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Original Article

Synthesis and Physical, Chemical, and Biological Testing of Bio-Enzymes made from Kitchen Waste

Santhosh Senthilkumar

Indus International School, Bangalore, India.

Corresponding Author : santhoshsenthil5070@gmail.com

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Abstract *- Sustainability has been a key focus for the past few decades. Resource management and environmental protection are essential to the survival of life and society. Food waste is prevalent, as around 1.3 billion tonnes get wasted in a year. In a world where food is scarce in many regions, it is crucial to reuse any organic material that is wasted or cannot be used for cooking. Making Bio-Enzymes is a potential way to reuse these wastes. Bio-enzymes also replace the environmental and health hazards of chemical fertilizers and cleaners. This study looked into the possibility of making a Bio-Enzyme solution from kitchen waste so that the reuse of waste occurs at a domestic level. Common household wastes of lemon peels and bananas were fermented in an airtight container for two months and then analyzed and tested against Bio-Enzyme standards. The pH of the Bio-Enzyme was acidic in nature, as desired for a Bio-Enzyme solution (pH 4.7). The sample had sufficient BOD, COD, TDS, and phosphates. These confirmed that the Bio-Enzyme had active enzymes capable of digesting stains. The microbiological analysis also showed that the Bio-Enzyme solution had the potential to be used for household cleaning purposes.*

Keywords *- Bio-Enzyme, Sustainability, Kitchen waste, Fermentation, Cleaners.*

1. Introduction

In today's world, where the importance of sustainable living is being increasingly acknowledged, the priority is on reducing our ecological impression while ensuring the well-being of future generations. Hence, a need for creative waste management solutions has become critical. According to the Food and Agriculture Organization (FAO) (2016), close to one-third of the food produced in a year, approximately 1.3 billion tons, gets wasted [1]. Additionally, 67 million tons of food is wasted in India every year, which is worth more than USD 14 billion [2]. Large volumes of peel trash are produced annually by domestic cooking and the fruit and vegetable industries. The majority of these wastes include high concentrations of important bioactive chemicals. The utilization of these frequent agricultural wastes for sustainable alternative products [3] is a huge step towards sustainability and a circular economy.

One such solution that is making a significant difference is Bio-Enzymes. While the existing cleaners are effective, they contain harmful chemicals such as 2-butoxyethanol (2-BE), phosphates, Nonylphenol ethoxylates (NPEs), and ammonia, that can threaten aquatic life, trigger eutrophication, and worsen air pollution [4, 5, 6, 7].

Bio-Enzyme is a multi-purpose, natural, toxic chemical liquid produced through a fermentation process from vegetables and fruit peel waste (usually citrus peels). Bio-enzymes are produced from the process of submerged fermentation, which is the addition of microorganisms to a liquid medium to produce enzymes. In recent years, the demand for Bio-Enzymes has surged due to their effective environmentally friendly applications in cleaning and organic farming due to their digestive properties. Produced by the breakdown of organic wastes by active microbes in media, such as yeast, these enzymes can be obtained without the need for high-energy reactors and chemical catalysts [8]. Committed to environmental stewardship and rectifying past environmental damages, scientists have conducted extensive research on these enzymes and their ability to perform the jobs of chemical cleaners and synthetic fertilizers. Besides environmental friendliness, a particular advantage is that this process produces Bio-Enzymes containing numerous hydrolases, allowing for diverse applications. Popularly produced Bio-Enzymes contain a mixture of hydrolases such as amylases, pectinases, proteases, mannanases, cellulases, and lipases [9]. This allows them to be effective cleaners as different enzymes can digest different stains, and together, they can digest most stains. For Instance,

- Amylases can digest starchy stains such as condiments.
- Lipases can digest fatty stains such as butter and other fats.

- Cellulases help in fabric smoothness and soil removal in clothing.
- Proteases are used to break down protein-based molecules like blood and food [10].

