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Optimization of *Saccharomyces cerevisiae* Dose in Eco-Enzyme Fermentation: Effects on pH, BOD, DO, Nitrite, and Nitrate

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Abstract. The contribution of Saccharomyces cerevisiae is expected to accelerate the fermentation process and enhance the microbial population involved in eco-enzyme production. This study aimed to analyze the potential role of S.cerevisiae in improving eco-enzyme production. A true experimental method was employed using a completely randomized design with four treatments and three replications. The results, based on the average matrix similarity test using the Wilks' Lambda Test, showed that the significance values for pH, DO, BOD, nitrite, and nitrate were < 0.05. Therefore, H0 was rejected, indicating that varying doses of S.cerevisiae had a significant effect. Furthermore, significant differences were observed among the treatments for each dependent variable. In conclusion, S.cerevisiae contributes to enhancing the overall quality of eco-enzymes by facilitating fermentation and accelerating the decomposition of organic matter.

Keywords: BOD, eco-enzyme, fermentation, *Saccharomyces cerevisiae*, wastewater valorization

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1. Introduction

Eco enzymes are liquids produced by the fermentation of organic materials and have significant benefits for environmental management. [1,2], Stating that eco enzymes support organic farming as biopesticides and organic fertilizers. Eco enzymes are currently produced from organic waste using molasses solution [3,4]. [5] stated that eco enzyme fermentation products have high microbial activity, so they can be used to inhibit microbial growth, especially pathogenic microbes. All eco enzymes showed gas production, pitera fungi, changes in aroma to sour, dark brown in color, and a pH value below 4. According to [6], the production of pitera fungi in eco enzymes began to appear within 2-10 days after production. Pitera fungi are good fungi derived from eco enzyme fermentation. The presence of pitera fungi indicates that eco enzymes contain more organic acids and have a lower pH, so the presence of Pitera fungi indicates good eco enzyme quality.

The bacteria found naturally in vegetables, fruits, and their peels help convert carbohydrates into simple ones that yeast can use [7]. *Saccharomyces cerevisiae* is a group of microbes that are classified as yeast. The content contained in yeast cells *Saccharomyces cerevisiae* consists of: crude protein, carbohydrates, fats, and minerals, which provide opportunities for these microorganisms to be utilized as fermentation agents in the provision of eco enzymes [8,9]. In line with the research results of [10], which stated that the kinetic parameters of single-cell protein production fermentation by yeast in non-dairy creamer liquid waste media had the highest protein content of 22.15 mg/l, specific growth rate (μ) 0.240868 (hour-1), which can be interpreted that these microbes can adapt and grow well in waste media.

The accumulation of organic waste from leftover feed and feces in fish farming causes sedimentation at the bottom of the pond, necessitating a decomposition process. If organic waste is not decomposed, the rearing medium will decompose anaerobically, producing toxic gases that negatively impact the cultivation process. [11] stated that ammonia levels in water for tilapia (*Oreochromis sp*.) is <0.02 mg/L. This is due to the presence of fish feces and the lack of water changes during maintenance. Water quality management to maintain good maintenance media is necessary to reduce organic waste discharged into public waters. One approach to water quality management is biological, utilizing bacterial activity to accelerate the decomposition of organic waste.

The study aims to examine the influence of providing *Saccharomyces cerevisiae* with different variations in eco enzyme performance. pH, DO, BOD, nitrite, nitrate levels were analyzed as indicators of eco enzyme quality. The use of eco enzymes as organic waste decomposers to reduce waste pollution can be pursued as a sustainable fish farming management technology.

