contained 10 % synthetic molasses wastewater. The mixture was incubated at 30 °C for 24 hours. The absorbance was then measured using a spectrophotometer at a wavelength of 660 nm, as in [12]. The desired value was 0.5.

To prepare the yeast solution, 5 % (by volume) of the previous culture was added to 30 mm of synthetic molasses wastewater containing 1 %, 2 %, and 3 % sucrose, lactose, and glucose. The mixture was incubated at room temperature for 1–7 days in a 200-rpm shaker.

At the end of each incubation period, a sample was taken and analysed by centrifuging at 5000 rpm for 20 minutes. The supernatant was collected and 1 mL of it was diluted with 0.1 M acetate buffer. The absorbances were then measured using a spectrophotometer at a wavelength of 475 nm.

### *2.2. Appropriate nitrogen sources*

The yeasts were cultured in MYGP Broth containing 10 % synthetic molasses wastewater, as mentioned in 2.1. The yeast solution was prepared by adding 5 % (by volume) of the previous culture to 30 mm of synthetic molasses wastewater, which had varying concentrations of urea, peptone, and yeast extract (1 %, 2 %, and 3 %). The mixture was then incubated at room temperature for 1–7 days in a 200-rpm shaker. The absorbance at 475 nm at the end of each period was also measured.

## 3. Experimental Results

After culturing the microorganisms in the molasses-containing samples for the designated duration, the absorption of colour was measured. This involved assessing the levels of colour absorption in the samples to evaluate the effectiveness of the microbial treatment in reducing intensity of colour.

### *3.1. Appropriate carbon sources*

The results of carbon sources on the reduction of the wastewater colour by *Citeromyces siamensis*, *Issatchenkia orientalis* and *Saccharomyces cerevisiae* was verified by measuring the colour absorption with spectrophotometer. Then calculate the percentage of colour reduction.

*3.1.1. Results of carbon sources on the reduction of wastewater colour by Citeromyces siamensis*

The results were presented in Fig. 1. During a period of 7 days, samples containing *Citeromyces siamensis* were collected, and the optical density (OD) was measured at 475 nm. It was observed that the colour absorption decreased during this period. Furthermore, it was found that the addition of 1 % glucose, sucrose and lactose resulted in the greatest reduction in colour absorption.

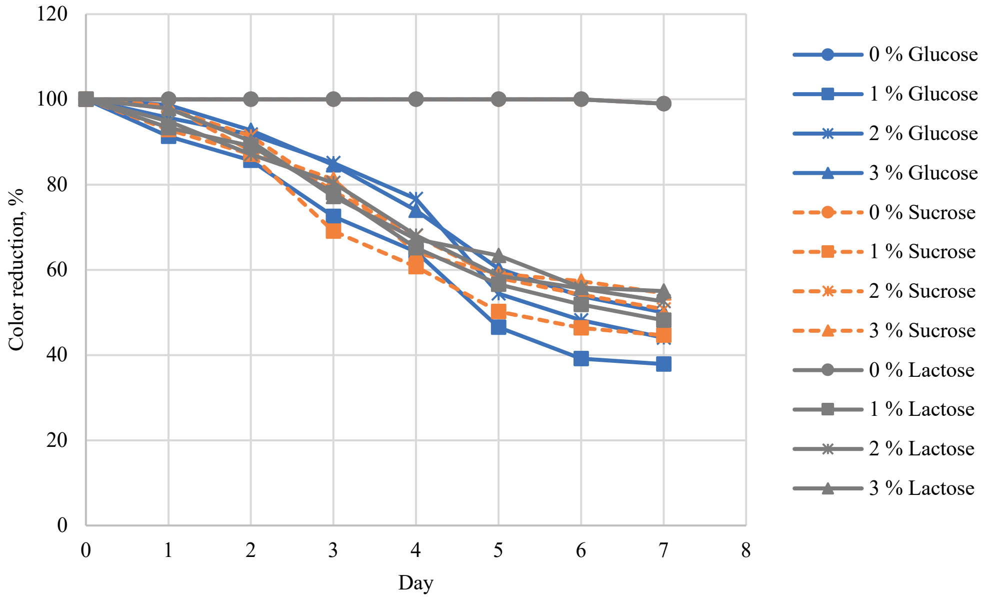


Fig. 1. Results of carbon sources on the reduction of the wastewater colour by *Citeromyces siamensis.*

