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Analysis Of Ammonia Assimilation Kinetics And Determination Of Substrate Inhibition Conditions In Variations Of Molasses Dose In A Batch Reactor System

Muhamad Iqbal Putra[a*], Rizky Ibnufaatih Arvianto[a], Suhirman[a], Adna Ivan Ardian[a], Denanda Clarasati Puteri[a], Dennis Farina Nury[a], Muhammad Zulfikar Luthfi[a], Maghfira Arum Lestari[a]

[a]Teknologi Proses Industri Petrokimia, Politeknik Industri

[*]Corresponding author: muhamad.iqbal@poltek-petrokimia.ac.id

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ABSTRACT. The purpose of this study is to find the inhibitory phenomena caused by an excessive organic load and to estimate the ideal molasses dosage for ammonia assimilation by Saccharomyces sp. The experiment was carried out utilizing a batch reactor system with different molasses doses of 0, 5, 10, 15, and 20 mL at a constant starting ammonia content of 50 mg/L. Ammonia assimilation followed a pseudo-first-order model with a significant coefficient of determination R2 > 0.90 in the active dose range, according to the kinetic studies. The results confirmed that a dose of 10 mL was the optimum condition, producing the highest reaction rate constant (k) of 0.5107 day-1 and an ammonia reduction efficiency of 93.58%. On the other hand, raising dosage 20 mL caused a substrate inhibition phenomenon, which was marked by a drop in the k value to 0.2268 day-1 and a low ammonia reduction efficiency of 58.42% because of the initial acidification. The ammonia removal rate and biomass concentration (MLSS) had a very strong positive linear connection (r=0.98) according to Pearson correlation analysis, indicating that nitrogen assimilation with biomass growth-rather than physical volatilization-is the primary mechanism of removal. To optimize ammonia absorption performance without causing environmental toxicity, this study suggests a dose of 10 mL.

1. INTRODUCTION

One important manufacturing sector that generates large amounts of wastewater with a high load of inorganic nitrogen pollutants, especially ammonia, is the petrochemical industry [1], [2]. When ammonia-containing wastewater is released into the environment without proper treatment, it can be poisonous to aquatic ecosystems and cause other major problems [3]. The development of efficient and economical ammonia removal technologies is needed to meet increasingly stringent environmental quality standards.

Formerly autotrophic yeast was used in the nitrification-denitrification process to remove ammonia. However, this approach has significant technical drawbacks, including the extremely slow growth rate of nitrifying yeast and the requirement for high alkalinity [4], [5]; the high alkalinity needed to maintain optimal pH during nitrification, which increases operational complexity and costs [6]; and unstable variations in dissolved oxygen levels and water quality, which also make it difficult to maintain process efficiency [7]. Over time, these issues will raise operational complexity and costs. A viable substitute is the application of yeast, such as Saccharomyces sp., via the heterotrophic absorption pathway. Saccharomyces sp. is more stable under a variety of operating circumstances because it grows more quickly than autotrophic yeast and is highly tolerant of harsh environmental conditions [8]. According to a different study, Saccharomyces sp. can assimilate ammonia and produce biomass that can be utilised as a substitute protein source [9].

The use of molasse as a carbon source has the potential to be useful for ammonia assimilation. However, the proper molasse dosage is required because carbon content might inhibit ammonia (substrate) and reduce ammonia degradation efficiency [10], [11]. According to research, molasse can significantly increase ammonia concentration, but the ratio of carbon to nitrogen must be optimised to provide the best possible results for microorganism growth and nitrogen depletion [10], [12]. In addition, molasse can speed up ammonia degradation by providing carbon that is easily metabolised by heterotrophic microorganisms, improving air quality and the growth of cultured organisms such as shrimp [13]. However, if the molasse dosage is higher, it may cause a pH

shift that detrimental to microbes [12]. Additionally, recent research suggests that complicated substrate inhibition events may be triggered by high concentrations of organic carbon [14], [15]. While Ullah et al. uncover a mechanism of toxicity of weak organic acids that might upset intracellular pH homeostasis, Chen et al.'s studies emphasise the danger of osmotic stress at high sugar concentrations [16], [17].

