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Inorganic ion removal from synthetic wastewater by *S. cerevisiae* under anaerobic conditions

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Abstract: Agricultural activities in Malaysia contribute significantly to wastewater pollution, leading to problems such as eutrophication, economic loss, and health risks. *Saccharomyces cerevisiae* (yeast) is a promising biological treatment option, but limited research exists on the use of baker's and brewer's yeast in tropical regions like Malaysia. This study evaluates the effectiveness of locally sourced baker's and brewer's yeast in removing nitrate, nitrite, ammonium, phosphate, and sulfate from synthetic agricultural wastewater, as well as their biomass production. Yeast cultures were incubated for 120 hours at 28 °C, and viable cell counts were measured before and after incubation using microscopy. Pollutant concentrations were analysed via spectrophotometry following standardized protocols. Both yeast types showed significant removal of phosphate, ammonium, and sulfate, but had limited effectiveness against nitrate and nitrite. Yeast cell counts increased significantly, with microscopic evidence of budding, indicating both strains could survive, grow, and reproduce under anaerobic conditions. Both baker's and brewer's yeast show potential for ion removal, though real-world application may be limited by environmental factors and wastewater variability.

Keywords: Ion removal, Remediation, Wastewater and treatment, Wastewater pollution, Yeast.

Introduction

Malaysia has developed a robust agricultural and livestock industry (Zayadi, 2021). However, these activities have led to the discharge of agricultural waste, such as animal feces, plant residues, pesticides, fertilizers, and animal feed remnants into the environment, particularly into water bodies. This has become a significant environmental concern (Chislock *et al.*, 2013). These waste materials are rich in eutrophication-causing ions, including nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), phosphate (PO_4^{3-}), and sulfate (SO_4^{2-}). If not treated properly, these pollutants can trigger eutrophication in water bodies, leading to reduced oxygen levels, death of aquatic organisms, and overall degradation of water quality and aquatic ecosystems (Chislock *et al.*, 2013).

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According to a report, 60% of lakes in Malaysia have experienced serious eutrophication since 2019, resulting in severe ecological damage and health risks to local communities (Koh *et al.*, 2019). Given this situation, finding an effective method to treat agricultural wastewater in Malaysia has become urgent. Traditional treatment methods include physical approaches such as membrane separation and electrodialysis, chemical techniques like coagulation and flocculation, and biological methods involving plants or bacteria (Singh *et al.*, 2024). However, physical and chemical treatments often have limitations, including high chemical consumption, labor costs, and relatively low effectiveness in removing specific ions (Akinnawo, 2023). Biological methods also present challenges-plants have a limited effective range, and bacteria may pose contamination risks.

An alternative biological approach is the use of yeast (*Saccharomyces cerevisiae*) for wastewater treatment. Yeast offers several advantages: it is cost-effective, sustainable, easy to obtain, and capable of functioning under anaerobic conditions (Al-Najar *et al.*, 2021). Moreover, yeast has demonstrated the ability to absorb a variety of compounds found in wastewater, including eutrophication-related ions such as phosphate (PO_4^{3-}) and ammonium (NH_4^+) (Nicula *et al.*, 2023). These attributes make yeast a promising candidate for bioremediation of agricultural wastewater in Malaysia, where such pollutants are common. Although various yeast strains have proven effective for wastewater treatment in other countries (Al-Najar *et al.*, 2021), their performance may vary depending on environmental and climatic conditions. Consequently, strains that are effective elsewhere may not perform as well in Malaysia. Additionally, there is currently a research gap regarding the use of baker's and brewer's yeast for wastewater treatment in tropical regions, including Malaysia.

To address this gap, the present study investigates whether baker's yeast and brewer's yeast commonly available in Malaysia can effectively remove eutrophication-causing inorganic pollutants—namely NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, and SO_4^{2-}-from wastewater. Given the well-established and large-scale production of these yeast types in Malaysia (Wresearch, n.d.), their availability and cost-effectiveness represent a major advantage for large-scale implementation.

However, their efficacy in removing inorganic pollutants remains underexplored. Therefore, this study aims to evaluate the effectiveness of Malaysian baker's and brewer's yeast in removing common eutrophication-related ions by measuring the reduction percentage of each ion in synthetic wastewater after 120 hours of incubation. Additionally, changes in viable cell counts will be monitored to assess yeast growth.

