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The Preliminary Study about Physico-Chemical Property of Bio-Enzyme Produced from Orange Fruits Waste Treated with Different Concentrations of Probiotic

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ABSTRAK

Limbah buah-buahan dilaporkan sebagai salah satu penyumbang utama produksi limbah makanan. Limbah buah mempunyai dampak terhadap lingkungan sehingga perlu ditangani. Limbah buah harus diolah terlebih dahulu untuk mengurangi dampak negatifnya sebelum dibuang ke lingkungan. Sementara itu, limbah buah telah dilaporkan mengenai sifat fungsional dan nutrisinya sebagai bio-enzim. Kajian pendahuluan ini bertujuan untuk memberikan informasi mengenai karakteristik fisika-kimia bioenzim meliputi pH, oksigen terlarut (DO), total padatan terlarut (TDS), warna, dan bau bioenzim dari limbah buah jeruk yang diberi perlakuan konsentrasi probiotik berbeda dalam fermentasi anaerobik. Bio-enzim merupakan larutan fermentasi yang terbuat dari campuran 15 g gula merah : 5 kg limbah buah jeruk : 12 L air. Rancangan penelitian yang digunakan adalah perlakuan fermentasi bioenzim dengan penambahan probiotik yaitu 0 ml (kontrol), 80 ml (P1), 160 ml (P2), 240 ml (P3), dan 320 ml (P4) untuk masing-masing drum plastik tertutup 25 volume L yang kemudian difermentasi selama 35 hari. Studi pendahuluan menghasilkan efisiensi gula merah dan efektivitas limbah buah dalam menghasilkan bio-enzim dengan pH 3,4-3,6, TDS 982,7-1152,5 ppm, DO 1,4-2,2 ppm, N total 0,017-0,035%, P 0,017-0,02%, K 0.046-0.192%, C organik 0.782-0.936%, rasio C/N 28.29-47.36, gula reduksi 0.005-0.134%, jumlah lempeng total 4.9 x 104 hingga 1.1 x 105 koloni/g, kapang & ragi 2.2 x 104 s/d 5,8 x 104, warna cairan coklat muda, sedikit asam hingga asam dan berbau segar, permukaan cairan tertutup jamur, Si 12,93-22,53%, Al 1,96-2,39%, Ca 3670-6940 ppm, dan Fe 214-806 ppm. Penelitian ini berlanjut hingga 3 bulan untuk mengetahui keanekaragaman metagenom mikroorganisme anaerobik pada fermentasi bioenzim, karakteristik enzim, produksi gula pereduksi, dan sifat kimia bioenzim limbah buah jeruk.

***Kata kunci*:** fermentasi anaerob, bio-enzim, limbah buah jeruk, fisika-kimia

ABSTRACT

Fruit wastes are reported as among the main contributors to the food waste production. They have impact on the environment so they need to be treated. To reduce the negative effects, fruit wastes must be treated before they are released into environment. Meanwhile, the fruit wastes have been reported about their functional properties and nutritional as bio-enzyme. This preliminary study aimed to provide information about the physico-chemical characteristics of bio-enzyme, including pH, dissolved oxygen (DO), total dissolved solids (TDS), color, and smell of bio-enzyme from orange fruit wastes treated different concentrations of probiotic in anaerobic fermentation. Bio-enzyme was a fermented solution made from a mixture of 15 g brown sugar : 5 kg orange fruit waste : 12 L water. We designed treatments of bio-enzyme fermentation with addition of probiotic, namely 0 ml (control), 80 ml (P1), 160 ml (P2), 240 ml (P3), and 320 ml (P4) for each closed plastic drums 25 L in volume and then were fermented for 35 days. The preliminary study resulted that efficiency of brown sugar and effectivity of fruit waste to produced bio-enzyme with pH 3.4-3.6, TDS 982.7-1152.5 ppm, DO 1.4-2.2 ppm, N total 0.017-0.035%, P 0.017-0.02%, K 0.046-0.192%, C organic 0.782-0.936%, C/N ratio 28.29-47.36, reduction sugar 0.005-0.134%, total plate number 4.9 x 10^4 to 1.1 x 10^5 colony/g, mold & yeast 2.2 x 10^4 to 5.8 x 10^4, light brown of liquid color, little sour to sour and freshness in odor, fungi covered surface of the liquid, Si 12.93-22.53%, Al 1.96-2.39%, Ca 3670-6940 ppm, and Fe 214-806 ppm. This research continues for up to 3 months to determine the metagenome diversity of anaerobic microorganism in bio-enzyme fermentation, characteristics of enzyme, reducing sugar production, and chemical properties of bio-enzyme from orange fruit wastes.