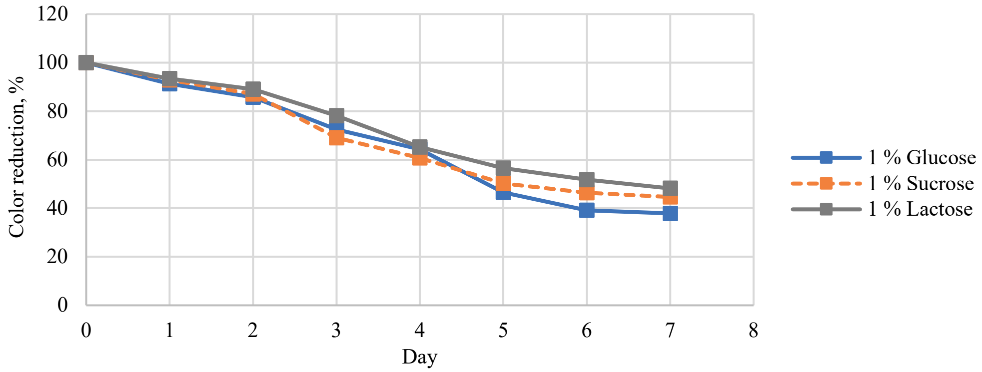


Fig. 2. Comparison different carbon sources on the reduction of the wastewater colour by *Citeromyces siamensis.*

The addition of 1 % glucose resulted in a reduction of adsorption by 37.87 %. Increasing the glucose concentration to 2 % reduced adsorption to 44.06 %, while a 3 % glucose addition resulted in a reduction of 50.00 %. The addition of 1 % sucrose led to reduction in the adsorption by 44.64 %. With 2 % sucrose addition, the adsorption decreased to 50.76 %, and when 3 % sucrose was added, it decreased at 54.53 %. When 1 % lactose was added, adsorption decreased to 48.17 %. With 2 % lactose addition, the adsorption value decreased to 52.59 %, and when 3 % lactose was added, it decreased to 55.01 %. When the best concentrations were compared, 1 % glucose had the highest absorption reduction (Fig. 2).

### *3.1.2. Results of carbon sources on the reduction of wastewater colour by Issatchenkia orientalis*

The decolourization of wastewater was examined by testing the influence of various carbon sources using *Issatchenkia orientalis*. The results were shown in Fig. 3. During 7-day, samples containing *Issatchenkia orientalis* were collected and measured optical density (OD) at 475 nm. It was observed that the colour absorption decreased over this time. The addition of 1, 2, and 3 % glucose reduced the absorption to 41.42, 43.66, 51.19 %, respectively. The addition of 1, 2 and 3 % sucrose reduced the absorption to 41.87, 50.43 and 54.91 %. For the addition of 1, 2 and 3 % lactose, the absorption was reduced to 47.95, 51.07 and 55.17 %. When comparing the different sources, it was observed that *Issatchenkia orientalis* in the samples supplemented with 1 % glucose exhibited the greatest decrease in colour absorption which closed to 1 % sucrose (Fig. 4).

