EFFECTS OF DIFFERENT OVEN DRYING METHODS ON THE NUTRITIONAL VALUE AND LACTIC ACID BACTERIA LOAD OF *Eudrilus eugeniae*

By

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ABSTRACT

EFFECTS OF DIFFERENT OVEN DRYING METHODS ON THE NUTRITIONAL VALUE AND LACTIC ACID BACTERIA LOAD OF *Eudrilus eugeniae*

Ng Yan Sze Jacquelyn

The vermicomposting industry is becoming popular among compost production companies due to its low cost and ease of management in utilizing earthworms as the composting agent. Vermicompsting synchronizes with the propagationof the earthworm creating a surplus of earthworm population. The surplus earthworms can be utilize in animal feed as an alternative for imported livestock feed to sustain a constant profitable animal farming industry. This leads to the primary aim of this study which was to compare the different drying methods effect on the earthworm's nutritional content and lactic acid bacteria (LAB) load for future reference to animal feed production. Since African Nightcrawler (ANC), *Eudrilus eugeniae* has a shorter duration of time to emerge from cocoonand optimal sexual maturity compared to the red and blue worm, it was chosen to be the earthworm source to study its potential as a supplementary animal feed. In this study, a few batches of live ANC (T1) were dried at 60 °C by conventional oven (T2) for 3 hours and vacuum oven (T3) for 2 hours. Thenutritional content and LAB load of live, T2 and T3 ANC were determined by proximate analysis and isolation of lactic acid bacteria for Colony Forming Unit (CFU) count and biochemical tests. Results showed that the nutritional content in terms of crude protein (CP) and dry matter (DM)

percentage of T3 (CP 55.03%, DM 22.79%) was significantly higher ($p < 0.05$) than T2 (CP 47.43%, DM 20.39%). The potential LAB CFU count for T2 (5.87×10^9 CFU/g) and T3 (4.90×10^9 CFU/g) did not differ significantly ($p \geq 0.05$). In conclusion, vacuum oven drying method (T3) is more efficient compared to conventional oven drying method (T2) in terms of reducing the drying duration, retaining higher nutrient composition and comparable amount of potential LAB that is essential in animal feed.

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DECLARATION

I hereby declare that this final year project report is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

NG YAN SZE JACQUELYN

APPROVAL SHEET

This final year project report entitled "**EFFECTS OF DIFFERENT OVEN DRYING METHODS ON THE NUTRITIONAL VALUE AND LACTIC ACID BACTERIA LOAD OF *Eudrilus eugeniae***" was prepared by NG YAN SZE JACQUELYN and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Agricultural Science at Universiti Tunku Abdul Rahman.

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PERMISSION SHEET

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I hereby give permission to the University to upload the softcopy of my final year project report in PDF format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

(NG YAN SZE JACQUELYN)

TABLE OF CONTENTS

		Page
ABSTRACT		**ii**
ACKNOWLEDGEMENTS		**iv**
DECLARATION		**v**
APPROVAL SHEET		**vi**
PERMISSION SHEET		**vii**
TABLE OF CONTENTS		**viii**
LIST OF TABLES		**xi**
LIST OF FIGURES		**xii**
LIST OF ABBREVIATIONS		**xiii**
CHAPTER		
1.0	INTRODUCTION	1
	1.1 Background of Study	1
	1.2 Problem Statement	3
	1.3 Objectives	4
2.0	LITERATURE REVIEW	5
	2.1 Common Drying Method in Feed Production	5
	2.1.1 Application of Conventional Oven and Vacuum Oven	6
	2.1.2 Different Duration and Temperature of Conventional Oven and Vacuum Oven Drying	7
	2.1.3 Nutritive Values and Microbial Load of Dried Earthworms	8
	2.2 Earthworms	10
	2.2.1 Types of Earthworms	12
	2.2.2 Different Composition of C/N ratio of Vermicompost	13
	2.3 Presence of Probiotics in Earthworms	14
	2.3.1 Desiccation and Thermal Tolerance of Lactic Acid Bacteria	14
	2.3.2 Method of Screening for Lactic Acid Bacteria	16
3.0	MATERIALS AND METHODS	18
	3.1 Introduction	18
	3.2 Preparation of Live African Nightcrawler	19
	3.3 Drying Treatments of African Nightcrawler	20
	3.4 Proximate Analysis	22
	3.4.1 Dry Matter and Moisture Determination	22
	3.4.2 Crude Protein Determination	23
	3.4.3 Crude Fat Determination	26
	3.4.4 Crude Ash Determination	28
	3.5 Lactic Acid Bacteria Isolation and Biochemical Test	29