The characteristics that restrict yeast performance in harsh environments have not received much attention in research to date; instead, it has mostly concentrated on final efficiency. In order to identify the crucial source point between bio-stimulation and substrate inhibition, this study intends to examine the kinetics of ammonia assimilation by Saccharomyces sp. with different molasses dosages. The novelty of this study lies in its holistic approach, linking assimilation kinetics (k) profiles with biomass growth dynamics (MLSS) and pH changes to provide operational recommendations for industrial wastewater treatment.

2. MATERIALS AND METHODS

2.1 Inoculum and Materials

The biological agent utilised in the study was a microbial consortium called Biofill, which contained Bacillus subtilis and nitrifying bacteria in addition to Saccharomyces sp. Molasses, which is derived from regional industrial waste, serves as Saccharomyces' primary source of carbon. Synthetic waste was utilised, and a 25% ammonia solution was diluted to a typical starting concentration of 50 parts per million. Ammonia in the solvent was found using Nessler's reagent (K_2Hgl_4) as an analytical reagent.

2.2 Experimental Design

A 5 L reactor was used in the study, which was carried out in a batch reactor setup. The system was run with five different molasses dose variations of 0 mL, 5 mL, 10 mL, 15 mL, and 20 mL to examine substrate inhibition and ideal meal levels. Fifteen grams of the microbial consortium were added to each reactor, which was then covered with aluminium foil, agitated until it was homogenous, and left for seven days.

2.3 Analysis Procedure

For physical and chemical examination, each sample was taken at prearranged intervals (days 1, 2, 3, 4, 5, and 6). The Nessler method was used to measure the ammonia levels. A UV-Vis device was used to perform backfilling. Shimadzu UV-1900 spectrophotometer operating at 425 nm. Each sample was taken for filtration, its volume was noted, it was dried at 105 °C, and it was weighed to do the biomass growth analysis. The biomass growth analysis's findings were expressed in milligrams per millilitre. An OHAUS Aquasearcher AB33M1 was used to calibrate pH variations with high precision and measure pH dynamics during the assimilation process.

2.4 Kinetic Model

To evaluate the assimilation rate and identify substrate inhibition, changes in ammonia concentration over time were measured and kinetic calculations were performed using a first-order kinetic model. The model was based on data on changes in ammonia concentration that occurred in the study, so that with this phenomenon, the initial assumption can be drawn that the assimilation rate is linear with the reactant concentration. The assimilation rate equation is written as equation (1) as follows:

$$\ln \frac{C_t}{C_o} = -kt \quad (1)$$

Where , C_t is the ammonia concentration at time t (mg /L), C_o is the initial ammonia concentration (50 mg /L), k is the kinetic rate constant (day^{-1}), t is the assimilation time (days). In addition to kinetic measurements, an analysis of the efficiency of each molasses dose was carried out using equation (2) as follows:

$$E(\%) = \frac{C_o - C_t}{C_o} \times 100 \quad (2)$$

2.5 Statistical Analysis

To explain the correlation between the ammonia assimilation rate and biomass growth (MLSS), a Pearson correlation analysis was performed. The Pearson correlation coefficient (r) was calculated to measure the direction and strength of the relationship between the kinetic rate constant and MLSS using equation (3) as follows:

$$r = \frac{\sum(x_i-\bar{x})(y_i-\bar{y})}{\sqrt{\sum(x_i-\bar{x})^2 \sum(y_i-\bar{y})^2}} \quad (3)$$

Where x represents MLSS, y represents k. To support the statistical analysis performed. The calculation of the coefficient of determination (R^2) is used to assess the suitability of the kinetic model. An R^2 value close to 1 indicates that the first-order kinetic model can describe the biological assimilation process with high precision.