Materials and Methods

Synthetic Wastewater Preparation

Synthetic wastewater was formulated based on Nicula *et al.* (2023) and consisted of the following components: 200 mg/L D-glucose, 200 mg/L sucrose, 66.73 mg/L $(NH_4)_2SO_4$, 70 mg/L yeast extract, 91 mg/L NH_4Cl, 4.43 mg/L KH_2PO_4, 21 mg/L $MgSO_4{\cdot}7H_2O$, 2.68 mg/L $MnSO_4{\cdot}H_2O$, 30 mg/L $NaHCO_3$, 19.74 mg/L $CaCl_2$, 0.14 mg/L $FeCl_3{\cdot}6H_2O$, 455 mg/L $NaNO_3$, and 359 mg/L $NaNO_2$. According to Nicula *et al.* (2023), yeast biomass growth (up to ~11-fold) was associated with improved contaminant removal. In this study, the addition of glucose and yeast extract acted as supplemental carbon and nutrient sources, supporting yeast metabolism and biomass growth during incubation, which in turn could enhance nutrient and ion uptake. The solution was prepared in a 1 L Schott bottle. Although this formulation does not perfectly replicate real wastewater, it contains essential chemical constituents that simulate a realistic wastewater environment.

Yeast culture preparation

Baker's and brewer's yeast were purchased online from Malaysia Trends, Take It Global Sdn. Bhd. Approximately 10 g of each yeast type was added to 100 mL of sterile deionized water in separate beakers (10% w/v) and left at room temperature for 15 minutes to allow activation and rehydration of the yeast cells (Guadalupe-Daqui *et al.*, 2023). The suspensions were then shaken to ensure homogeneous distribution of the yeast cells. Subsequently, 1 mL of each suspension was aseptically transferred using a sterile pipette into 100 mL of Yeast Extract Peptone Dextrose (YPD) medium in a sterile conical flask. Yeast cultures were prepared to reach mid-logarithmic phase following the University of Florida protocol (n.d.). Cultures were diluted to an OD_{600} of 0.05–0.1 and incubated at 30 °C, 180 rpm. When OD_{600} reached approximately 0.4, the cultures were in mid-log phase and were harvested at this point. The yeast concentration was estimated to be approximately 1.2×10^7 cells/mL, based on the assumption that each 0.1 unit of OD_{600} corresponds to 3×10^6 cells/mL (Thermo Fisher Scientific, n.d.).

Baker's and brewer's yeasts were cultured in synthetic wastewater under anaerobic conditions using 1 L conical flasks sealed with parafilm. Parafilm sealing has been used in laboratory experiments as a simple, low-cost way to reduce gas exchange (including oxygen) and thus approximate limited-oxygen conditions (Bhardwaj *et al.*, 2019). Each flask contained 445 mL of synthetic wastewater inoculated with 5 mL (1% v/v) of yeast suspension (1.2×10^7 cells/mL), as described by Nicula *et al*. (2023). The flasks were incubated at 28 °C and 120 rpm in a shaking incubator for 120 hours. A control flask containing 450 mL of synthetic wastewater without yeast served as a negative control to assess baseline ion concentrations.

Yeast Cell Concentration and Viability Assessment

Yeast cell concentration was determined before and after incubation using a Neubauer counting chamber in combination with methylene blue staining, following the method of Matsumoto *et al*. (2022). A 0.5 mL aliquot of the yeast suspension was diluted with 14.5 mL of deionized water and stained with 3% methylene blue solution. A 200 µL portion of the stained suspension was then loaded onto the counting chamber, and viable cells (appearing colourless or white) were counted under a light microscope. The viable cell concentration (cells/mL) was calculated using the following formula:

Viable cells (cells/mL) = Average cell count per square $\times 10^4 \times$ dilution factor

The percentage increase in cell biomass over the incubation period was calculated as: Increase (%) = [(Final cell count – Initial cell count) / Initial cell count] × 100

In addition to cell quantification, morphological characteristics and budding activity were observed microscopically.

Ion removal

The removal of nitrogen compounds (NO_3^-, NO_2^-, NH_4^+), phosphorus (PO_4^{3-}), and sulfur (SO_4^{2-}) by the yeast cultures was evaluated after 120 hours using spectrophotometric methods specific to each ion as below. Measurements were compared against the initial ion concentrations in untreated synthetic wastewater. Ion removal percentage was calculated using the formula:

Removal (%) = $[(C_{initial} - C_{final}) / C_{initial}] \times 100\%$,

where $C_{initial}$ is the concentration before incubation and C_{final} is after 120 hours of incubation.