2. Methods

The design used in this study was a completely randomized design, with 4 treatments and 3 replications, to obtain optimal eco enzyme results. The treatments tested were:

P0 = 1 kg organic waste media + no dosage given *S.cerevisiae*
P1 = 1 kg organic waste media + *S.cerevisiae* 2%
P2 = 1 kg organic waste media + *S.cerevisiae* 4%
P3 = 1 kg organic waste media + *S.cerevisiae* 6%

Referring to research [12]

Indicators of successful eco enzyme formation are a dark brown color and a fresh, sour aroma typical of fermentation, and an acidic pH <4.1 [13,14]. Contains nitrite and nitrate, which are required by the media as nutrients [15]. Nitrite and nitrate content can be measured using a spectrophotometric method, media pH using a pH meter, DO content measurement using a DO meter, and BOD through an incubation method [13].

3. Results and Discussion

Table 1. pH measurement

Treatment	Repetition		
	1	2	3
P0	2,59	2,69	2,79
P1	2,90	3,10	3,00
P3	3,00	3,20	3,10
P4	3,10	3,30	3,20

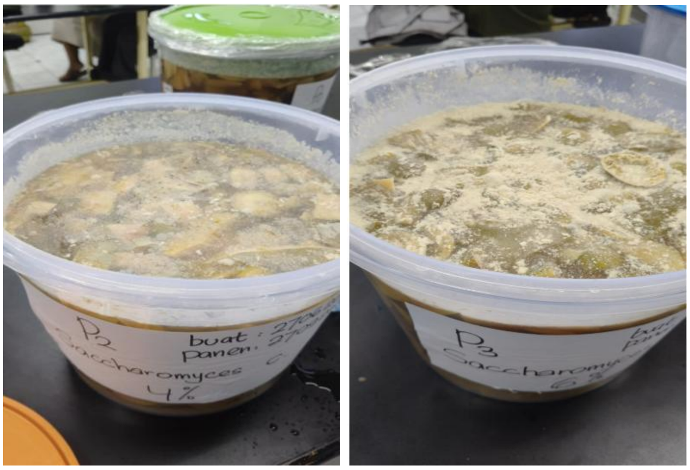

Figure 1. Eco enzymes grown on Pitera fungi

Table 2. Average Summary

Dependent Variable	Treatment			
	P0 (without saccharomyces)	P1 (Saccharomyces 2%)	P2 (Saccharomyces 4%)	P3 (Saccharomyces 6%)
Nitrit	0,00700	0,00433	0,00367	0,00200
Nitrat	2,267	3,500	3,667	3,800
DO	60,67	64,67	75,33	77,67
BOD	532,00	516,00	494,67	460,00