***Keywords*:** anaerobic fermentation, bio-enzyme, orange fruit waste, physico-chemical

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1. INTRODUCTION

The fruit agribusiness, production and processing sector, including post-harvest and processing stages until retail and final consumption produces food waste or food by-products (Plazzotta et al. 2017). This organic waste contributes to environment quality that cause an undesirable impact on the environment. Fadlilla et al. (2023) explain that organic waste contributes to environmental pollution due to an unpleasant odor, water and soil contamination, and beauty reducing of environment when the fruit wastes are disposed in open landfills without further processing.

The fruit wastes have been reported about their functional properties and nutritional, it contains a large number of bioactive compounds with functional ingredients (Lucarini et al. 2021; Zhu et al. 2023). It contains a variety of bioactive compounds such as dietary fiber, flavonoids, phenolic compounds, antioxidants, polysaccharides, and several other health-promoting nutrients and phytochemicals. These bioactive compounds can be extracted and used as value-added products in different industrial applications (Nirmal et al. 2023). One of extracted product from fruit wastes is bio-enzymes are also called as eco-enzyme or garbage enzyme (Lakra et al. 2022).

Bio-enzyme have a multiple functionality like use as bio-fertilizer, reducing contamination of ground water and use as natural detergent (Patel et al. 2023). One of organic waste source that can be processed into bio-enzyme is orange fruit. Orange fruit has unique properties, such as phytochemicals, polyphenols, vitamins (a good source of vitamin C), minerals, dietary fibers, essential oils, amino acids, pectin, carotenoids, flavonoids, a high acidity value, to smell and a strong flavor (Benny et al. 2023).

One of bio-enzyme application is to treat domestic waste water and to clean water body, mostly ponds (Penmatsa et al. 2019; Kerkar & Salvi, 2020). We want to implement bio-enzyme in ex-tin mining pond waters that have acidic water and heavy metal contaminations. However, the quality of bio-enzyme must be analyzed to make sure its effectiveness.

In this preliminary research, we focus to observe the physico-chemical characteristics of bio-enzyme from orange fruit waste like pH, dissolved oxygen (DO), total dissolved solids (TDS), elements, color, and smell. This study will support the further analysis about biochemical characteristics and metagenome diversity of microorganism of the bio-enzyme.

2. METHODOLOGY

This research was conducted from July to August 2023 in Laboratory of Aquaculture, Faculty of Agriculture, Fishery, and Biology, Bangka Belitung University, Indonesia.

The process of making bio-enzyme was begun with collecting orange fruit waste from fruits seller. The orange wastes were washed cleanly and put into the plastic drum 25 L in volume. Bio-enzyme was a fermented solution made from a mixture of 15 g brown sugar : 5 kg orange fruit waste : 12 L water. We designed treatments of bio-enzyme fermentation with addition of probiotic, namely 0 ml (control), 80 ml (P1), 160 ml (P2), 240 ml (P3), and 320 ml (P4) for each closed plastic drums. The drums were tightly closed and stored in a place that was not exposed to direct sunlight or rain and orange fruit wastes were fermented for 35 days in this closed plastic drums.