3. RESULTS AND DISCUSSION

3.1 Ammonia Assimilation Profile

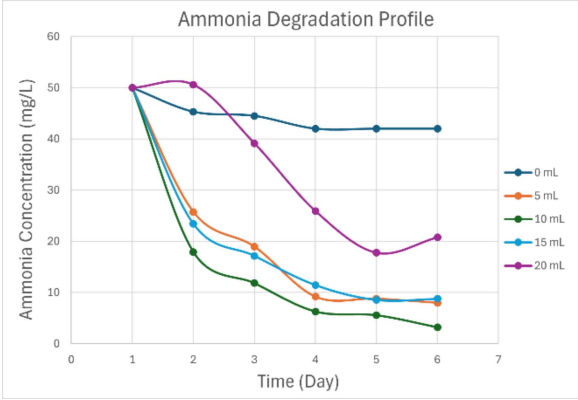

Figure 1. Ammonia Assimilation Profile

Fig. 1 shows the ammonia assimilation profile. The assimilation profile occurring at each dose indicates that the loss of ammonia from the system is dominated by the heterotrophic nitrogen assimilation mechanism by Saccharomyces. sp. by utilizing ammonium ions as the main nitrogen source for the synthesis of amino acids and cellular proteins through the Glutamate enzymatic pathway Dehydrogenase (GDH), Glutamine Synthetase (GS), and Aspartate Aminotransferase (AST). In Saccharomyces sp., the main pathway is the GDH enzyme which acts as a reaction catalyst:

$$NH^{4+} + \alpha\text{-ketoglutarate} \rightarrow \text{L-glutamate} \quad (4)$$

In addition, AST plays a role in converting nitrogen to L-aspartate. The GS pathway exists but is not dominant at high ammonia concentrations. The results of this study indicate that the ammonia assimilation phenomenon that occurs is in harmony with literature studies. Literature studies show that nitrogen products produced by the GDH/AST mechanism include glutamate, aspartate, glutamine, ornithine, spermidine, and trigonelline, which function as basic materials for the synthesis of proteins, nucleotides, and polyamines. This mechanism not only supports cell growth but also converts ammonia into non-toxic organic nitrogen, thus contributing to the control of nitrogen pollution in the aquaculture environment [8]. The optimal dose in this ammonia assimilation profile is shown at a dose of 10 mL molasses. This is the optimum dose that provides abundant carbon for yeast to convert ammonia into non-toxic organic nitrogen products. Conversely, at low doses of 0-5 mL, the assimilation process is hampered by carbon limitations, so that the remaining ammonia cannot be converted.

3.2 Growth Biomass (MLSS)

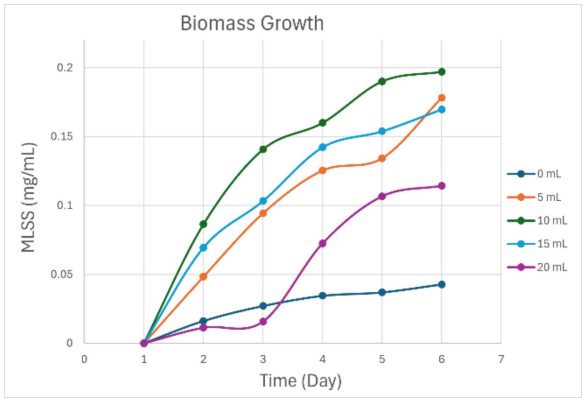

Figure 2. Biomass Growth with Various Molasses Doses

Fig. 2 shows an increase in biomass growth that is directly proportional to the assimilation rate. Optimal biomass growth was found at a molasses dose of 10 mL, where on the last day the biomass growth was obtained at 0.1971 mg/mL. This high cell production is empirical evidence that the loss of ammonia from the system is dominated by the assimilation mechanism of Saccharomyces sp. Saccharomyces sp. utilizes the carbon source from molasses to convert ammonium ions into non-organic nitrogen compounds. Meanwhile, the lowest biomass growth was found at a molasses dose of 20 mL, where on the last day the biomass growth was 0.1142 mg/mL. Data from the 20 mL dose showed a substrate inhibition phenomenon characterized by a decrease in performance in the ammonia assimilation profile and a decrease in biomass growth. This concept is in accordance with the literature study of Chen et al., that high sugar concentrations can create high osmotic pressure, forcing cells to enter a long lag phase before they can start nitrogen metabolism due to osmotic pressure adjustments for survival (osmoregulation) [16].