Similarly, Bio-Enzymes produced for plant fertilization have properties that enhance plant growth, repel pests, and improve soil quality [11]. These enzymes break down different components of the soil to produce nutrients that can be absorbed by the plants for effective growth. The variety of enzymes that can be produced allows us to prepare different types of Bioenzymes for different purposes. Variations in the fruit and vegetable wastes introduced can be used to customize the enzymes generated since different organic waste sources release different enzymes. Most existing research studies mainly focus on Bio-Enzyme production from citrus wastes since citrus fruits tend to release an adequate amount of effective enzymes such as amylases, pectinases, proteases, lipases, and cellulases. A study conducted by Parul et al. 2022 [12] reported the production of Bio-Enzymes from both Citrus and non-citrus fruits.

Similarly, research conducted by Kanchana et al., 2023 [13] used basic household waste such as fruit peels, vegetable peels, and flowers to produce a multiutility Bio-Enzyme solution. Recent studies have shown that agro-industrial waste, such as fruit peels and molasses, can be efficiently used as substrates for bioenzyme production, resulting in sustainable and cost-effective enzyme synthesis. According to Patel et al. (2022), utilizing orange peels as the major substrate resulted in a considerable increase in the production of cellulase and pectinase enzymes. The research demonstrated the potential of bioenzyme manufacturing from waste as a sustainable industrial practice by highlighting the high effectiveness of the enzymes produced in applications, including wastewater treatment and textile processing.

While much study has been done on bioenzyme manufacturing utilizing diverse substrates, there has been a conspicuous lack of emphasis on the use of household kitchen waste as a potential source for enzyme synthesis. Most studies have focused on agro-industrial waste or specialized feedstocks, leaving the enormous potential of household organic waste almost unexplored. Given the growing volume of home garbage created worldwide and the difficulties involved with its disposal, this poses a crucial knowledge gap in both trash management and bioenzyme production. The novelty of this study is that it explores kitchen waste as an undiscovered resource for bioenzyme production. The work not only tackles the environmental impact of kitchen waste, but it also presents a low-cost and environmentally friendly approach to bioenzyme synthesis, with the potential to convert waste into a valuable resource for a variety of uses. The current research aims to find effective Bio-Enzymes that can be prepared using daily kitchen wastes such as peels and spoiled vegetables/fruit.

2. Materials and Method

2.1. Materials Used

- Lemon peels from old lemons
- Overripe bananas (with the peel)
- Household brown sugar (granulated)
- 300 mL of RO water
- Baker's dry active yeast
- 2 Litre airtight plastic bottle

2.2. Methodology

The present analysis and characterization can be divided into five main steps:

- Preparation of Bio-Enzyme solution
- Filtration of Bio-Enzyme solution
- Characterization of Bio-Enzyme solution
- Test for microbial properties

2.2.1. Preparation of Bio-Enzyme solution

Lemon peels and overripe whole bananas were collected from kitchens for Bio-Enzyme production. To enhance surface area, the lemon peels and whole bananas were chopped into smaller slices. Jaggery (100 g), lemon peels (150 g), whole bananas (150 g), and water (1000 mL) were put together in an airtight plastic container in the proportions 1:3:10 and properly mixed.

A teaspoon of yeast was added to boost the rate of fermentation and shorten the fermentation period [14]. The yeast utilized is Saccharomyces cerevisiae or baker's yeast. Then, the containers were kept undisturbed for 2 months for the fermentation to complete.

Gases build up in the bottle due to fermentation, and the pressure needs to be released to prevent the bottle from bursting, so the bottle lid was opened for 1 minute every day. The contents were also agitated every day. The raw bio-enzymes are the liquid portion that needs to be filtered to separate it [11]. After two months, the container contained the liquid Bio-Enzyme solution and the remaining undigested organic waste that had to be filtered out.

2.2.2. Filtration of Bio-Enzymes

The Bio-Enzyme was filtered using a sieve to obtain the liquid Bio-Enzyme. The residual organic waste can be used to speed up new Bio-Enzyme fermentation setups, so it was collected and stored [15]. The Bio-Enzyme solution was stored separately in an airtight bottle before a sample of 1 L was collected for testing.