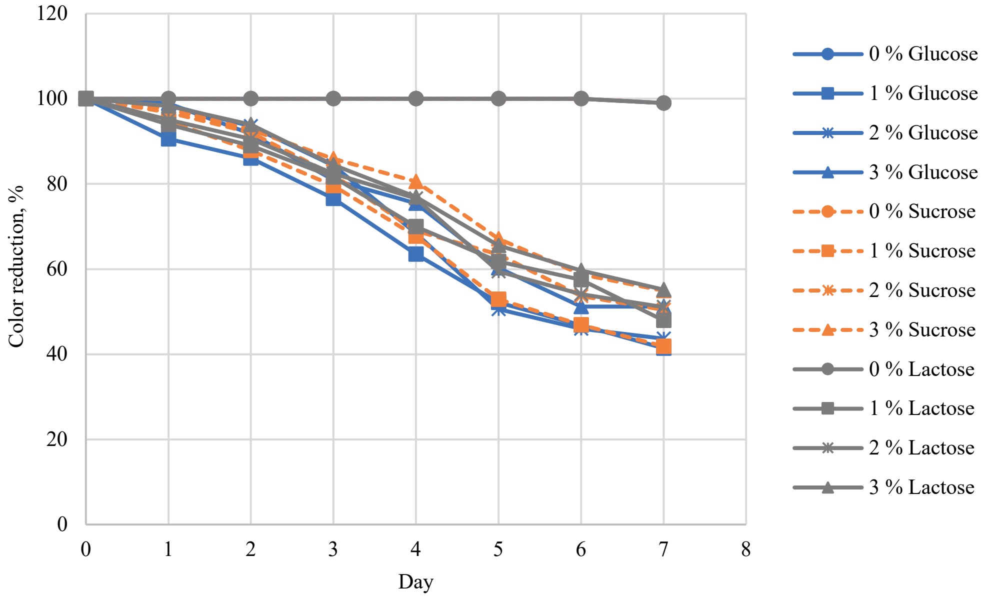


Fig. 3. Results of carbon sources on the reduction of the wastewater colour by *Issatchenkia orientalis.*

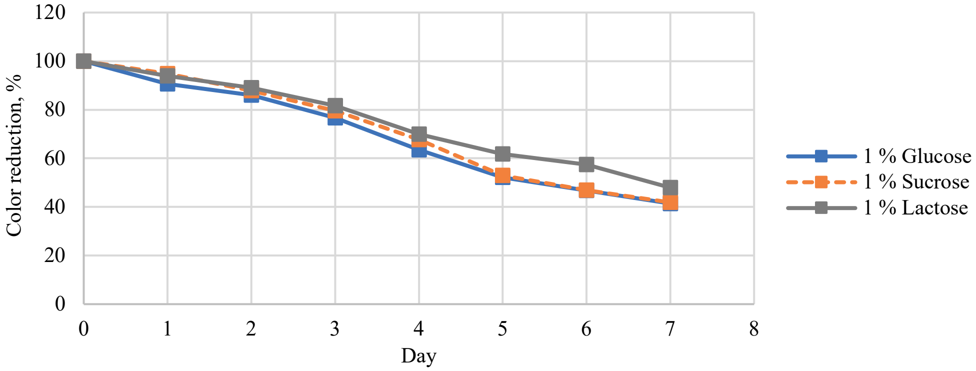


Fig. 4. Comparison different carbon sources on the reduction of the wastewater colour by *Issatchenkia orientalis.*

### *3.1.3. Result of carbon sources on the reduction of wastewater colour by Saccharomyces cerevisiae*

The results in Fig. 5 were for the addition of *Saccharomyces cerevisiae* under the same conditions as *Citeromyces siamensis* and *Issatchenkia orientalis*.