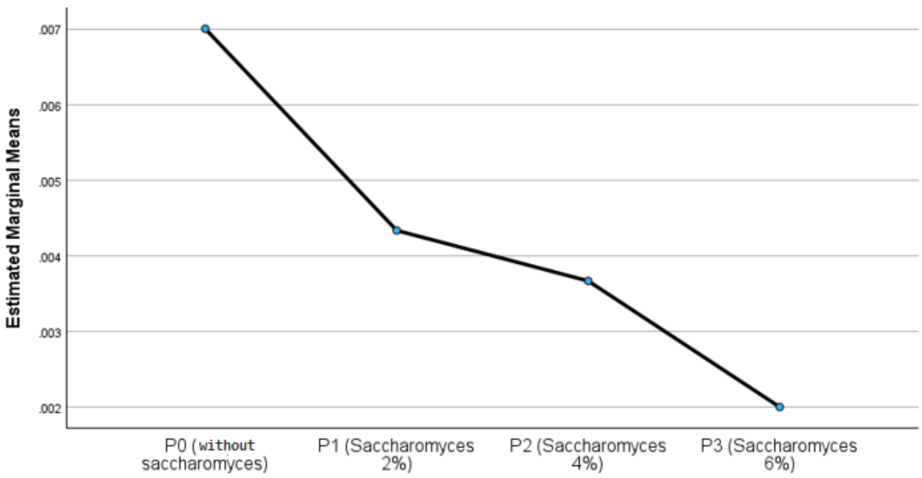

Figure 2. Estimated Marginal Means of Nitrit

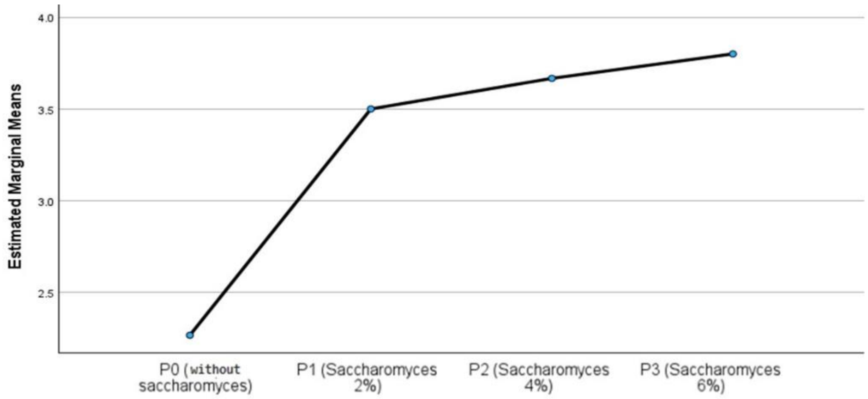

Figure 3. Estimated Marginal Means of Nitrat

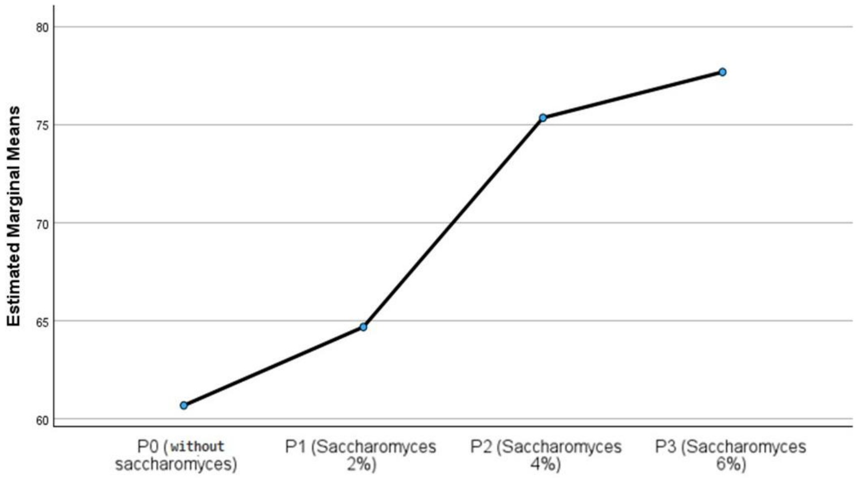

Figure 4. Estimated Marginal Means of DO

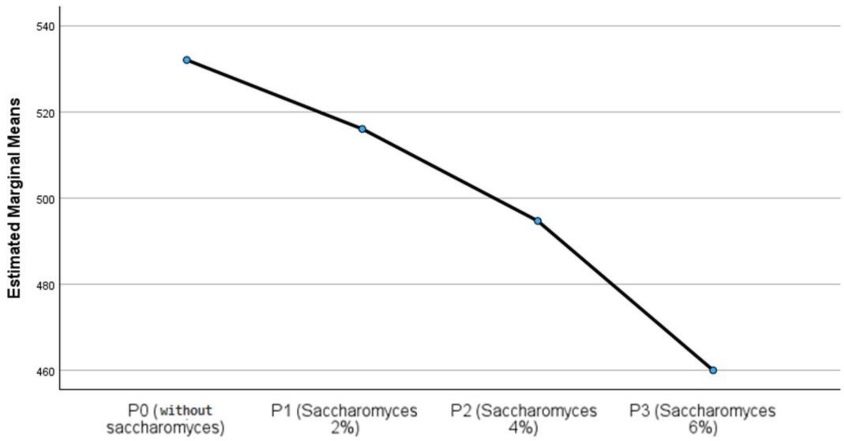

Figure 5. Estimated Marginal Means of Nitrat

This study involved four dependent variables, namely Nitrite, Nitrate, DO, and BOD. Based on the test of the similarity of the mean matrix using the Wilks Lambda Test, the sig. value was <0.05, so H_0rejected. Therefore, it can be seen that there are significant differences between the treatments for each dependent variable. Furthermore, to determine which dependent variable showed a significant difference, a post-MANOVA test was conducted, namely ANOVA for each dependent variable. Based on the post-MANOVA test, namely ANOVA for each dependent variable, it was found that significant differences occurred in the four dependent variables. This is indicated by the Sig. value < 0.05, so H_0 rejected for each dependent variable. Next, a post-ANOVA test was conducted, namely LSD (*The least significant difference*) to see which treatment resulted in a significant difference in each dependent variable.

Based on the post-ANOVA test using LSD (Least Significant Difference), it was observed that treatment P0 had higher nitrite levels compared to treatments P1, P2, and P3, while treatments P1 and P2 showed no significant difference but both had higher nitrite levels than P3. For nitrate, treatment P0 had lower levels than treatments P1, P2, and P3, with no significant differences observed among P1, P2, and P3. In terms of dissolved oxygen (DO), treatments P0 and P1 were not significantly different, but both had lower DO levels than treatments P2 and P3, which also did not differ significantly from each other. For biochemical oxygen demand (BOD), treatments P0 and P1 showed no significant difference and had higher BOD levels than P2 and P3, while treatment P2 had a higher BOD level than P3.