In the early 35 days of first month, the drum lid is routinely opened for observation day, namely 0 day, 1 day, 5 days, 10 days, 15 days, 20 days, 25 days, 30 days, and 35 days. This preliminary study was done for measuring the physico-chemical of bio-enzyme as preliminary research, including pH, dissolved oxygen (DO), total dissolved solids (TDS), color, odor, the presence of fungi, and elements.

3. RESULT AND DISCUSSION

Bio-enzymes or eco-enzyme was discovered by Dr. Rosukon from Thailand. Bio-enzyme was the result of fermentation for 3 months from a mixture of sugar, fruit and vegetable wastes, and water with a ratio of 1 : 3 : 10 (Rasit et al. 2019; Galintin et al. 2021). The manifestation of ratio was production of 10 L garbage enzyme, used 3 kg of fruit wastes was fermented together with 1 kg brown sugar and 10 L water for three months (Jabhade et al. 2022). However, this ratio was not efficient, especially using brown sugar with high concentration. We made new bio-enzyme formulation with mixing brown sugar 15 g : 5 kg fruit wastes : 12 L water and we added commercial probiotic in different concentration for research treatments. Addition of probiotic aimed to increase abundance of anaerobic fermentation microorganism and effectivity decomposition process of organic waste.

Sugar are nutrients as a carbon source for microorganisms in the process of making bio-enzyme (Gumilar et al. 2023). In this research, brown sugar was used in lower concentration than other researches. We think that brown sugar only was used in adaptation and first growth of fermentation microorganism. When microorganism have grown, furthermore microorganism can use fruit that content simple sugar as shorter chains of carbohydrate such as disaccharide and monosaccharide as their carbon source. On the other hand, we used quantity of fruit wastes in higher concentration than other researches, to increase organic wastes that can be decomposed in fermentation process. This research resulted there was a difference of TDS value where the higher TDS found at some references use ratio 1 : 3: 10. Meanwhile, pH value indicated there was no significant differences between ratio of this research with ratio 1 : 3 : 10. Even more, our fermentation day was 35 days compared 3 months in other researches. The comparation of pH and TDS value between treatment ratio of this research with 1 : 3 : 10 from references were shown in Table 1.

Based on data of TDS from 0 day to 35 days for all treatment, it showed regression equations for control (y = 93,535x + 128,2) and P4 (y = 120,27x + 118,48) (Figure 1). Base on the regression equations, the simulation for x value was 90 days or 3 months, TDS of control was 8546,4 mg/L and TDS of P4 was 10943,0 mg/L.

The TDS value of treatment in this research was higher than Muliarta et al. (2023) and Permatananda & Pandit (2023), however lower than Rasit et al. (2019) and Galintin et al. (2021). The differences of TDS value between results of this research with others can be caused by brown sugar concentration. Retnowati and Kusnadi (2014) explain the TDS value probably increased along with the percentage of brown sugar addition. Brown sugar concentration can stimulate lactic acid bacteria (LAB) growth and then the lactic acid was secreted from LAB will be accumulated and measured as total dissolved solids.

Even though TDS value was lower than others, We proposed new formula in making bio-enzyme with taking a mixture of 15 g brown sugar: 5 kg fruit waste: and 12 L water. We considered that the amount of fruit waste used was greater than in other studies so that the amount of organic waste that can be reduced from the environment can be increased. In addition, the efficiency of using brown sugar was a consideration during the fermentation process and the addition of high brown sugar can cause the color of bio-enzyme become more dark or brown. Organic substances indicated the enzyme solution contained large amounts of organic matters which due to the fruit wastes and brown sugar that being added as substrate in fermentation process. Furthermore, these fermentation materials that considered as organic solid wastes were also the factor for high TDS in bio-enzyme.

During batch culture, microorganism grow in five phases of growth, namely lag phase, exponential phase, stationary phase, stop replicating phase, and death phase. Lag phase is an important phase for microorganism growth due to it describes the adaptation required for bacterial cells to begin the life in new environmental conditions (Rolfe et al. 2012). In the bio-enzyme production, sugar is an energy source for microorganism in carrying out fermentation, while microorganism that carry out fermentation require energy which is generally obtained from glucose (Arun & Sivashanmugam, 2015; Viza, 2022; Permatananda & Pandit, 2023). Reischke et al. (2015) explain adding organic substances of monosaccharide like glucose will trigger a microbial response and Reischke et al. (2014) also explain that adding monosaccharide resulted an initial lag phase of stable respiration and bacterial growth.