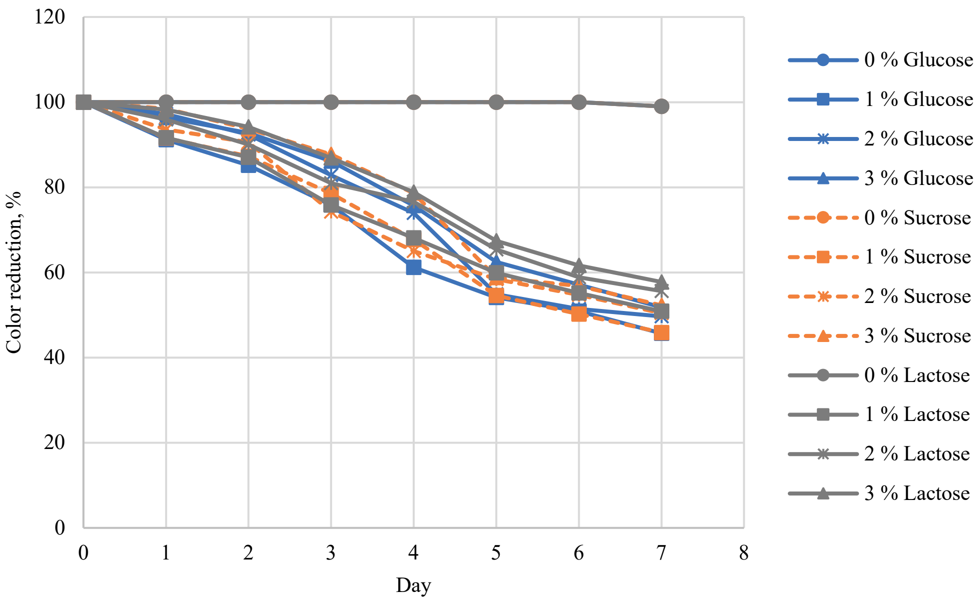


Fig. 5. Results of carbon sources on the reduction of the wastewater colour by *Saccharomyces cerevisiae.*

The results showed that when glucose was added to the samples, the absorption decreased. OD at 475 nm. decreased to 45.69, 49.67 and 51.81 % when 1, 2 and 3 % glucose were added. Absorption decreased to 45.89, 50.43 and 52.27 % from the effect of adding 1, 2 and 3 % sucrose. The addition of 1, 2 and 3 % lactose resulted in the OD values being reduced to 50.86, 55.63 and 57.76 %, respectively. When comparing the optimal concentrations of each type of sugar, it was found that 1 % glucose resulted in the highest reduction in absorption. Decreasing value closed to 1 % sucrose (Fig. 6).

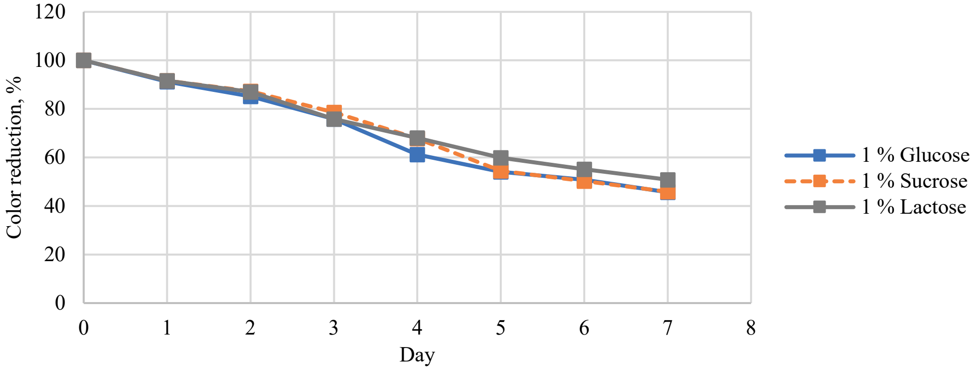


Fig. 6. Comparison different carbon sources on the reduction of the wastewater colour by *Saccharomyces cerevisiae.*

### *3.2. Appropriate nitrogen sources*

The impact of nitrogen sources on the reduction of wastewater colour by *Citeromyces siamensis*, *Issatchenkia orientalis*, and *Saccharomyces cerevisiae* was assessed by measuring colour absorption using a spectrophotometer. Subsequently, the percentage of colour reduction was calculated to evaluate the effectiveness of the nitrogen sources in colour reducing colour.

#### *3.2.1. Results of nitrogen sources on the reduction of wastewater colour by Citeromyces siamensis*

The results in Fig. 7 showed the effects of the addition of different nitrogen sources on the colour reduction of *Citeromyces siamensis* inoculated wastewater.