Phosphate (PO_4^{3-}) Removal

A vanadate-molybdate reagent was prepared following Libretexts (2023). The solution A was prepared by dissolving 40 g of ammonium molybdate tetrahydrate, $(NH_4)_6Mo_7O_{24}.4H_2O$ in 400 mL of deionized water. Solution B was prepared by dissolving 1 g of ammonium vanadate, NH_4VO_3 in 300 mL deionized water and adding 200 mL of 65% nitric acid. Solution B was first poured into a 1000 mL volumetric flask, followed by Solution A. The flask was then filled to the mark with deionized water. To measure phosphate level, 25 mL of yeast-treated wastewater was centrifuged (2000 rpm, 5 min, room temperature) using the Heraeus® Megafuge® 1.0 R centrifuge to remove cells. Then, the supernatant was transferred to a 50 mL volumetric flask and 10 mL of vanadate-molybdate reagent was added and diluted to the mark with deionized water. After complete reaction at room temperature, absorbance was measured at 470 nm. A standard curve was prepared using phosphate standards (0–20 mg/L), yielding the equation:

$$A_{470} = 0.204 \times [PO_4^{3-}] - 0.0071.$$

Ammonium (NH_4^+) Removal

The determination of ammonia was conducted using the Nessler method, as described in the Giovannelli Lab Protocols (n.d.). Nessler reagent (commercially obtained) and Rochelle salt solution were used. Rochelle salt was prepared by dissolving 50 g of potassium sodium tartrate in 30 mL of distilled water, followed by boiling to remove any residual ammonia. The solution was then cooled and diluted to a final volume of 100 mL with distilled water. For sample preparation, 5 mL of each sample was centrifuged, and 1 mL of the resulting supernatant was transferred to a clean test tube. To this, 100 µL of Rochelle salt solution and 100 µL of Nessler reagent were added. After an incubation period of 15 minutes at room temperature, the absorbance was measured at 420 nm using a UV-Vis spectrophotometer. A standard curve was generated based on known ammonium concentrations, yielding the linear relationship:

$$A_{420} = 0.158 \times [NH_4^+] + 0.0067$$

Sulfate (SO_4^{2-}) Removal

The removal of sulfate ions (SO_4^{2-}) was analyzed using the barium chloride ($BaCl_2$) turbidity method, as described by Dash *et al*. (2016). A 20 mL aliquot of each sample was centrifuged using a Heraeus® Megafuge® 1.0 R centrifuge. The supernatant was transferred to a 100 mL volumetric flask and diluted to volume with deionized water. Subsequently, 2 g of $BaCl_2$ was added to each flask and mixed thoroughly. After 1 minute of reaction time, turbidity was measured spectrophotometrically at 420 nm. A calibration curve was prepared using standard sulfate solutions ranging from 10 to 50 mg/L, resulting in the equation:

$$A_{420} = 0.0317 \times [SO_4^{2-}] + 0.0165$$

Nitrite (NO_2^-) Removal

Nitrite concentration was determined using the Griess method, as described by Nerdy & De Lux Putra (2018). Two reagents were prepared in 15% acetic acid: sulfanilic acid solution (3.4 g/L) and N-(1-naphthyl)ethylenediamine dihydrochloride solution (1.4 g/L). For each sample, 10 mL was centrifuged at 2000 rpm for 5 minutes at room temperature using a Heraeus® Megafuge® 1.0 R centrifuge. A 2.5 mL aliquot of the supernatant was then mixed with 2.5 mL of sulfanilic acid solution and allowed to react for 5 minutes. Subsequently, 2.5 mL of the second reagent was added. The final mixture was diluted to 100 mL with distilled water. After 15 minutes of color development, the absorbance was measured at 540 nm. A standard calibration curve was constructed using sodium nitrite standards, resulting in the following linear equation:

$$A_{540} = 0.9185 \times [NO_2^-] + 0.00009521$$

Nitrate (NO_3^-) Removal

This step was performed only after the direct measurement of nitrite (NO_2^-), and it involved the reduction of nitrate (NO_3^-) to nitrite using zinc powder and hydrochloric acid (HCl), as outlined by Nerdy & De Lux Putra (2018). For the preparation of 1 mol/L HCl solution, 41.7 mL of concentrated hydrochloric acid was carefully diluted with distilled water in a 500 mL volumetric flask up to the calibration mark. For the procedure, 3.5 mL of yeast-inoculated wastewater was centrifuged for 5 minutes at 2000 rpm at room temperature using a Heraeus® Megafuge® 1.0 R centrifuge. The supernatant was transferred to a 100 mL volumetric flask and diluted to the mark with distilled water, followed by the addition of 0.1 g of zinc powder and 1 mL of 1 mol/L HCl solution. The flask was left at room temperature for 10 minutes to allow complete reduction of NO_3^- to NO_2^-.