3.5.1 Serial Dilution 30
3.5.2 Pour Plating 30
3.5.3 Colony Forming Unit Count 31
3.5.4 Subculture 31
3.5.5 Gram Stain 32
3.5.6 Catalase Test 33
3.5.7 Bile Tolerance Test 34
3.5.8 Acid Tolerance Test 34
3.6 Statistical Analysis 35

4.0 RESULTS 36
4.1 External Morphology and Proximate Analysis of African Nightcrawler (ANC) With and Without Drying Treatments 36
4.1.1 Dry Matter, Moisture, Crude Protein, Crude Fat and Crude Ash Percentage of Different African Nightcrawler (ANC) Treatments 37
4.2 Potential Lactic Acid Bacteria Isolates 39
4.2.1 Characteristics of Potential Lactic Acid Bacteria Isolates 39
4.2.2 Colony Forming Unit Per Gram of Potential Lactic Acid Bacteria Isolates 41
4.2.3 Optical Density (OD) Reading of Growth in Bile Tolerance Test of Potential Lactic Acid Bacteria Isolates 42
4.2.4 Optical Density (OD) Reading of Growth in Acid Tolerance of Potential Lactic Acid Bacteria Isolates 44
4.2.5 Growth Increment Percentage (%) in Bile and Acid Tolerance of Potential Lactic Acid Bacteria Isolates 49

5.0 DISCUSSION 51
5.1 External Morphology and Proximate Analysis of African Night Crawler (ANC) With or Without Drying Treatments 51
5.1.1 Dry Matter and Moisture Content of African Night Crawler (ANC) With or Without Drying Treatments 52
5.1.2 Crude Protein ,Crude Fat and Crude Ash Content of African Night Crawler (ANC) With or Without Drying Treatments 53
5.2 Potential Lactic Acid Bacteria Isolates 56
5.2.1 Characteristics of Potential Lactic Acid Bacteria Isolates 56
5.2.2 Colony Forming Unit Per Gram of Potential Lactic Acid Bacteria Isolates 58
5.2.3 Optical Density Reading of Growth and Growth Increment in Bile and Acid Tolerance Test of Potential Lactic Acid Bacteria Isolates 59
5.3 General Discussion 60
5.3.1 Suggestion for Future Study 62

6.0 CONCLUSIONS 64

REFERENCES 65

APPENDICES 76

LIST OF TABLES

Table		Page
4.1	Summary of Drying Method Parameters	8
4.2	Nutritional Composition of African Nightcrawler (ANC) between Different Treatments	38
4.3	Characteristics of Potential Lactic Acid Bacteria Isolates	40
4.4	Average Colony Forming Unit (CFU) Per Gram of Potential Lactic Acid Bacteria Isolates	42
4.5	Treatment 1, 2 and 3 Optical Density 600 nm Reading of Growth at Each Hour for Bile Tolerance Test	43
4.6	Treatment 1 Optical Density 600 nm Reading of Growth at Each Hour for Acid Tolerance Test	45
4.7	Treatment 2 Optical Density 600 nm Reading of Growth at Each Hour for Acid Tolerance Test	46
4.8	Treatment 3 Optical Density 600 nm Reading of Growth at Each Hour for Acid Tolerance Test	48

LIST OF FIGURES

Figure		Page
3.1	Overview of research methodology	18
3.2	ANC in a sieve during washing process	19
3.3	Weight taking of ANC using an electronic balance	20
3.4	Metal mesh secured on top of metal tray with ANC	21
3.5	Crude protein determination methodology	25
3.6	End of protein digestion by BUCHI^{TM} Speed Digester	25
3.7	Titration	26
3.8	End of fat extraction by SOXTHERM®	27
3.9	Crucibles with samples in furnace	28
3.10	Serial dilution	30
3.11	Catalase test	33
4.1	ANC treatments	36
4.2	Nutritional Composition of African Nightcrawler (ANC) between Different Treatments	38
4.3	Gram staining	40
4.4	Elevation of colony	41
4.5	OD Reading of Growth in Bile Tolerance Test of Potential Lactic Acid Bacteria Isolates	43
4.6	C1 isolate OD Reading of Growth in Acid Tolerance Test	45
4.7	C2 isolate OD Reading of Growth in Acid Tolerance Test	46
4.8	C3 isolate OD Reading of Growth in Acid Tolerance Test	47
4.9	C4 isolate OD Reading of Growth in Acid Tolerance Test	47
4.10	C5 isolate OD Reading of Growth in Acid Tolerance Test	48
4.11	C6 isolate OD Reading of Growth in Acid Tolerance Test	49
4.12	Growth Increment Percentage of Potential Lactic Acid Bacteria Isolates	50