Table 1. Comparation TDS and pH Value of Fermentation for 35 Days and 90 Days with different Ratio Between Sugar, Fruit Wastes, and Water

Parameters	Fermentation for 35 days in this research (ratio 15 g brown sugar : 5 kg fruit wastes : 12 L water)					Fermentation 3 months in other researches (ratio 1 : 3 : 10)			
	Control (0 ppm)	P1 (80 ml)	P2 (160 ml)	P3 (240 ml)	P4 (320 ml)	*a)	*b)	*c)	*d)
TDS (mg/L)	982,7	991,3	999,0	1012,5	1152,5	15900	1102	278	14000
pH	3.6	3.6	3,4	3,5	3,4	3,07	3,67	3,3	2,86

Sources: a)Galintin et al. (2021); b)Muliarta et al. (2023);c) Permatananda & Pandit (2023); d)Rasit et al. (2019)

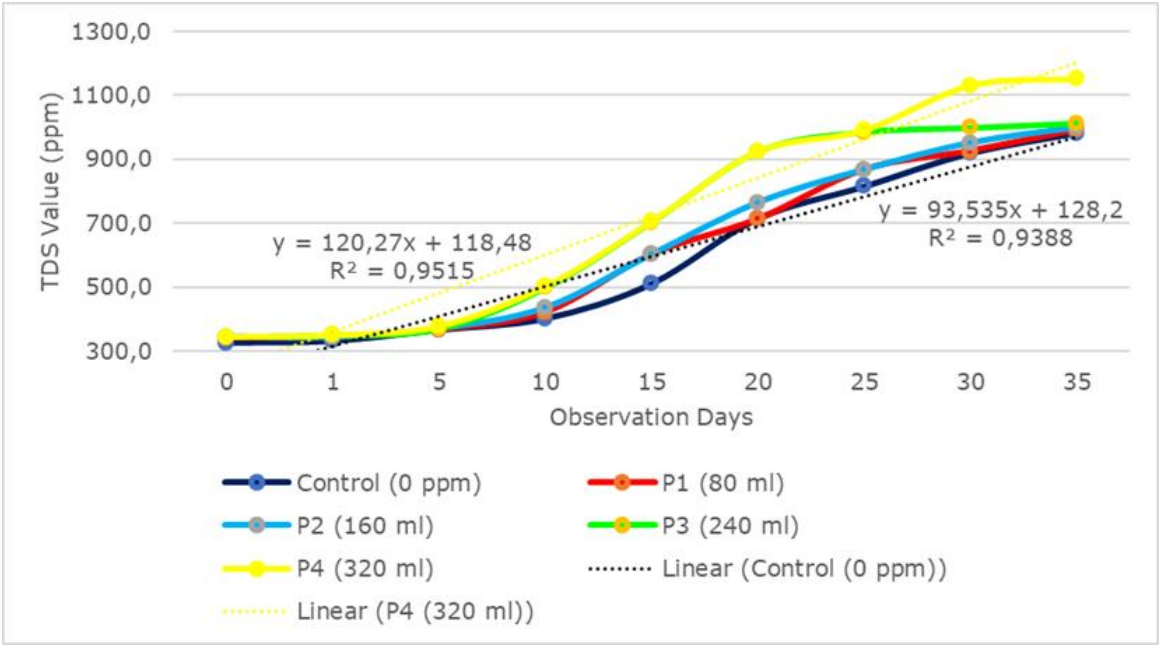

Figure 1. TDS Value for 35 Days Fermentation

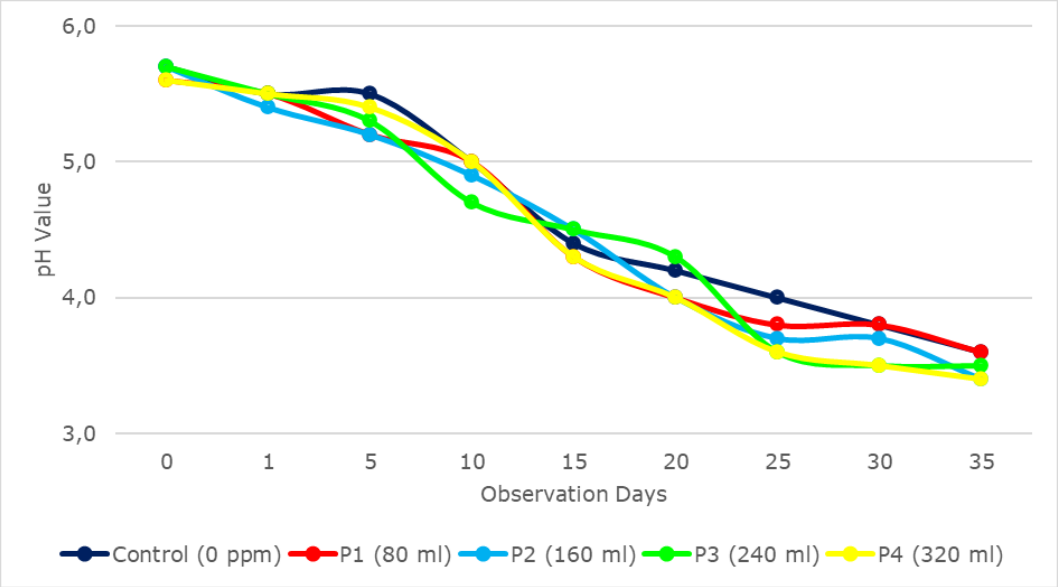

Figure 2. pH Value for 35 Days of Anaerobic Fermentation

In the next phase, exponential growth, it is characterized by the quickest growth rate, the shortest generation time, and exponential population expansion. The exponential growth rate requires some factors to be present in excess in the growth medium, including sources of carbon, nitrogen, phosphate, and certain trace elements (Rolfe et al. 2012). Decomposition process in fermentation is the breakdown of organic matter into simpler organic or inorganic stuff such as simple sugars (Benny et al. 2023). The main nutrients in oranges include carbohydrates (9.3-11.1 g), protein (0.8-1 g), fiber (0.3-1.7 g), fat (0.1-1 g), mineral (0.3-0.7 g), vitamins (30-68 mg), phosphorous (10-30 mg), iron (0.1-2.3 mg), calcium (26-90 mg), and energy (43-57 K.cal) per 100 g of edible portion (Abobatta 2019). Carbohydrates