Following the reduction step, 2.5 mL of sulfanilic acid solution was added to the flask and mixed thoroughly. After 5 minutes, 2.5 mL of N-(1-naphthyl) ethylenediamine dihydrochloride solution was added and the mixture was shaken again. The final solution had a dilution factor of 10×. The absorbance was then measured at 540 nm using a UV-Vis spectrophotometer to determine the total nitrite concentration. The standard calibration curve used was: $A_{540} = 0.9185 \times [NO_2^-] + 0.00009521$. A blank solution was prepared by mixing 2.5 mL of each Griess reagent and diluting to 100 mL with distilled water using the same procedure. The concentration of nitrate (NO_3^-) was determined by subtracting the previously measured nitrite concentration from the total concentration obtained after reduction (assuming complete conversion of NO_3^- to NO_2^-).

Statistical Analysis

Biomass production and Ion removal data between baker's and brewer's yeast were analysed using the Statistical Package for the Social Sciences (SPSS). One-way analysis of variance (ANOVA) was used to determine significant differences between groups. Depending on the homogeneity of variances, either the Least Significant Difference (LSD) test or Dunnett's T3 post hoc test was applied. All statistical analyses were conducted at a 95% confidence level ($p < 0.05$).

Results

Viable Cell Increase and Morphology

According to Figure 1, the brewer's yeast treatment group exhibited a higher increase in viable cells—approximately 599.4% ± 89.45%-compared to the baker's yeast group, which showed an increase of 558.15% ± 55.66%. However, statistical analysis indicated no significant difference between the two groups, suggesting that both baker's and brewer's yeast possess similar growth rates and viability under the tested conditions.

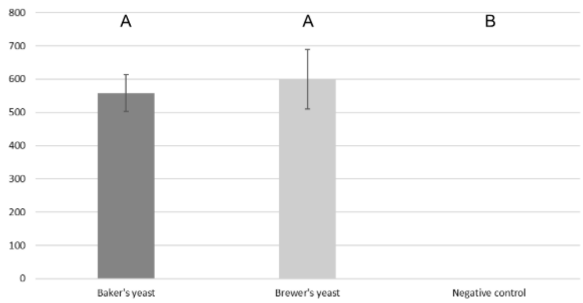

Figure 1: Viable cells increase percentage of yeast-inoculated treatment groups after 120 hours of incubation. Note: a, ab, b: different alphabet in each column shows the different significant means (Post Hoc Test, LSD, $P<0.05$).

Both baker's and brewer's yeast treatment groups exhibited the presence of white-stained (viable) cells and budding cells after 120 hours of incubation in synthetic wastewater, indicating that the yeast cells remained viable and actively dividing under the experimental conditions. Brewer's yeast samples showed a higher number of both white (viable) and blue (non-viable) cells compared to baker's yeast, suggesting a higher overall cell density but with a mixed population of live and dead cells.

Phosphate (PO_4^{3-}) Removal

According to Figure 2, the treatment group inoculated with brewer's yeast exhibited the highest phosphate (PO_4^{3-}) removal efficiency, reaching 98.619% ± 0.094% after 120 hours of incubation. This was followed closely by the baker's yeast treatment, which achieved a removal efficiency of 98.425% ± 0.230%. In contrast, the negative control (consisting of synthetic wastewater without any yeast inoculation) showed a significantly lower removal efficiency of 3.884% ± 1.712%. These results suggest that both baker's and brewer's yeast are equally effective in removing phosphate from synthetic wastewater.