2.2.3. Characterisation of Bio-Enzymes

The filtered liquid component was used to characterize bio-enzymes. A number of characteristics were measured and recorded, including appearance, pH, Total Dissolved Solids (TDS), chemical and Biological Oxygen Demands (BOD and COD), phosphate concentration, and ammonical nitrogen concentration [11].

2.2.4. Test for Microbial Properties

The Bio-Enzyme’s heterotrophic plate count and total coliform were tested using the IS 5402 standard. These tests were conducted to ensure that the Bio-Enzyme is safe to use and meets the standard to be classified as a Bio-Enzyme.

3. Results & Discussion

Table 1. Result of chemical and microbial testing

Testing Parameter	Acceptable Criteria	Result Obtained
Appearance	Should be a brownish-yellow turbid liquid	Brownish-yellow turbid liquid
pH @25°C	2.5 to 4.5	4.7
Total Dissolved Solid	NLT 500 mg/L	3350
Biological Oxygen Demand	NLT 500 mg/L	12440
Chemical Oxygen Demand	NLT 2000 mg/L	99568
Ammonical Nitrogen	NLT 2 mg/L	39
Phosphate	NLT 1 mg/L	9.7
Heterotrophic Plate Count	NLT 5000 CFU/g	88,000
Total Coliform	<10 CFU/g	<10

Key: NLT: Not Less than
CFU: Colony Forming Unit

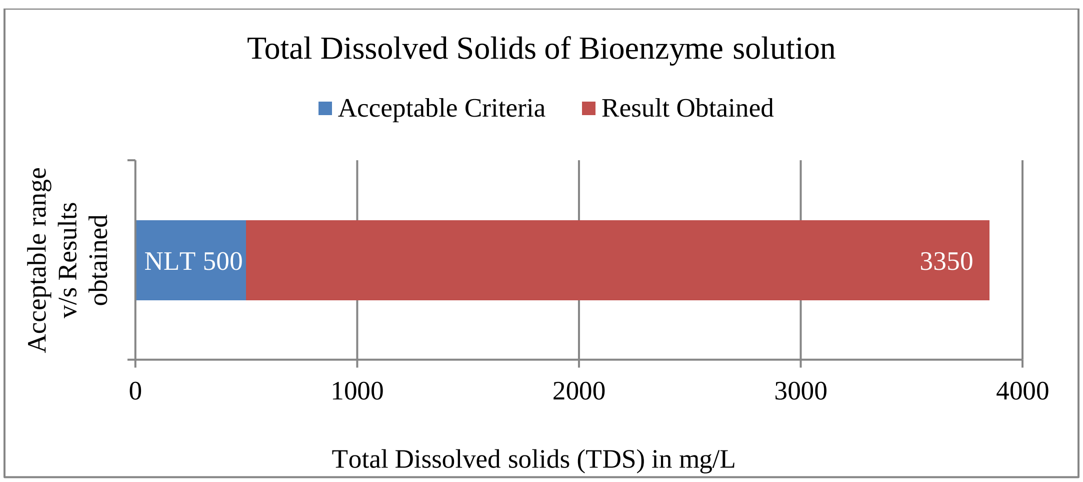

Fig. 1 TDS in Bio-Enzyme solution

3.1. Appearance

According to IS 3025, the acceptable appearance of a Bio-Enzyme is a ‘brownish yellow turbid liquid.’ This characteristic appearance is expected as microorganisms break down the organic food waste during the fermentation period, which produces pigments and releases natural pigments present in the waste. The pigments, chlorophyll, carotenoids, and anthocyanins are present in lemons [16], and alpha (α) and beta (β) carotene are present in bananas [17]. Assessing for appearance is a visual and initial indicator to gauge whether fermentation has occurred properly. From Table 1, it can be seen that the appearance of the Bio-Enzyme solution under study is brownish-yellow turbid, which indicates that fermentation has occurred properly.

3.2. pH

The pH of the Bio-Enzyme solution was measured at 25 degrees Celsius using a pH sensor. The optimum pH for Bio-Enzymes ranges between 2.5 and 4.5, which can be attributed to the acids released during the fermentation of raw materials, such as citrus peels.