LIST OF ABBREVIATIONS

ANC	African Nightcrawler
AOAC	Association of Official Analytical Collaboration
BLAST	Basic Local Alignment Search Tool
BSFL	Black soldier fly larvae
CFU	Colony forming unit
DNA	Deoxyribonucleic acid
ECM	Extracellular matrix
et al.	et alia
FCR	Feed conversion ratio
LAB	Lactic acid bacteria
MRS	de Man, Rogosa, and Sharpe
MW	Meal worm
OD	Optical density
PCR	Polymerase chain reaction
pH	Potential of Hydrogen
ROS	Reactive oxygen species
SPSS	Statistical Package for the Social Sciences
T1	Treatment 1: live ANC
T2	Treatment 2: conventional oven-dried ANC
T3	Treatment 3: vacuum oven-dried ANC
TABRS	Thiobarbituric acid reactive substance

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The earthworm, *Eudrilus eugeniae* common name is African Nightcrawler (ANC) (Blakemore, 2015). It is native to tropical regions in West Africa but are found cultivated in several other countries including Malaysia as bioreactors to produce vermicompost (Dominguez, et al., 2001). The distinct characteristic of ANC is that they are nocturnal, which means they are only actively composting organic wastes during the night when there is no sunlight (Balbuena, 2016; Blakemore, 2015). The most prominent external morphology of ANC is the purple-grey sheen colour of their skin (Blakemore, 2015; Dominguez, et al., 2001). In addition, the posterior segments of ANC were observed to be tapered and their clitellum are always contrasting with the surrounding either darker or lighter in colour (Balbuena, 2016; Blakemore, 2015).

The most common application of ANC in vermicomposting is as bioreactors for converting organic wastes into composts due to their ease in adaptability towards environmental changes, rapid consumption and digestion, as well as with relatively high fecundity (Vuković,5 et al., 2021; Sarimong and Legaspi., 2019). In addition, the excreta of the worms, also known as vermicasts gives

better supply of plant nutrients when used as an organic fertilizer when compared to conventional compost, as earthworms' gut are rich in beneficial microbial flora that breaks down organic matter efficiently that enables plants to utilize the nutrients readily (Rehman, et al., 2023).

The ANC was observed to be one of the most productive composting earthworms compared to *Eisenia fetida* (red worm) and *Perionyx excavatus* (blue worm) due to its shorter duration to emergence from the cocoon (15 to 30 days) and optimal time to sexual maturity (30 to 95 days) under desirable conditions (Hallat, et al., 2015; Othman, et al., 2012). Earthworms that are used in vermicompost production were also used as animal feed and studied for their nutritional and microbial load especially the red worm, which has longer duration to emergence from cocoon and to sexual maturity when compared to ANC (Othman, et al., 2012).

The most common way of processing earthworms into animal feed is through oven drying. There are several methods of drying feed including conventional oven drying and vacuum oven drying (Kröncke, et al., 2018). The drying rate of vacuum oven is faster than that of conventional oven. Vacuum oven is known to dry materials in a shorter duration due to its vacuum environment created by the vacuum pump that allows rapid moisture evaporation compared to conventional oven (Ngamwonglumlert and Devahastin, 2018). The advantages of using vacuum drying are to reduce or eliminate the oxidation and thermal stress on

feed materials that are easily oxidized and degraded at high temperatures in the presence of oxygen (Ngamwonglumlert and Devahastin, 2018).