In line with the research results of [16], a good eco enzyme fermentation solution has a brownish color depending on the composition of sugar and organic materials used. In addition, eco enzymes have a fresh, sour aroma resulting from fermentation originating from the acetic acid (H_3COOH) content. The results of the study showed a pH value ranging from 2.59 to 3.3. [17] stated that the low pH value of this eco enzyme is caused by the high content of organic acids, such as acetic acid and citric acid. Acetic acid is produced through the bacterial metabolic process that occurs anaerobically, namely the bacteria's effort to obtain energy in the absence of oxygen. The acetic acid and citric acid content in eco enzymes function to inhibit the growth of certain types of fungi. This is in accordance with research conducted by [18] which showed that acetic acid and citric acid significantly inhibit the growth of *Colletotrichum sp*. and several pathogenic fungi in plants. This inhibition increases with decreasing pH from 6.0 to 4.0. The use of acetic acid for control *Colletotrichum sp*. conducted in the research was also found to be safer than the use of chemical pesticides.

Eco enzymes also contain propionic acid, amylase, lipase, trypsin, and protease enzymes. The propionic acid content in eco enzymes is produced from sugar fermentation which functions to lower the pH so that microbes cannot live [19]. Eco enzyme solutions produced from organic materials such as fruits produce acidic liquids with a low pH value. Some fruit peels are used in the manufacture of eco enzymes, including pineapple peels. Pineapple peels themselves contain vitamin C, carotenoids and flavonoids. In addition, pineapple peels contain tannins, oxalates, and phytates [20,21]. Flavonoids are the most abundant phenolic compounds found in nature. Flavonoids have uses including as antibacterials, antimicrobials, and antivirals [22]. Flavonoids can also inhibit fungal growth by inhibiting cell division and disrupting cell membranes [23]. Furthermore, citrus peels contain several secondary metabolites, including alkaloids, flavonoids, tannins, phenols, saponins, and steroids. Furthermore, cucumbers also contain flavonoids, which act as antifungal agents [24]. The eco enzyme fermentation process produces compounds that are useful in agriculture. Eco enzymes contain acetic acid (H_3COOH), which can kill germs, viruses, and bacteria that cause infections. They also contain lipase and amylase, which can prevent the development of pathogenic bacteria. They also contain NO_2 (nitrite) and NO_3 (nitrate), which are needed by the media as nutrients [25].