From Table 1, it can be seen that the Bio-Enzyme understudy had a pH of 4.7, which is slightly higher than the optimal range for Bio-Enzymes.

This could probably be due to the use of banana waste, which has a pH range of 4.5 - 5.3, resulting in a slightly higher pH than the permissible range.

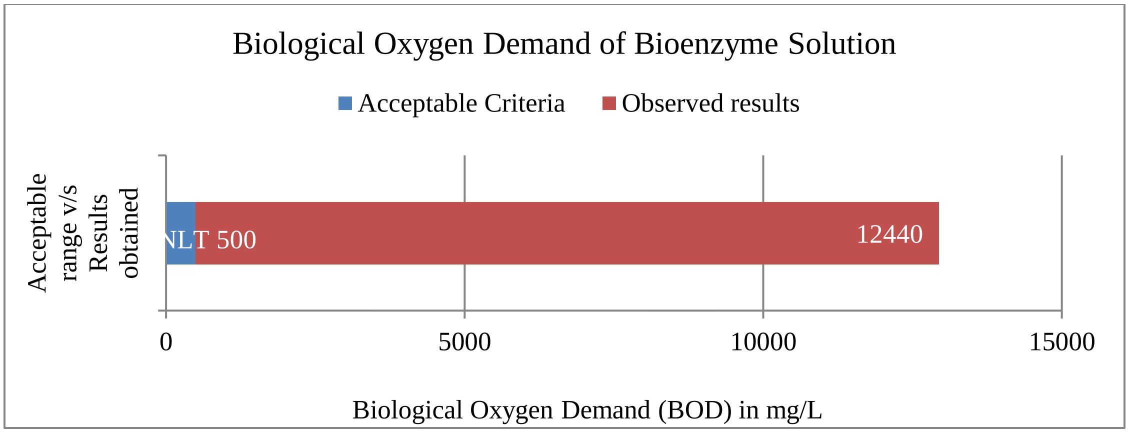

Fig. 2 BOD in Bio-Enzyme solution

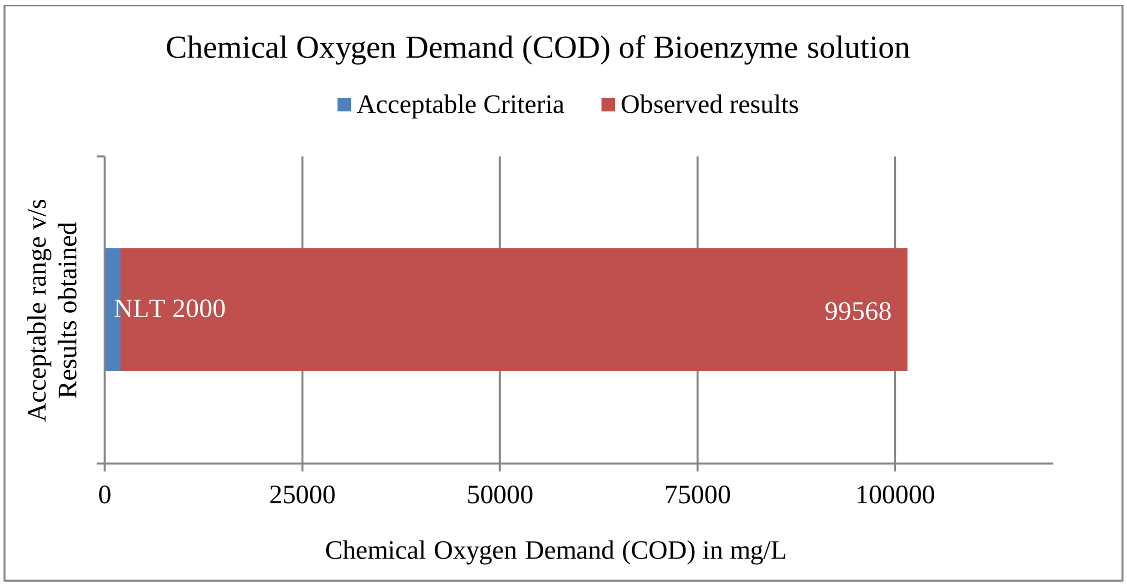

Fig. 3 COD in Bio-Enzyme solution

3.3. Total Dissolved Solids (TDS)

TDS is a measure of the total dissolved organic and inorganic compounds present in a liquid. According to the IS 3025 standard, the TDS of a Bio-Enzyme solution should be above 500 mg/L. Digestive enzymes are water soluble and organic so they could contribute to a higher TDS concentration in the Bio-Enzyme. The sample meets the minimum requirement as it is above the minimum value, i.e. 3350 mg/L.

3.4. Biological Oxygen Demand (BOD)

BOD indicates how much oxygen is consumed by microorganisms to break down organic matter. According to the IS 3025, this value for Bio-Enzymes should be greater than 500 mg/L. Higher BOD indicates higher organic content, and an abundance of enzymes could contribute to higher organic content, leading to a higher BOD. The recorded value of 12440 mg/L seen in Fig. 2 is higher than the minimum requirement, indicating the presence of active enzymes.

3.5. Chemical Oxygen Demand (COD)

Bio-enzymes are utilized in breaking down organic matter, and the chemical oxygen demand must be higher than a certain value for the efficiency of oxidation to be high enough for the complete breakdown of the organic waste. Based on the IS 3025 standard, the COD of the Bio-Enzyme is within range as the requirement is for the demand to not be less than 2000 mg/L, and the measured value shown in Figure 3 is a much higher 99568 mg/L.

3.6. Ammonical Nitrogen

The ammonia nitrogen content in the bioenzyme solution was determined to be 39 mg/L, which is far beyond the permissible limit. The presence of ammonical nitrogen in bioenzyme solutions is typically caused by the breakdown of organic materials employed in synthesis. These organic compounds frequently comprise kitchen garbage, fruits, and vegetables, which include proteins and other nitrogenous chemicals.