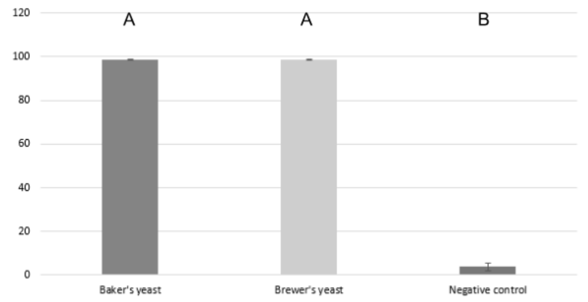

Figure 2: Phosphate (PO_4^{3-}) removal percentage in synthetic wastewater after incubation for 120 hours. Note: a, ab, b: different alphabet in each column shows the different significant means (Post Hoc Test, Dunnett T3, *P*<0.05).

Ammonium (NH_4^+) Removal

According to Figure 3, the treatment group inoculated with baker's yeast demonstrated the highest ammonium (NH_4^+) removal efficiency, achieving 67.978% ± 0.919%. This was followed by the brewer's yeast treatment, which showed a removal efficiency of 58.670% ± 6.286%. The negative control, which consisted of synthetic wastewater without yeast inoculation, exhibited the lowest removal efficiency at 24.600% ± 8.935%. These results suggest that both baker's and brewer's yeast are equally effective in removing ammonium from synthetic wastewater.

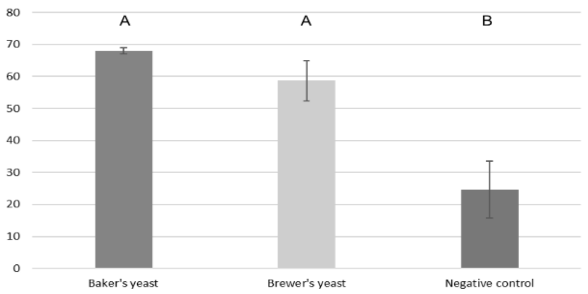

Figure 3: Ammonium (NH_4^+) removal percentage in synthetic wastewater after incubation for 120 hours. Note: a, ab, b: different alphabet in each column shows the different significant means (Post Hoc Test, LSD, *P*<0.05).

Sulphate (SO_4^{2-}) Removal

According to Figure 4, the treatment group inoculated with baker's yeast exhibited the highest sulfate (SO_4^{2-}) removal efficiency, achieving 54.811% ± 6.619%. This was followed by the brewer's yeast treatment, which showed a removal efficiency of 30.700% ± 1.546%. In contrast, the negative control demonstrated a much lower removal efficiency of 8.762% ± 7.501%. Statistical analysis indicated sulphate removal efficiency of baker's yeast was significantly higher than that of brewer's yeast, suggesting that baker's yeast is more effective in removing sulphate from synthetic wastewater.

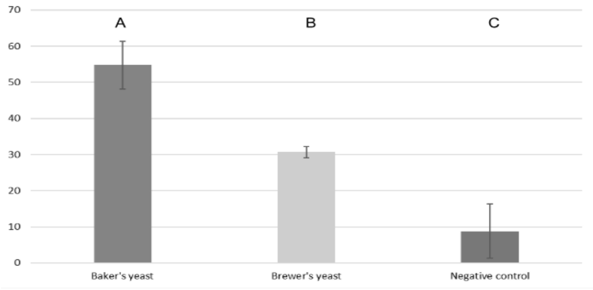

Figure 4: Sulphate (SO_4^{2-}) removal percentage in synthetic wastewater after incubation for 120 hours. Note: a, ab, b: different alphabet in each column shows the different significant means (Post Hoc Test, LSD, *P*<0.05).

Nitrate (NO_3^-) Removal

According to Figure 5, all three treatment groups exhibited relatively low nitrate (NO^-) removal efficiencies with no significant differences among the baker's yeast, brewer's yeast, and negative control groups ($p > 0.05$). The treatment group inoculated with baker's yeast showed the highest removal efficiency at 0.291% ± 0.126%, followed by the brewer's yeast group at 0.242% ± 0.176%. The negative control group demonstrated the lowest removal efficiency, at 0.175% ± 0.100%. These results suggest that both baker's and brewer's yeast have minimal to no effect on nitrate removal, and that their removal efficiencies do not differ from one another.