1.2 Problem Statement

The management of agricultural waste has been one of the most crucial concerns. This is because agricultural wastes contribute to about 82% of food waste emissions reported by Ritchie (2019). The likelihood of increase in agricultural waste is high due to the ever-increasing human population that contributes to the demand of agriculture production for food (Capanoglu, et al., 2022). Thus, there is in dire need to intensify the recycle and composting of wastes in a sustainable way to prevent further contribution to global pollution. This is where vermicompost industry plays a major role in composting organic wastes by utilizing naturally existing composting earthworms (Rastegari, et al., 2023). Consequently, the growing adoption of vermicomposting for these waste materials also supports the proliferation of earthworm populations (Ghorbani and Sabour, 2021). This eventually leads to the surplus of earthworms, ANC in this case, could be turned into animal feed since the cost of existing feed ingredient such as maize and soybean meal is high in Malaysia due to the reliance on importation (Malaysian Investment Development Authority, 2023; Wahab, 2019). To convert ANC into animal feed meal, usually drying is required to enable longer shelf life and ease in logistic. Most of studies on the effect of

conventional and vacuum drying method were focused on the red worms (Gunya, et al., 2016; Suarez-Hernandez, et al., 2016; Bou-Maroun, et al., 2013; Rożen, et al., 2015) and there was lack of studies on ANC as processed animal feed on its nutritive values and microbial load.

1.3 Objectives

The objectives of this research encompasses: -

1. To compare between conventional oven and vacuum oven drying method effects on the nutritional value of processed ANC meals.
2. To evaluate the effects of the drying methods on the lactic acid bacteria count presence in the ANC meals.

CHAPTER 2

LITERATURE REVIEW

2.1 Common Drying Method in Feed Production

The mechanism that lies behind drying approach consists of two routes. The initial route is the movement of moisture from the cell in the internal body diffusing onto the surface then, from the surface the moisture will begin to change its physical matter from liquid to vapour and evaporate to the surrounding (Calín-Sánchez, et al., 2020). Thus, drying method is a form of obtaining dry mass in a solid form. The main purpose of drying in the feed industry is to preserve and prolong the shelf life of the feed and ease the storage of feed hence, reducing post-harvest loss (Xiao and Mujumdar, 2019). This is because moisture removal reduces the proliferation of micro-organisms that causes majority of the feed spoilage and contamination (Xiao and Mujumdar, 2019).

In addition to that, moisture removal reduces water content in the cell which in turn reduces intracellular water potential required by microbes for enzymatic activity to metabolize nutrients (Wolińska and Stępniewska, 2012). There are several types of drying methods available for animal feed including conventional

oven, vacuum oven, freeze-drying, microwave-oven, and sun-drying (Dada, et al., 2023; Kröncke, et al., 2018; Rożen, et al., 2015).

2.1.1 Application of Conventional Oven and Vacuum Oven

Oven drying is one of the most common drying methods in producing feed meal through the application of heat transfer according to several studies. Dada, et al. (2023) reported to have dried *Alma millsoni*, an earthworm species derived from the tropical wetland, using the conventional oven drying method to study its nutritional and microbial qualities as feed supplement for livestock and therapeutic medicine. Gunya, et al. (2016) also reported to have oven-dried earthworm *Eisenia fetida*, the common red worm, to study its nutritional values and fatty acid profile to be used in feed formulation.

Other than that, Suarez-Hernandez, et al. (2016) processed earthworm flour utilizing oven drying to study its effects on crude protein content compared to other drying methods with respect to the values of drying kinetics, temperature, air speed and amount of sample. Next, Bou-Maroun, et al. (2013) did a study on oven dried *Eisenia fetida* protein powder and the effects on the protein content and solubility, and the volatile compounds present. The compilation of a few studies in utilizing conventional oven drying shows that it is a relatively common method to process earthworms.

However, there are lack of studies in utilizing vacuum oven to process earthworms. According to a study done by Kröncke, et al. (2018) using meal worms, *Tenebrio molitor* was dried with vacuum oven and conventional oven to compare both drying method effects on the nutritional contents. Rożen, et al. (2015) added that vacuum drying was used as a pre-treatment to preserve different species of invertebrates including earthworms prior to analysing their elemental composition.

2.1.2 Different Duration and Temperature of Conventional Oven and Vacuum Oven Drying

The temperature used to dry the *Alma millsoni* earthworms in the conventional oven by Dada, et al. (2023) was at 70°C for 24 hours prior to homogenizing into powder form to analyse the nutritional content and microbial present. Gunya, et al. (2016) adjusted the oven temperature to 90°C to dry the *Eisenia fetida* earthworms for 4 hours prior to milling the dried form into powder using pestle and mortar. Suarez-Hernandez, et al. (2016) to have dried at four different temperatures, 50, 65, 90 and 100°C for about 7 hours for each temperature parameter.