The addition of 1 % peptone resulted in the reduction in absorption by 43.50 %. Increasing the concentration to 2 % led the absorption reduction to 51.19 %, while the addition of 3 % peptone resulted in reduction at 54.70 %. The addition of 1 % urea led to a reduction in absorption by 48.39 %. With 2 % urea, the absorption decreased to 51.92 %, and when 3 % urea was added, it decreased to 57.27 %. When 1 % yeast extract was added, absorption decreased by 45.92 %. With 2 % yeast extract addition, the absorption value decreased to 50.54 %, and when 3 % yeast extract was added, it decreased to 49.25 %. When comparing the optimal concentrations, it was found that 1 % peptone resulted in the highest reduction in absorption (Fig. 8).

#### *3.2.2. Result of nitrogen sources on the reduction of wastewater colour by Issatchenkia orientalis*

The results in Fig. 9 showed the effects of the addition of different nitrogen sources on the colour reduction of *Issatchenkia orientalis* inoculated wastewater.

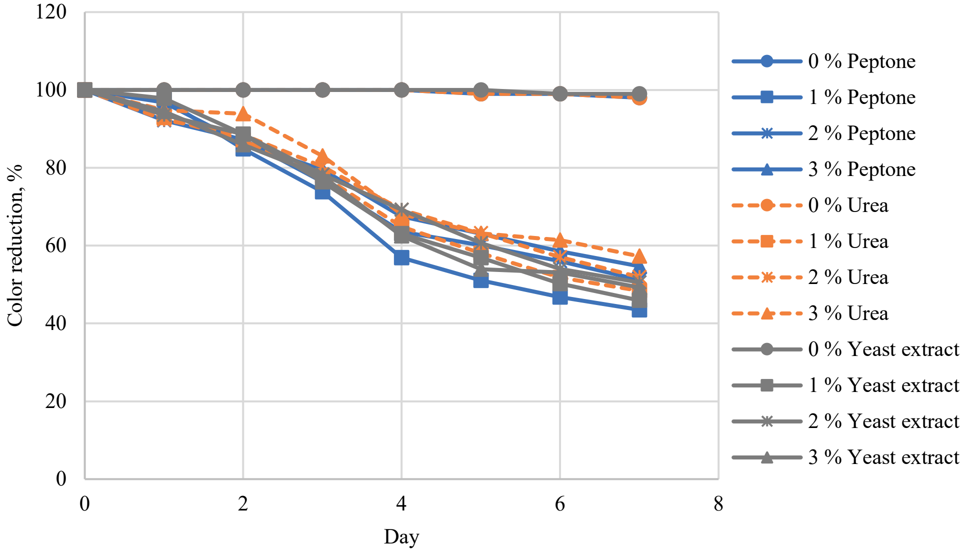


Fig. 7. Results of nitrogen sources on the reduction of the wastewater colour by *Citeromyces siamensis.*

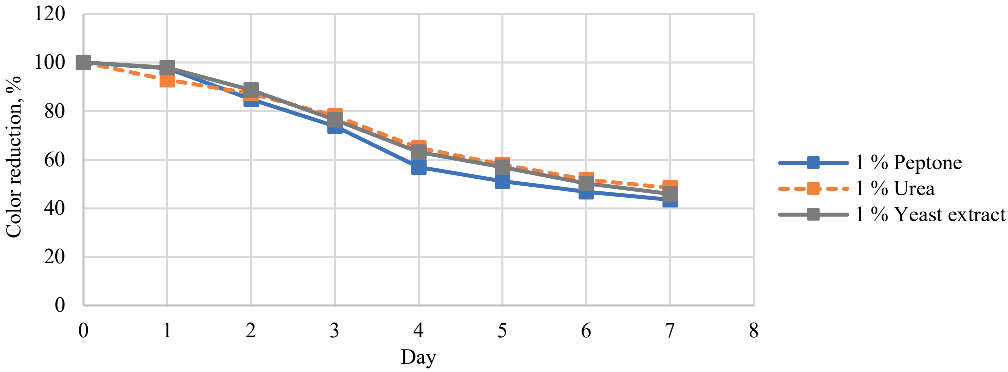


Fig. 8. Comparison different nitrogen sources on the reduction of the wastewater colour by *Citeromyces siamensis.*