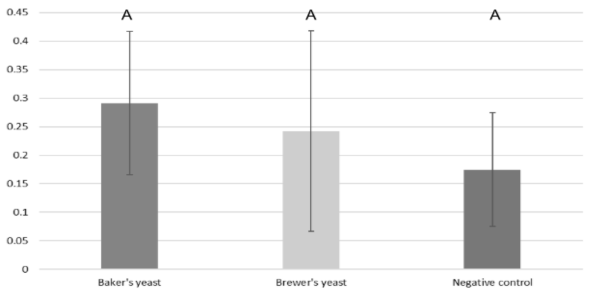

Figure 5: Nitrate (NO_3^-) removal percentage in synthetic wastewater after incubation for 120 hours. Note: a, ab, b: different alphabet in each column shows the different significant means (Post Hoc Test, LSD, *P*<0.05).

Nitrite (NO_2^-) Removal

According to Figure 6, the negative control group exhibited the highest nitrite removal efficiency at approximately 66.506% ± 0.925%, followed by the brewer's yeast treatment group with 54.666% ± 7.644%, and the baker's yeast group with the lowest removal efficiency at 51.099% ± 8.33%. Statistical analysis indicated no significant difference between brewer's yeast and either baker's yeast or the negative control. These findings suggest that both baker's and brewer's yeast exhibited limited or no effective nitrite removal.

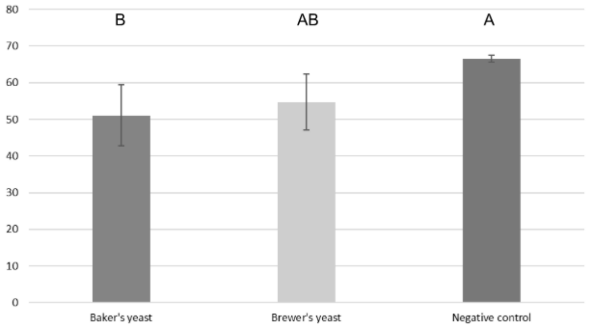

Figure 6: Nitrite (NO_2^-) removal percentage in synthetic wastewater after incubation for 120 hours. Note: a, ab, b: different alphabet in each column shows the different significant means (Post Hoc Test, LSD, *P*<0.05).

Discussion

According to Figure 1, the minor variations observed may reflect natural biological variability such as differences in metabolic activity. Compared with the anticipated growth rate of 733.33% ± 1533% reported by Nicula *et al*. (2023), the values in this study were lower, likely reflecting strain-dependent growth potential. Commercial yeast products differ in genetic background, metabolic capacity, and stress tolerance, which influence their performance in specific environments (Bai *et al*., 2022). Brewer's yeast possesses adaptive mechanisms against ethanol stress (e.g., altered membrane lipids, enhanced protein regulation, and stress pathway activation) and is better adapted to low-oxygen environments through regulation of genes such as ATF1 and ATF2 (Meng *et al*., 2017; D'Amore & Stewart, 1987; Pires *et al*., 2014). These traits may explain its apparent growth advantage under anaerobic incubation, although no statistically significant difference in viable cell counts was detected ($p > 0.05$). Morphological observations revealed more frequent clustered budding in brewer's yeast, consistent with colony morphology reports where incomplete daughter cell separation leads to clusters (Foley *et al*., 2005). This may relate to flocculation driven by FLO-encoded proteins under stress (Jaeger *et al*., 2020), underscoring brewer's yeast resilience in anaerobic wastewater conditions.

Phosphate (PO_4^{3-}) removal was efficient in S. cerevisiae, consistent with its central role in nucleic acid synthesis, membrane phospholipids, and ATP metabolism. Uptake is mediated by the PHO pathway, where Pho84 functions as a high-affinity transporter and the VTC complex enables polyphosphate storage; expression is further enhanced by the transcription factor Pho4 under phosphate limitation (Takado *et al*., 2023). Since phosphate was the sole phosphorus source in the synthetic wastewater, yeast growth depended on its uptake, explaining both the significant PO_4^{3-} removal and the concurrent increase in viable cells. Brewer's yeast showed slightly higher uptake than baker's yeast, though the difference was not statistically significant ($p > 0.05$), likely reflecting strain-level variability in nutrient uptake and metabolism (Nicula *et al*., 2023). Some phosphate loss also occurred in the negative control, attributable to abiotic processes such as precipitation with divalent cations (Ca^{2+}, Mg^{2+}) under certain conditions. Similar abiotic removal via adsorption has been reported by Mekonnen *et al*. (2020), who achieved up to 80% reduction using coal particles.