Saccharomyces cerevisiae The fungus used as a starter in the production of eco enzymes functions as a fermentation agent that converts sugar (glucose) into alcohol (ethanol), carbon dioxide, and energy. This process occurs anaerobically, meaning it does not require free oxygen. This fungus also plays a role in producing enzymes that help speed up biochemical reactions in the eco enzyme production process.

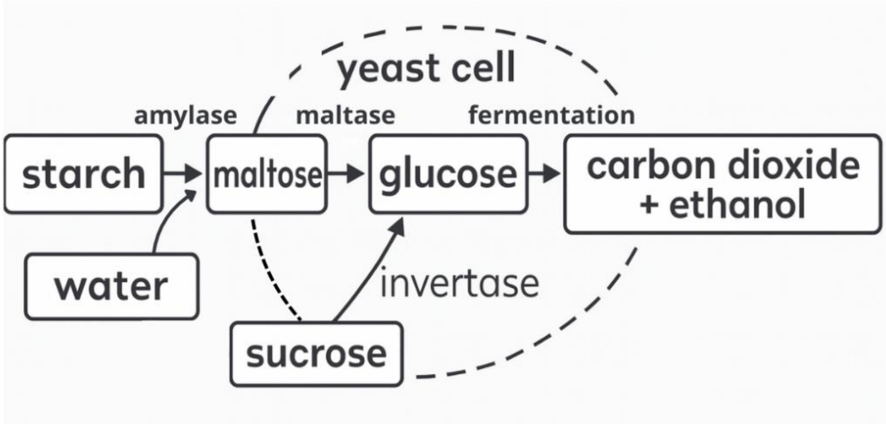

Figure 6. Metabolic process in *Saccharomyces cerevisiae*

The amylase enzyme functions to convert starch into maltose and glucose with the following reaction equation:

$$(C_6H_{10}O_5)n \xrightarrow{\boxed{\text{Enzim amilase}}} C_{12}H_{22}O_{11} + C_6H_{12}O_6$$

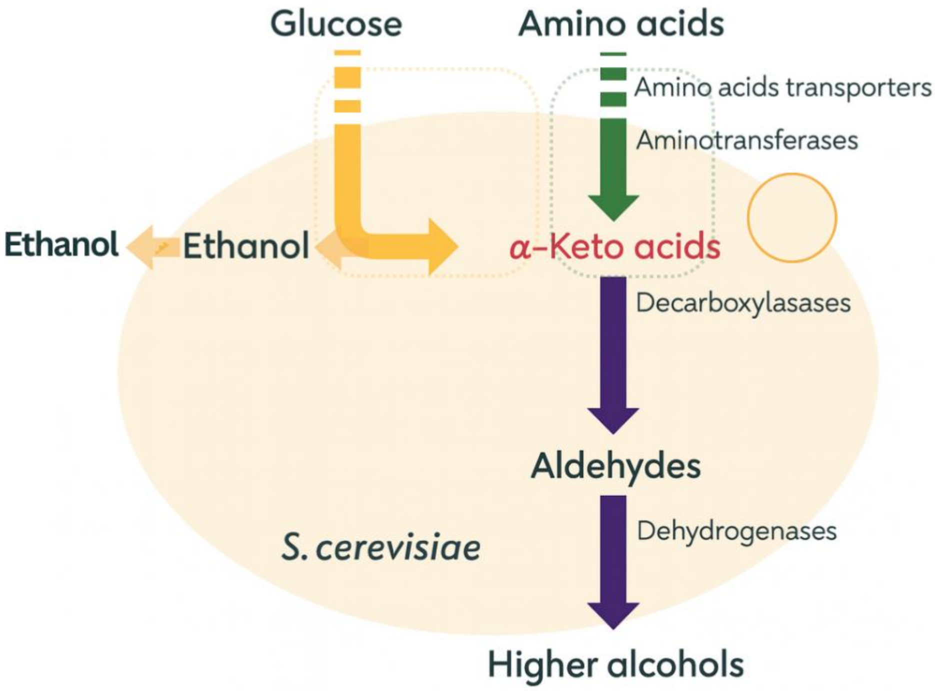

Figure 7. Metabolic Pathway of Higher Alcohol Formation in *Saccharomyces cerevisiae*