Next, Bou-Maroun, et al. (2013) dried three forms of *Eisenia fetida* earthworm fraction; juice, pulp and whole worm at 60°C for 4 hours prior to processing it further into powder form using a crusher for dried whole worm. Kröncke, et al.

(2018) reported to have conventional and vacuum oven dried *Tenebrio molitor*, meal worms at 120°C for1 hour and 60°C for 24 hours, respectively. Vacuum oven was adjusted to 50°Cby Rożen, et al. (2015) to dry the *Dendrobaena veneta* earthworms for 48 hours as a pre-treatment prior to analysing their elemental composition.

Table 4.1: Summary of Drying Method Parameters.

Author	**Species**	**Drying Method**	**Parameters**	
			Temperature (°C)	**Duration (h)**
Dada, et al. (2023)	*Alma millsoni*	CO	70	24
Gunya, et al. (2016)	*Eisenia fetida*	CO	90	4
Suarez-Hernandez, et al. (2016)	-	CO	50, 65, 90, 100	7
Bou-Maroun, et al. (2013)	*Eisenia fetida*	CO	60	4
Kröncke, et al. (2018)	*Tenebrio molitor*	CO and VO	CO: 120 VO: 60	CO: 1 VO: 24
Rożen, et al. (2015)	*Dendrobaena veneta*	VO	50	48

Key: CO conventional oven dried, VO vacuum oven dried.

2.1.3 Nutritive Values and Microbial Load of Dried Earthworms

Dada, et al. (2023) reported the proximate composition of oven-dried earthworms with moisture at 9.07%, ash at 5.93%, fat at 12.70%, protein at 61.93%, crude fibre at 0.17% and total carbohydrate at 10.22%; total viable microbial count of 68.67×10^3 CFU/g. The following few conventional oven dried earthworms did not determine the microbial load thus, no report on microbial count is available. The proximate composition of the milled

earthworms reported by Gunya, et al. (2016) were 10.5% for moisture, 9.5% for fat and 59.7% for crude protein. Suarez-Hernandez, et al. (2016) reported to have obtained humidity, ash, fat and crude protein at 10.31%, 9.45%, 3.49% and 61.73%, respectively for 65°C of conventional oven-dried earthworm while, 100°C oven-dried earthworm was reported to have 9.90%, 7.83%, 3.88% and 62.86%, respectively.

Bou-Maroun, et al. (2013) only reported on the water and protein content of the three fractions of dried earthworms. The water and protein content were 5.24% and 67.31%, respectively for whole worms, 1.14% and 54.35%, respectively for juice fraction and 3.99% and 64.24%, respectively for pulp fraction. The moisture content, fat and protein reported by Kröncke, et al. (2018) for conventional oven-dried meal worms were 1.50%, 27.10% and 57.00%, respectively while it was 1.65%, 31.40% and 54.80%, respectively for vacuum oven-dried meal worms. The proximate analysis was not performed by Rożen, et al. (2015) as the research focus was on the preservation of invertebrates and their macro- and micro-elements. There is clearly a lack of study on the proximate composition of vacuum oven-dried earthworms, in particular the ANC. Overall, Gunya, et al. (2016) and Bou-Maroun, et al. (2013) was observed to have optimal nutritional content in terms of crude protein and moisture content for conventional oven method while, optimal nutritional content in terms of protein and moisture in vacuum oven method for processing meal worms can be observed from the research done by Kröncke, et al. (2018).

2.2 Earthworms

Earthworms such as ANC are categorized under macro-invertebrates in Kingdom Animalia and further classified into class Clitellata then, narrowed down to phylum Annelida which, distinguishes them from the microscopic worm, nematode (Engelmann, et al., 2016). According to Engelmann, et al. (2016) earthworms have a complete circulatory system and coelom and often mistaken to be similar to nematodes physiological wise due to resemblance in the external structures but differ significantly in size as nematodes are microscopic while earthworms can be seen by the naked eyes. Earthworms are productive creatures that thrives naturally in the Earth's soil. Philips, et al. (2021) mentioned that earthworms are the chief in providing major functions and services to the ecosystem by nutrient cycling, promoting beneficial microbial growth and aeration for better soil fertility and healthier plant growth.