are commonly referred to as polysaccharides that can be decomposed to be disaccharides (e.g sucrose and lactose) and monosaccharide (e.g glucose and fructose), being used as an energy source (Awuchi & Amagwula, 2021) for microorganism in fermentation process (Wang et al. 2012). The use of fruit waste in large quantities according to the formula we have offered has the potential to provide organic matter such as carbohydrates and others as nutrition for microorganism growth. Thus, brown sugar was only used as a starter in the adaptation (lag) phase and then microorganisms will utilize the fruit waste as nutrients in the exponential (log) phase until the nutrients were depleted as long as decomposition processing.

Based on this rationality reasons, we still measured physico-chemical property of bio-enzyme produced from orange fruits waste treated with different concentrations of probiotic in this preliminary study. pH valuie indicated fermentation was occurred in acidic condition with pH between 3.4 to 3.6 in 35 days of fermentation process (Figure 2).

Organic acid is important key in determination of acidity. It means that the higher the organic acid concentration determine the lower pH value. In this preliminary research, bio-enzyme had low pH value as a result of organic acid that was produced. Orange fruits are one of organic acids source including citric acid (679.2 mg/100 mg orange juice) and ascorbic acid (58.0 mg/100 mg orange juice) (Carvalho et al. 2020). The pH of bio-enzymes has been reached below pH 4.0 and it was taken 3 months for production from organic wastes (Rahayu et al. 2021).