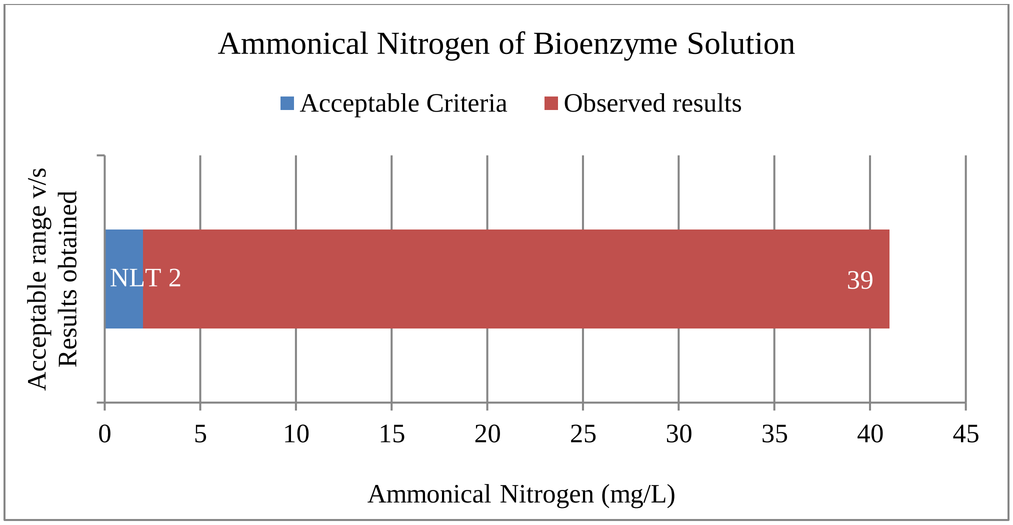

Fig. 4 Ammonical nitrogen in Bio-Enzyme solution

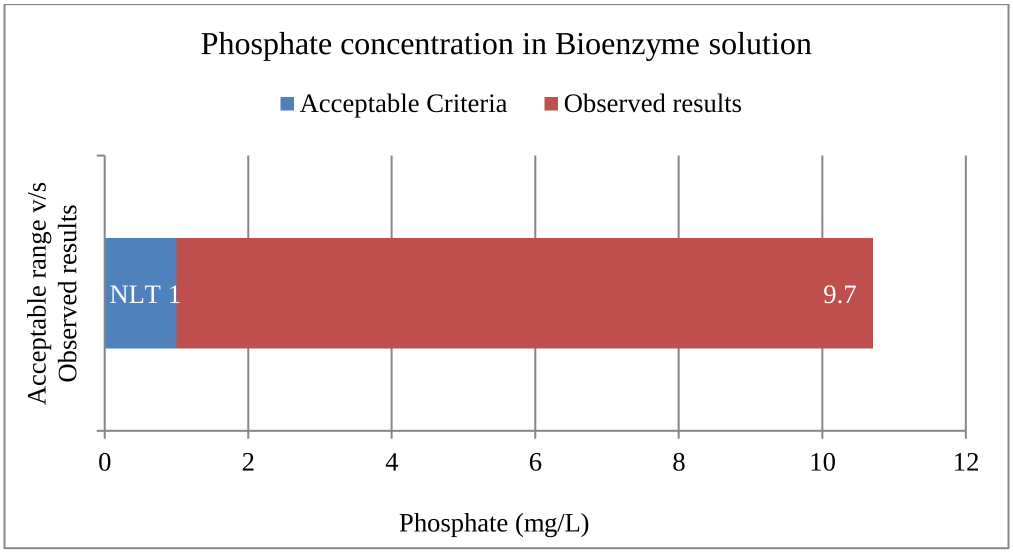

Fig. 5 Phosphate concentration in Bio-Enzyme solution

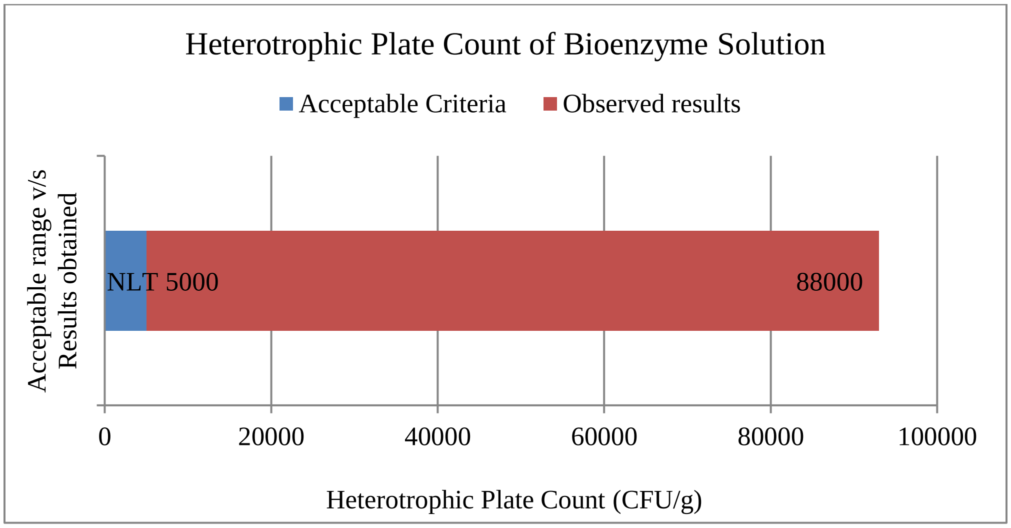

Fig. 6 Heterotrophic plate count of Bio-Enzyme solution

3.7. Phosphate Concentration

Phosphate concentration gives an idea about the presence of phosphatases in the Bio-Enzymes. Phosphatases break down organic phosphorus compounds into inorganic phosphates [18]. High phosphate concentration could result from the high concentration and activity of phosphatase enzymes.

Higher phosphate concentration would be exceptionally beneficial for fertilizer Bio-Enzymes as phosphate is a crucial plant nutrient that is common in chemical fertilizers [19]. The Bio-Enzyme solution under study was prepared using lemon and banana wastes. Both fruits have phosphates so these phosphates could have also contributed to the high phosphate concentration of 9.7 mg/L seen in Figure 5 [20, 21].