The addition of 1 % peptone resulted in the reduction in absorption by 41.33 %. Increasing the concentration to 2 % led the absorption reduction to 48.60 %, while the addition of 3 % peptone resulted in reduction at 51.82 %. The addition of 1 % urea led to a reduction in absorption by 46.75 %. With 2 % urea, the absorption decreased to 51.19 %, and when 3 % urea was added, it decreased to 55.97 %. When 1 % yeast extract was added, absorption decreased by 46.22 %. With 2 % yeast extract addition, the absorption value decreased to 52.25 %, and when 3 % yeast extract was added, it decreased to 56.68 %. When comparing the optimal concentrations, it was found that 1 % peptone resulted in the highest reduction in absorption, same as *Citeromyces siamensis* (Fig. 10).

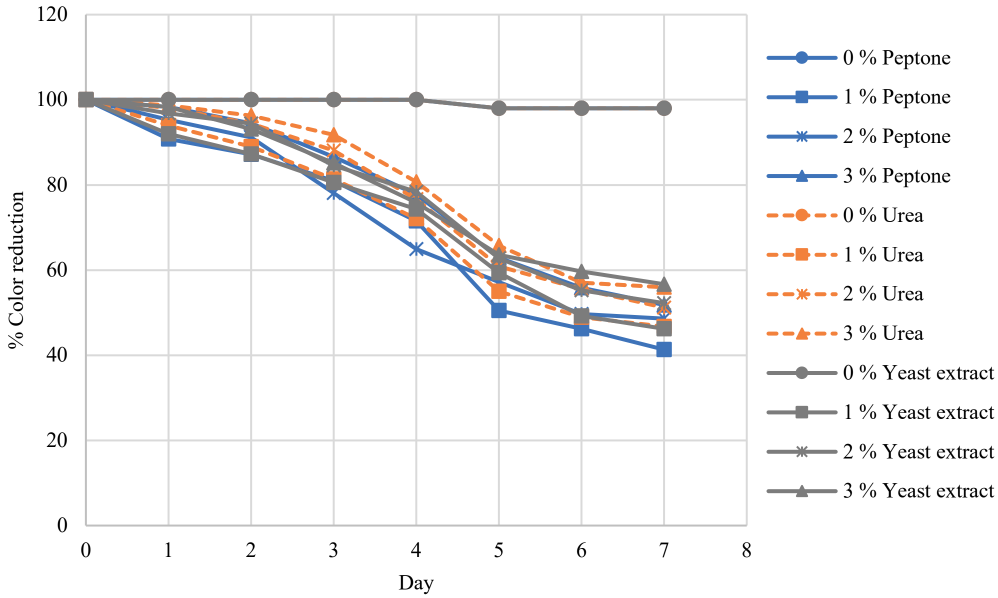


Fig. 9. Results of nitrogen sources on the reduction of the wastewater colour by *Issatchenkia orientalis.*