Nitrites and nitrates in eco enzymes, which are formed during the fermentation process, actually have no primary function within the eco enzyme itself. Eco enzymes are better known for the microorganisms and enzymes they produce during fermentation, which are beneficial for various purposes. Nitrites and nitrates are more likely byproducts of the process. Fermentation carried out by *Saccharomyces cerevisiae* Produces various enzymes that help in the decomposition of organic matter and the process of maturation of eco enzymes.

Based on the results of the study of dose variations *Saccharomyces cerevisiae* 6% gives the best results, with a nitrite content of 0.00200 ml/l, nitrate 3,800, DO 77.67 and BOD 460.00. The low nitrite content is because nitrite is converted to nitrate, this change is important considering that nitrite can act as a poison. In line with research by [26] which states that high nitrite concentrations in water can harm aquatic organisms, while Nitrate is a macro nutrient that is not toxic in normal concentrations. Nitrate is one of the nutrients needed by organisms to support their growth.

The BOD levels in eco enzymes decrease as the dose increases. *S.cerevisiae,* this is because the organic material in the eco enzyme is increasingly being broken down by microorganisms, which then produces metabolic products of microorganisms so that they are able to stop the biological activity of the microorganisms themselves in the eco enzyme. This statement is in line with the statement expressed by [27], which states that the decrease in BOD levels is caused by the metabolic process of microorganisms in the eco enzyme which then produces alcohol and acetic acid compounds which are divided into 2 reactions, namely the alcohol fermentation reaction, namely the reaction of converting glucose into ethanol (ethyl alcohol) and carbon dioxide. The first thing that happens is the hydrolysis of pyruvate with water molecules, releasing carbon dioxide from pyruvate and converting it into two-carbon acetaldehyde. Acetaldehyde is reduced by NADH to ethanol, thereby regenerating NAD + which is needed for glycolysis. The microorganisms involved are generally *Saccharomyces cerevisiae* and the reaction that occurs in this fermentation is anaerobic. Ethanol is the main product of this fermentation, in addition to other products such as acetic acid. Furthermore, the acetic acid fermentation reaction is carried out by acetic acid bacteria (*Acetobacter aceti*) with ethanol as a substrate. If given sufficient oxygen, these microorganisms can produce acetic acid from organic materials, namely fruit peel waste.

Enzymes produced by *Saccharomyces cerevisiae* It also plays a role in reducing unpleasant odors that may arise during the fermentation process. By assisting the fermentation process and the decomposition of organic materials, *Saccharomyces cerevisiae* contribute to the overall improvement of eco enzyme quality. *Saccharomyces cerevisiae* is a key component in eco enzymes that aids in fermentation, enzyme production, and improving the quality and effectiveness of eco enzymes. Characteristics of successful eco enzymes are dark brown in color and have a strong, sweet and sour fermentation odor. White mold is present. Meanwhile, characteristics of failed eco enzymes can be identified by a foul odor and the appearance of black mold on the surface.

4. **Conclusion**

Saccharomyces cerevisiae as a starter, it is a key component in eco enzymes that helps in fermentation, enzyme production, and improving the quality and effectiveness of eco enzymes. The results of the study showed that based on the average matrix similarity test using the Wilks Lambda Test, it was found that DO, BOD, NO_2 (nitrite), NO_3 (nitrate) Sig. values <0.05, so that H_0 rejected.

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