The fermentation process of organic waste increased the decomposition of organic substance and microorganism activity to produce enzyme as they provided an acidic environment. pH value of this study with ratio of 15 g brown sugar: 5 kg fruit waste: and 12 L water with different treatments was below 4.0 for 35 days (Figure 2). It indicated the fermentation process was correct, bio-enzyme product was good quality, and organic acids (acetic acid) was produced with optimally function as well as Hafsah & Mushonev (2022). The pH of the bio-enzyme will also depend on the amount of water added and the fermentation time. Generally, the longer the fermentation time impact the lower the pH (Benny et al. 2023). Furthermore, sugar type (Sai et al. 2023) and type of organic waste also impact to pH value (Juma'at et al. 2021).

This fermentation was occurred in facultative anaerobic condition with indication low dissolved oxygen value. The DO of bio-enzyme production decreased to 1.4-2.2 ppm in 35 days of fermentation (Figure 3). Low pH value and dissolved oxygen can support the acidophilic and facultative anaerobic microorganism to grow optimally. The microorganism, including bacteria and fungi and the content of organic waste were the important key in determining acidity and anaerobic condition. The presence of microorganism was shown in Figure 4 that visualize fungi growth along fermentation days.

Nutrient-rich content of fruit wastes as organic waste is an alternative resource for microorganisms (Ling et al. 2023). Tang et al. (2018) explain during the fermentation processes, organic wastes will be hydrolyzed into dissolved organic matter and then acidified into organic acids. Microorganisms to use solid wastes and enhancing denitrification. Organic wastes are decomposed into organic acids and soluble polysaccharides in the fermentation liquid as carbon sources and could increase microbial diversity. Mahdia et al. (2022) explain bio-enzymes from fresh citrus waste after a 3-month fermentation period contained bacteria and fungi of 1.9 x 106 CFU/ml and 8.5 x 105 CFU/ ml, respectively, with a pH of 3.39 ± 0.023.