3.3 pH Dynamics in the Assimilation Process Ammonia

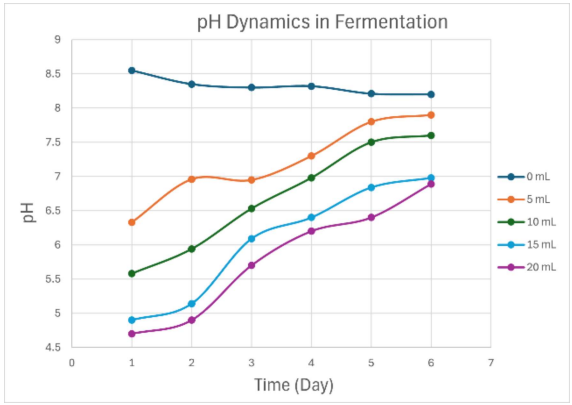

Figure 3. pH Dynamics in the Ammonia Assimilation Process

Fig. 3 shows that the addition of molasses lowered the initial pH, followed by a gradual recovery towards the neutral range over six days during the assimilation process. At 10 mL, the initial pH of 5.58 increased to 7.60 on day 6, consistent with conditions that support fermentation activity and cell recovery. This alkalinization phenomenon indicates the cells' success in metabolizing organic acids from molasses and assimilating ammonium ions. This gradually neutral pH condition creates an optimal environment for the activity of the Glutamate enzyme. Dehydrogenase (GDH) for the conversion of ammonia to amino acids [8]. In contrast, at doses of 15 mL and 20 mL, the system experienced acid stress with an initial pH of 4.90 and 4.70, respectively. This concept is in accordance with the literature study of Ullah et. al., where the initial pH determines the ability of cells to restore intracellular pH (pHi) to ensure optimal [17] cell function in microorganisms. The consequences of acidosis This stress is directly linked to the failure of the assimilation profile and the low MLSS of 0.1142 mg/mL at a dose of

20 mL:

1. Energy Diversion: Cells are forced to use energy to restore intracellular pH to ensure optimal cell function in yeast, instead of using it for protein biosynthesis via the GDH/AST pathway.
2. Enzyme Inhibition: Decreasing pH destabilizes the structure of the GDH and Glutamine enzymes. Synthetase (GS), so that the pathway for converting ammonia into biomass stops.

Therefore, the long lag phase and low efficiency at a dose of 20 mL are due to the failure of cells to cope with the double load: osmotic pressure due to high sugar, as explained in the literature study by Chen et al. and acid poisoning due to low pH, which synergistically inhibits the ammonia assimilation pathway [16].

3.4 Reaction Kinetics and Effects Dose Molasses

Based on the first-order kinetic parameters in Table 1, a dose of 10 mL was identified as the optimum condition, recording the highest reaction rate constant (k) of 0.5107 day^{-1}. At this optimum condition at a dose of 10 mL, the assimilation process can reach an efficiency of 93.58% within 6 days, with a coefficient of determination R^2 of 0.9497. The high value of this reaction rate is linearly correlated with the MLSS peak data and pH stability, amounting to 0.1971 mg/mL and 7.6. The high value of R^2 indicates ideal environmental conditions without stress. Acid and enzyme activity Glutamate Dehydrogenase (GDH) can be explained by first-order kinetics, where the substrate reduction is directly proportional to the remaining reactant concentration. Conversely, increasing the dose to 20 mL causes a drastic decrease in performance. The k value drops significantly to 0.2268 day^{-1} with a final efficiency of only 58.42%.

Table 1. First Order Kinetic Analysis of Ammonia Assimilation Rate

Dosage (mL)	k (day^{-1})	R^2	Efficiency (%)
0	0.0331	0.8085	16.00
5	0.3745	0.9097	84.00
10	0.5107	0.9497	93.58
15	0.3465	0.9087	82.46
20	0.2268	0.8799	58.42

The assimilation curve at a dose of 20 mL in Figure 1 shows a slope in the initial phase, confirming substrate inhibition. The lag that occurs in this initial phase indicates metabolic disturbances, including:

1. Environmental Barriers: Low environmental pH (4.70) forces cells to divert ATP energy for osmoregulation, so that the activation energy for the ammonia assimilation reaction is not met.
2. Biomass Limitation: The low k value is a logical consequence of the minimal amount of active biomass in MLSS of 0.1142 mg/mL so that it is not optimal in carrying out enzymatic reactions.

