

I. INTRODUCTION

Economic growth and social progress contributes to the rapidly increasing rate of food waste at 1.12% incline annually (Cruz & Barceló, 2015). Even so, food production is expected to significantly increase to fulfill the world's food security needs (Hitaj, et al., 2019). This poses a great concern as a large amount of the food harvested is discarded as waste for different reasons, totaling to around 931 million tonnes each year with 570 million tonnes of which occurring at the household level (Batista, 2021; Nicastro & Carillo, 2021). This further proves the unhealthy habit of the food sector, significantly disposing a huge amount of its production as waste. Amongst the globally produced food waste, fruit and vegetable waste (FVW) takes up most of the portion. Around 50% of the fruits and vegetables manufactured worldwide are not consumed and are thrown away as waste (Sagar, et al., 2018).

As a developing country, Malaysia has also been struggling with this problem. According to Salin, Singh, & Raghavan (2017), 75% of the household food waste is a consequence of overconsumption and 25-30% is attributed to the waste during cooking. Additionally, with the ever-changing market demand for freshness, overstocking, and inaccurately predicting the product's shelf-life problems, the waste produced has been growing at an increasing rate over the past few years (Riesenegger & Hübner, 2022). This poses a threat to the environment as fruit vegetable waste (FVW) will end up decomposing and producing different dangerous greenhouse gasses such as methane and carbon dioxide (Biruk, 2022). This is why a new and efficient approach should be adopted to lessen the environmental impacts caused by FVWs and use the resources for a better purpose.

FVWs are organic, biodegradable, and rich in microorganisms, with high contents of moisture and nutrients. This enables them to be an alternative resource for microorganisms residing in the waste to metabolize into value-added products through anaerobic fermentation as an eco-enzyme (Mehmood, et al., 2021; Ling, et al., 2023). According to Novianti & Muliarta, (2021), eco-enzyme is a versatile yet complex liquid, usually made of organic waste, sugar, and water, currently popularly used as a fertilizer and natural cleaning agent. The solution consists of different protein chains, mineral salt, as well as juvenile hormones, whose interaction holds antimicrobial properties and are able to break down different macromolecules (Mushtaq, et al.,

2022). Eco-enzymes also bring many benefits to the environment, as they reduce the usage of chemicals whilst also valorizing waste, reducing its amount and repurposing it to treat other environmental problems (Sreekala, Ismail, & Nathan, 2022).

For this, microbial diversity is an essential factor as the synergic interactions between these microorganisms are the ones producing a variety of alcohol, acids, salts, and enzymes, enabling the eco-enzymes with different yet advantageous properties for household and industrial purposes (Tyc, et al., 2017; Mandpe, et al., 2021). Additionally, because of their practicality and price, eco-enzymes have now become a budget-friendly substitute for many different treatments for sustainability problems and are a good option to reduce environmental pollution (Sreekala, Ismail, & Nathan, 2022). Especially when using fruit & vegetable waste as the main ingredient, eco-enzymes will help reduce atmospheric carbon dioxide, mitigating the greenhouse effect and helping to resolve global warming.

From precedent research, there are many different microorganisms that have been identified to dwell and grow in FVW eco-enzymes (Prabhurajeshwar & Chandrakanth, 2017). Amongst them, yeast and *Lactobacillus* are known to be amongst two of the most heavily found microorganisms with many different strains identified as they are able to digest and break down the nutrients and minerals available in the waste (Singh, et al., 2021). Because of this finding, a lot of current research has been trying to utilize the presence and the nature of these microbes to treat different sustainability problems currently emerging and happening in the world, one of which is wastewater treatment. Specifically in the eco-enzyme generated from the fruit and vegetable waste in UCSI university, a member of the research team, through phenotypic and genotypic methodologies, have identified three different yeast strains namely *Saccharomyces sp.*, *Pichia sp.*, *Yarrowia sp.*, and two different *Lactobacillus* strains namely *Lactobacillus acidophilus* and *Lactobacillus plantarum* to be present in the eco-enzyme. That said however, most are still unable to determine how long the anaerobic fermentation process should take place to produce the most efficient solution.

Microorganisms grow following the same growth phases. Starting from its lag phase, advancing into its exponential phase, staying in its stationary phase to maximize its growth, before advancing to its death phase (Rolfe, et al., 2012). Although they follow the same stages,

different bacteria grow at different rates, some faster than others, while some slower than others. Lactobacillus species for example, has a faster growth rate in comparison to other microorganisms, as it only stays in its lag phase for 6 hours before entering its exponential growth phase at hours 25-45 (Rezvani, Ardestani, & Najafpour, 2017). Yeast on the other hand, grows slower than others. With a 90 minute doubling time, they usually take around 3-4 days until the end of their growth phase (Cregg, et al., 2009). Taking this into account, different fermentation periods are used as one of the parameters in the experiment to see which colonies of yeast and Lactobacillus have matured and/or have died during that period. As the effectiveness and the results of the treatment done in the research are highly affected by the presence of the different microbial communities, harvesting the eco-enzyme at three different times will justify the experiment's results.

Through this research, eco-enzyme generated from FVW fermented at three different harvest times will be analyzed for their yeast and lactic-acid bacteria content through morphological identification. The research will result in a list of possible microorganisms and their ability to break down different chemicals present in the synthetic wastewater, which identification was done to somehow justify the results of the wastewater treatment. The eco-enzyme was used to treat synthetic wastewater for 6 weeks, and 12 measurements were done in that timespan. The results determined which of the three eco-enzyme were the most effective to treat synthetic wastewater in terms of their microbial diversity as well as fermentation duration.

1.2 Research Question

1. Are yeast and lactic-acid bacterial communities present in the eco-enzyme derived from the fermented fruit and vegetable?
2. Does different harvest time affect the growth, presence, and variety of different yeast and lactic-acid colonies in the eco-enzyme?
3. Are yeast and Lactobacillus able to break down chemicals present in wastewater and be a possibility for a new and sustainable wastewater treatment method?

1.3 Hypothesis

1. Different yeast and lactic-acid bacteria will be present in the eco-enzyme.
2. Different harvest times will affect the growth and presence of different yeast and lactic-acid colonies in the eco-enzyme as different microorganisms have different growth periods, in which some will mature faster or slower than others, hence it is expected that the eco-enzyme fermented for the longest to have the most microbial diversity.
3. Based on their properties and nature, yeast and lactic-acid bacteria have the possibility of being able to break down different types of chemicals present in wastewater, hence is a probable sustainable approach for wastewater treatment.