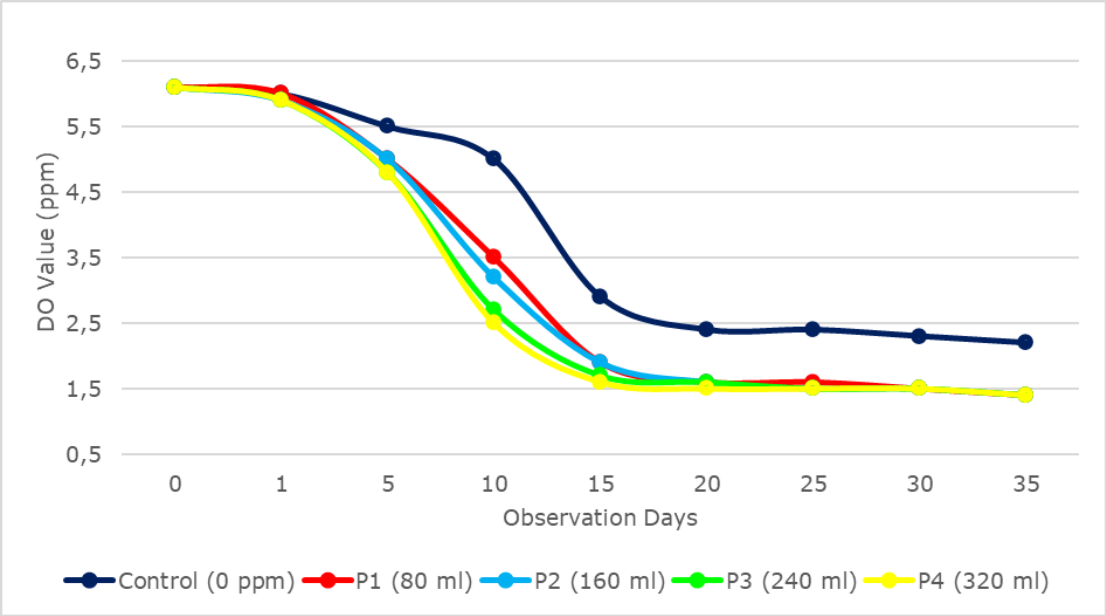

Figure 3. Dissolved Oxygen (DO) Value for 35 Days of Anaerobic Fermentation

Figure 4. Visualization of Bio-Enzyme Production from Orange Fruit Wastes by Anaerobic Fermentation: 0 Day (a); 1 Day (b); 5 Days (c); 10 Days (d); 15 Days (e); 20 Days (f); 25 Days (g); 30 Days (h); and 35 Days (i)

Table 2. Physico-Chemical of Bio-Enzyme with Ratio 15 g Brown Sugar : 5 kg Fruit Wastes : 12 L Water in 35 Fermentation Days

Parameters	Fermentation for 35 days in this research (ratio 15 g brown sugar : 5 kg fruit wastes : 12 L water)				
	Control (0 ppm)	P1 (80 ml)	P2 (160 ml)	P3 (240 ml)	P4 (320 ml)
N total (%)	0.022	0.031	0.017	0.035	0.027
P as P_2O_5 (%)	0.017	0.019	0.018	0.019	0.02
K as K_2O (%)	0.057	0.046	0.052	0.056	0.192
C organic	0.782	0.877	0.805	0.936	0.927
C/N ratio	35.35	28.29	47.36	26.76	34.34
Reduction sugar (%)	0.038	0.048	0.134	0.005	0.033
Total Plate Number (colony/g)	9.1×10^4	8.2×10^4	6.9×10^4	1.1×10^5	4.9×10^4
Mold & Yeast	4.2×10^4	3.6×10^4	2.7×10^4	5.8×10^4	2.2×10^4
Liquid Color	Light brown	Light brown	Light brown	Light brown	Light brown
Odor	Little sour and freshness	Little sour and freshness	Little sour and freshness	sour and freshness	sour and freshness
Presence of fungi	+++ and covered the surface of the liquid	++ and covered the surface of the liquid	++ and covered the surface of the liquid	+++ and covered the surface of the liquid	+++ and covered the surface of the liquid
Elements					
Si (%)	16.53	12.93	21.32	22.53	20.10
Al (%)	1.99	2.39	2.17	2.02	1.96
Ca (ppm)	4380	3670	6940	6280	5940
Fe (ppm)	561	806	547	290	214

During the fermentation process, the acids produced act on the organic material extracting the extracellular enzymes from them (Sai et al. 2023) and organic acid content are the best source to produce bio-enzymes (Benny et al. 2023). After three months fermentation process will produce enzyme, including protease, amylase, and lipase which can be used to decompose proteins, carbohydrates, and lipids (Vama & Cherekar, 2020; Ginting et al. 2021). In this preliminary study, we also observed the physico-chemical parameters including color, odor, presence of fungi, and elements (Table 2). Verma et al. (2019) explain the fermentation yielded a light brownish yellow colored liquid which was separated from the solids and white mold colonies were observed on the top surface of the solution.

4. CONCLUSION

Bioenzymes are produced by fermenting organic wastes, including fruit wastes. We have characterized the physical properties of bio-enzyme from orange fruit wastes by anaerobic fermentation.

We proposed new formula to increase efficiency and effectivity in bioenzyme production from fruit wastes. The preliminary study resulted information about acidic condition and low dissolved oxygen in this fermentation, light brown of liquid color, little sour to sour and freshness in odor, and fungi covered surface of the liquid. Furthermore, the fermentation process will be end in the 3rd month and it was used for the next analysis, including metagenome diversity of anaerobic microorganism in bio-enzyme fermentation, characteristic of enzyme, reducing sugar production, and chemical properties of bio-enzyme from orange fruit wastes.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest in this publication.

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