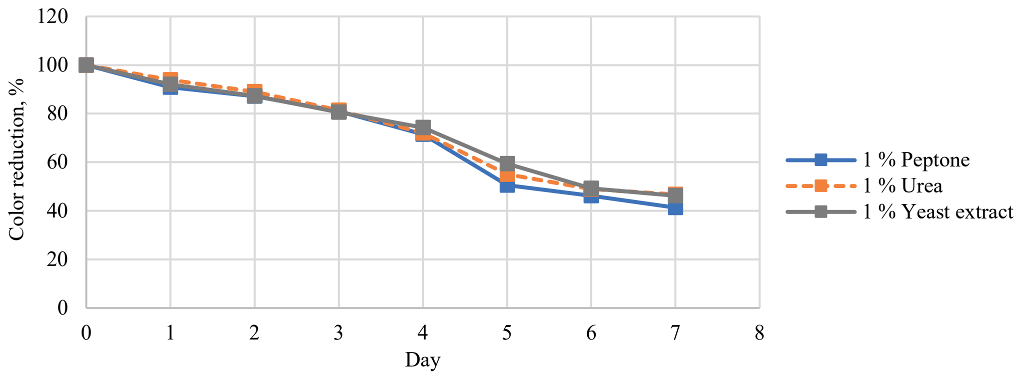


Fig 10. Comparison different nitrogen sources on the reduction of the wastewater colour by *Issatchenkia orientalis.*

### *3.2.3. Result of nitrogen sources on the reduction of wastewater colour by Saccharomyces cerevisiae*

The results in Fig. 11 showed the effects of the addition of different nitrogen sources on the colour reduction of *Saccharomyces cerevisiae* inoculated wastewater, under the same conditions as *Citeromyces siamensis* and *Issatchenkia orientalis.*

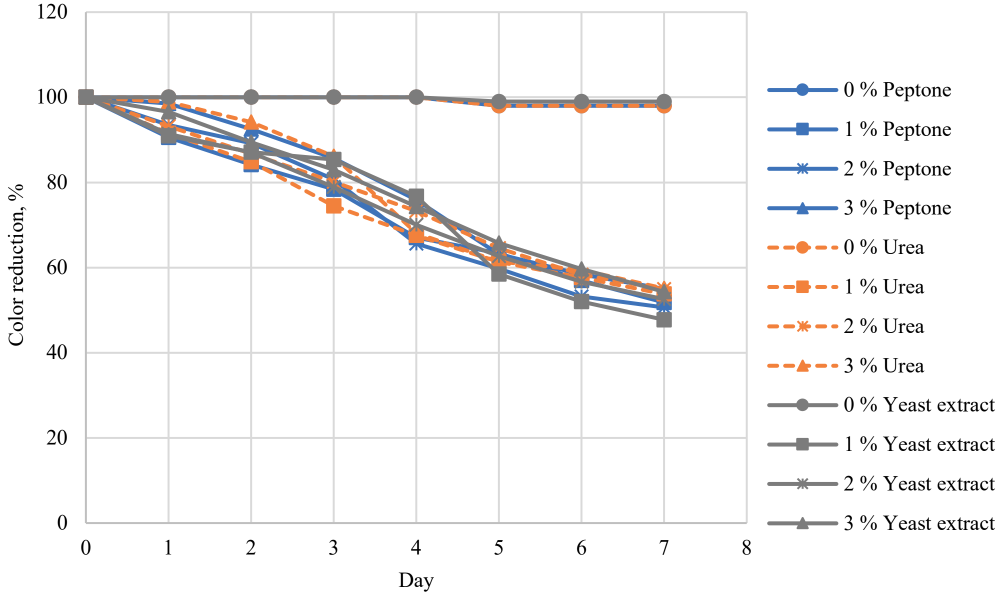


Fig. 11. Results of nitrogen sources on the reduction of the wastewater colour by *Saccharomyces cerevisiae.*

The addition of 1 % peptone resulted in the reduction in absorption by 51.82 %. Increasing the concentration to 2 % led the absorption reduction to 50.65 %, while the addition of 3 % peptone resulted in reduction at 55.01 %. The addition of 1 % urea led to a reduction in absorption by 53.78 %. With 2 % urea, the absorption decreased to 54.91 %, and when 3 % urea was added, it decreased to 55.10 %. When 1 % yeast extract was added, absorption decreased by 47.74 %. With 2 % yeast extract addition, the absorption value decreased to 52.59 %, and when 3 % yeast extract was added, it decreased to 54.31 %. When comparing the optimal concentrations, it was found that 1 % yeast extract resulted in the highest reduction in absorption, different from *Citeromyces siamensis* and *Issatchenkia orientalis* (Fig. 12).