Ammonium (NH_4^+) removal was substantial because *S. cerevisiae* preferentially assimilates NH_4^+ and can even rely on it as the sole nitrogen source under nitrogen-limited conditions (Nair & Sarma, 2021). Uptake is mediated by transporter proteins (Mep1p–Mep3p), with Mep2p showing the highest affinity (Km ~1–2 µM) (Marini *et al*., 1997). Although both baker's and brewer's yeast removed significant amounts of ammonium, the efficiency was lower than expected (~90% removal in other studies). This reduction likely reflects the presence of alternative organic nitrogen sources (amino acids, peptides) in the yeast extract used in the synthetic wastewater, which reduced the reliance on inorganic NH_4^+ (Duong *et al*., 2019). Baker's yeast showed slightly higher removal, consistent with its greater total nitrogen content (10.8–11.2%) compared to brewer's yeast (8.2%), which increases its demand for ammonium uptake (Tao *et al*., 2022). Ammonium loss also occurred in the negative control, likely through abiotic processes such as volatilization of NH_3 under elevated pH or temperature. Cueto Rojas *et al*. (2016) further reported that nitrogen metabolism in yeast can involve a "futile cycle," where NH_3 leakage into the extracellular environment contributes to apparent ammonium loss. The observed ammonium uptake coincided with higher viable cell percentages, reflecting its assimilation into amino acids and proteins for cell division and growth. Even under nitrogen scarcity, *S. cerevisiae* can maintain intracellular NH_4^+ (~3.6 mM) to sustain amino acid and protein biosynthesis essential for proliferation (Usaite *et al*., 2006).

Sulphate (SO_4^{2-}) removal following yeast inoculation is consistent with the known sulphur assimilation pathway (SAP) in *S. cerevisiae*. Uptake occurs via transporters such as SUL1, SUL2, and SOA1, after which sulphate is reduced through a series of enzymatic steps to sulfide, which is then incorporated into cysteine and methionine (Holt *et al*., 2017; Asghari-Paskiabi *et al*., 2020). Baker's yeast showed higher removal efficiency than brewer's yeast, likely due to its greater total sulphur content (0.28–0.86% vs. 0.19–0.65%) (Maw, 1963), reflecting higher demand for sulphur-containing compounds. Sulphate loss in the control (~8.8%) can be attributed to abiotic processes, such as precipitation with calcium, magnesium, or ferric ions, which form insoluble salts like gypsum or ettringite (Benatti *et al*., 2009; Dou *et al*., 2017). While both strains demonstrated significant sulphate removal, the practical application in real wastewater may be limited. Yeast preferentially assimilates organic sulphur sources (cysteine, methionine) over inorganic sulphate because of lower metabolic energy costs (Eschenbruch, 2017). Given that industrial and municipal wastewaters are typically rich in organic nitrogen and sulphur (Duong *et al*., 2019), yeast-based sulphate removal may be less effective in such complex environments.

Nitrate (NO_3^-) removal in *S. cerevisiae* is inherently limited, as this species lacks both the transporters (e.g., YNT1) and enzymes (e.g., nitrate reductase) required for nitrate assimilation, which are present in nitrate-assimilating yeasts such as *Pichia angusta* (Siverio, 2002). Consequently, *S. cerevisiae* is metabolically incapable of using nitrate as a nitrogen source, consistent with the minimal removal observed in this study.

Nitrite (NO_2^-) removal by *S. cerevisiae* is not expected, as this species lacks the transporters and enzymes needed for nitrite assimilation. The substantial removal observed across all groups, including the control, likely reflects abiotic processes. In particular, ferric ions (Fe^{3+}) present in the medium may have promoted Fenton-like redox reactions, converting nitrite into other nitrogenous species such as NO, NO_3^-, NO_2, or N_2O (Xiao *et al*., 2024). These transformations explain the decline in nitrite concentration independent of yeast metabolism.

Conclusion

The results demonstrated that both Malaysian baker's and brewer's yeast have the potential to remove certain inorganic ions under anaerobic conditions in synthetic wastewater. Among the ions tested, phosphate (PO_4^{3-}) showed the highest removal efficiency, followed by ammonium (NH_4^+) and sulphate (SO_4^{2-}). In all cases, the yeast treatment groups outperformed the negative control, indicating an active biological contribution to ion removal. In contrast, nitrate (NO_3^-) and nitrite (NO_2^-) removal was minimal and did not differ significantly from the control, aligning with the known absence of nitrate assimilation pathways in *Saccharomyces cerevisiae*.

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