3.8. Heterotrophic Plate Count

Heterotrophic plate count is a chemical test that measures the number of live, culturable heterotrophs. The extent to which fermentation has occurred could possibly be measured by obtaining the heterotrophic plate count, as heterotrophic bacteria are responsible for fermentation. More fermentation can relate to higher concentration and quality of enzymes released from the organic waste.

According to the IS 5402 standard, the count should not be less than 5000 CFU/g for a Bio-Enzyme, and the measured value shown in Fig. 6 exceeds this as it is 88,000 CFU/g. Figure 7 shows the growth of the heterotrophic organisms using a nutrient agar plate.

Fig. 7 Heterotrophic plate count plate of Bio-Enzyme solution

3.9. Total Coliform

Total coliforms are a type of bacteria that is widely used to determine the hygienic status of water and other solutions. They exist in the environment as well as in warm-blooded animals' gut. A total coliform concentration of fewer than 10 CFU/g indicates that the bioenzyme solution is microbiologically safe to use in household cleaning applications. It reduces the possibility of spreading pathogenic microorganisms while in use.

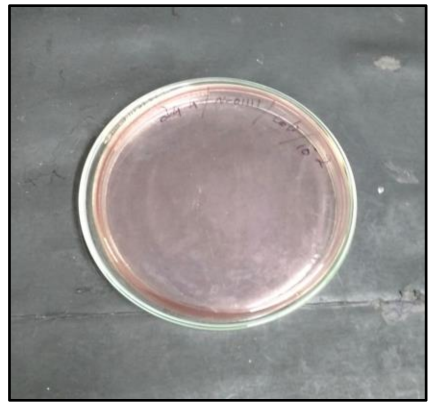

Fig. 8 Total coliform plate of Bio-Enzyme solution

4. Conclusion

This study aimed to test and produce a Bio-Enzyme from daily kitchen waste. Leftover parts of lemons and bananas were used for the extraction of Bio-Enzymes. Lemons and other citrus fruits are common in Bio-Enzymes, but Bananas are not. Bananas were added as they are regularly wasted and contain many digestive enzymes required in a Bio-Enzyme. The Bio-Enzyme met all parameters except for the pH requirement, as the pH was 4.7, which is 0.2 higher than the permissible limit. It could be due to the equal ratio of bananas to lemons used. An improvement would be to change this ratio. Despite this, the Bio-Enzyme is still usable as the solution remains acidic. The acidic range gives Bio-Enzymes a shelf-life of close to three years, maintains a good smell, and prevents degradation in the quality of enzymes.

A Bio-Enzyme with a pH of 4.7 would work but with a shorter shelf-life and potentially lower cleaning effectiveness over time. This Bio-Enzyme can be used for household cleaning purposes and is capable of removing most stains due to the classes of digestive enzymes present. There were certain limitations in the study, such as the use of only two types of kitchen waste, the Bio-Enzyme production not being done in optimal conditions (the jar was opened every day, which should have been stopped post the start of anaerobic respiration), and the study not testing different ratios of bananas and lemons.

One notable weakness of this study is to include antibacterial experiments employing the disc diffusion method to assess the isoenzymes inhibitory effects on various microbial strains. This would not only improve our understanding of bio enzymes' functional qualities, but it would also offer new possibilities for their use in industries where antibacterial activity is crucial. Testing different ratios could have helped develop a more effective Bio-Enzyme with a pH within the permissible range. The future scope of this study would be to conduct specific biochemical tests to identify the specific Bio-Enzymes present in the sample. Variate the ratio of the raw materials to optimize the condition and standardize the procedure to produce an effective Bio-

Enzyme solution utilizing kitchen waste. Additionally, the potential of Bio-Enzymes as an alternative to fertilizers could be an interesting topic of study. Such a process, if optimized, can utilize the kitchen wastes produced at the village level to produce Bio-Enzyme solutions in a bulk quantity, thereby solving the problem of waste management and generation of eco-friendly products.

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