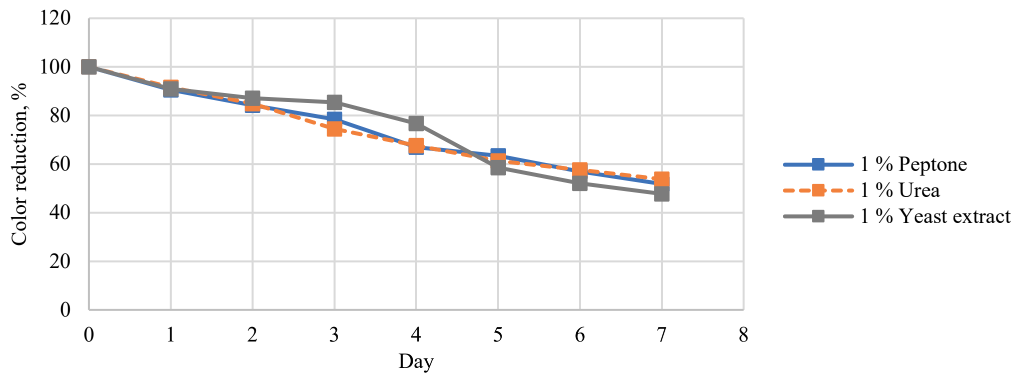


Fig. 12. Comparison different nitrogen sources on the reduction of the wastewater colour by *Saccharomyces cerevisiae.*

## 4. Conclusion

Based on the experimental results, it was demonstrated that 1 percent glucose was the most effective carbon source for reducing the colour of molasses wastewater. This carbon source exhibited a beneficial effect on all three types of microorganisms studied. Interestingly, increasing the concentrations of glucose, sucrose, and lactose did not lead to a significant enhancement in the decolourization of the wastewater samples. Specifically, for *Citeromyces siamensis*, glucose yielded the best results, followed by sucrose and lactose. On the other hand, for *Issatchenkia orientalis* and *Saccharomyces cerevisiae*, glucose and sucrose showed similar effectiveness, while lactose demonstrated the least beneficial impact.

In the investigation of nitrogen sources, it was determined that the most effective nitrogen source for reducing the colour of molasses wastewater in *Citeromyces siamensis* and *Issatchenkia orientalis* was 1 percent peptone. For *Saccharomyces cerevisiae*, 1 percent yeast extract proved to be the best nitrogen source. It was also observed that increasing the concentration of these nitrogen sources did not result in improvements in decolourization.

Cultivation of the three microorganisms without the addition of additional carbon or nitrogen sources showed very little reduction in colour, consistent with the earlier research conducted by [10]. When comparing the colour reduction abilities of all three types of microorganisms, it was observed that *Citeromyces siamensis* in 1 % glucose was the most effective. It was capable of reducing the intensity of the wastewater colour by up to 62.13 %.

The substance that yielded the best results in this study was glucose, which is readily available. However, it may be more expensive compared to sucrose, which was found to be the second most effective carbon source. The nitrogen sources also demonstrated positive results. Among the nitrogen sources tested, peptone exhibited notable effectiveness in promoting microbial decolourization. However, it is known to be expensive. On the other hand, urea, which is already utilized as a food source for microorganisms, is a much more affordable nitrogen source with comparable effectiveness in promoting decolourization. The utilization of sucrose or urea as additives for microbial decolourization presents a cost-effective alternative for treating molasses wastewater. These options offer affordability while maintaining effectiveness in the decolourization process.

However, the primary objective of this experiment was to examine whether the nitrogen source or the carbon source alone promotes decolourization in the wastewater. Adding multiple substances to enhance microbial activity can increase the cost of wastewater treatment. Therefore, this study focused on assessing the individual impact of nitrogen and carbon sources on the decolourization process. If there is an interest in investigating the combined effect of nitrogen and carbon sources, further research can be conducted.

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