This kinetic analysis validates that a dose of 20 mL produces a reaction rate that is no longer controlled by the substrate but is limited by the environment.

3.5 Pearson Correlation Analysis between Operational and Response Variables

To find the main influence that controls the rate of ammonia assimilation, a Pearson correlation analysis was conducted on the operational variables of the amount of molasses dose and the response variables MLSS, k, and Efficiency.

Table 2. Statistical Relationship between Molasses Dosage, k, Efficiency, and MLSS

	Molasses Dosage	**k**	**Efficiency**	**MLSS**
Molasses Dosage	1.0000	0.3169	0.4213	0.3390
k	0.3169	1.0000	0.9731	0.9804
Efficiency	0.4213	0.9731	1.0000	0.9959
MLSS	0.3390	0.9804	0.9959	1.0000

Table 2 shows the Pearson correlation matrix of operational and response variables having a strong relationship with an r value of 0.9804, between MLSS and k. Statistically, the Pearson correlation value approaching +1 indicates the dominance of the ammonia assimilation process is influenced by microbial activity, meaning that the rate of ammonia assimilation is directly proportional to the number of Saccharomyces cells. active sp. The

relationship between MLSS and efficiency shows an r value of 0.9731. This confirms that biomass is a variable that has a strong influence on the performance of the ammonia assimilation system. Analysis of the relationship between molasses dosage and the k parameter shows a weak Pearson correlation value of 0.3169. This explains that the relationship between the addition of molasses dosage and k is parabolic. This is clearly shown that in the profile of increasing molasses dosage up to 10 mL, assimilation performance tends to increase, but when added up to 15-20 mL, assimilation performance decreases due to the inhibitory effects of the substrate and acid. stress.

3.6 Comparative Study

This study shows a kinetic rate constant (k) of 0.5107 day^{-1}. This value significantly outperforms the autotrophic nitrification system reported by Bhattacharya et. al.

Table 3. Comparative Study of Biological Agent Performance on Ammonia Assimilation

Biological Agents	Carbon Source	Kinetics (k)	Efficiency (%)	Source
Mixed heterotrophs	Glucose	1.17 mg /l/h	97.00%	[18]
Pilot scale biofilter for AN (ammonia nitrogen)	Glucose, Sodium Carbonate	1.04 days $^{-1}$	91.90%	[19]
Submerged activated sludge	*Palm Oil Clinker* (POC)	5 days $^{-1}$	92.00%	[20]
Autotrophic nitrification	Glucose, Organic Carbon	0.008 days $^{-1}$	89.00%	[21]
Acinetobacter sp. JQ1004 (HN-AD)	*Heterotrophic Nitrification Medium* (HM) and *Denitrification Medium* (DM)	7.93 mg /l/h	54.60%	[22]

This difference explains the hypothesis that the heterotrophic assimilation pathway by yeast is faster in converting nitrogen than the autotrophic bacterial oxide pathway. The efficiency recorded in this study was 93.58%. This is lower when compared to the studies of Ray et al. and Meng et al. of 91% and 97%. This difference lies in the carbon source where in this study used a cheap and environmentally friendly by-product, while other studies used pure glucose. Overall, the study places the molasses-based Saccharomyces sp. assimilation method as a fast and economical solution for treating ammonia waste.

4. CONCLUSION

This research has succeeded in proving that Saccharomyces sp. with molasses as a substrate is a biological agent that can be used to optimally assimilate ammonia. The assimilation performance is influenced by the dose of molasses used, with a dose of 10 mL recorded the best performance with assimilation efficiency and kinetic constant of 93.58% and 0.5107 days^{-1}. These results are supported by Pearson statistical analysis which recorded a value of r = 0.98 between the assimilation rate constant and biomass growth. Overall, this method offers an economical waste treatment solution because it uses molasses as a cheap and environmentally friendly carbon source to convert ammonia into non-toxic nitrogen products.

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