Earthworms are able to perform the aforementioned ecosystem function and services due to their composting nature to feed on decaying organic matter and their movement in the ground creates tiny, narrow burrows within the soil for aeration (Philips, et al., 2021). Other than that, Medina-Sauza, et al. (2019) and Dionísio, et al. (2018) reported that earthworms also feed on harmful microbes and nematodes that are present in the soil. Philips, et al. (2021) then mentioned

that earthworms are widely used in composting organic wastes in the vermicomposting industry. Rehman, et al. (2023) reported that the end product of vermicomposting is called vermicasts that is excreted by the earthworms. These vermicasts are rich in readily available plant nutrients and beneficial microbes. Hence, vermicomposting main aim was to produce effective organic fertilizer for crops and ornamental plants. However, vermicomposting process has also simultaneously propagated these prolific earthworms leading to an increase of earthworm population. The increment of population could lead to insufficient number of organic wastes being fed per time as it becomes difficult in managing the vermicompost due to factors such as, lack of labour or logistic disruption. Thus, there are several research on studying the nutritional values and microbial load of earthworms as feed in powder form and mostly on the common red worm (Dada, et al., 2023; Gunya, et al., 2016; Suarez- Hernandez, et al., 2016; Bou-Maroun, et al., 2013).

2.2.1 Types of Earthworms

According to Ahmed and Al-Mutairi (2022), the annelid earthworms are categorized into three definite groups: anecic, endogeic and epigeic according to their feeding and burrowing habits and behaviour. Anecic earthworms thrives deep in the soil as they burrow vertically to haul organic matter deep into the soil to feed and thrive, and they excrete the casts on the soil surface (Ahmed and Al- Mutairi, 2022; and Medina-Sauza, et al., 2019). These earthworms are relatively large in size and sometimes have cast piles surrounding the entryway of their burrows (Ahmed and Al-Mutairi, 2022; Medina-Sauza, et al., 2019). Endogeic earthworms differ from anecic as reported by Ahmed and Al-Mutairi (2022) and Medina-Sauza, et al. (2019), as most of them do not burrow as deep and they move horizontally through the soil and feed mostly on partly decomposed organic matter and soil minerals. Whereas epigeic earthworms as reported by Ahmed and Al-Mutairi (2022) and Medina-Sauza, et al. (2019), most do not burrow and thrive mostly on soil surfaces to feed on organic litters that are partially decomposed. Medina-Sauza, et al. (2019) and Balbuena (2016) reported that ANC is categorized under epigeic earthworms because they are known to feed on surface litter. Although, when compared to the common nightcrawler, the Canadian Nightcrawler, *Lumbricus terrestris* reported by Steckley (2021), ANC does have relatively similar behaviour as the name suggested, it is a nightcrawler. This means that it is only actively feeding on the leaf litters and surface organic matter when there is no sunlight similar to that of the Canadian Nightcrawler.

2.2.2 Different Composition of C/N ratio of Vermicompost

Earthworms requires ideal growth conditions provided by feedstock for efficient productivity. The feedstock conditions depend highly on the substrates used as it alters the carbon to nitrogen (C/N) ratio of the vermicompost as mentionedby Rostami (2011). Biruntha, et al. (2020) reported the importance of initial C/N ratio of the feedstock determines the final pH when feedstock has been composted by the earthworms. Carbon contributes to the acidity while nitrogen ammonification contributes to the alkalinity of the feedstock (Biruntha, et al., 2020). According to Singh, et al. (2020), earthworms normally thrive in neutral soil pH but able to tolerate acidity and alkalinity of pH 5 and pH 8. respectively. Thus, it is more ideal to incorporate more carbon than nitrogen in a feedstock because carbon has a pH of about 5.6 while nitrogen when converted to ammonia at the end of vermicomposting has a pH of about 11 to 12. This way, the pH of the feedstock at the end can achieve a roughly neutral pH. According to several studies, the C/N ratio of 3:1 in cow manure and empty fruit bunches feedstock was observed to be one of the most ideal feedstocks for earthworm growth and productivity (Mashur, et al., 2021; Katakula, et al., 2021; Hayawin, et al., 2012).

2.3 Presence of Probiotics in Earthworms

Das, et al. (2021) reported to have isolated *Streptomyces antibioticus*, a strainof probiotic from the gut of *Eisenia fetida*, the common red worm with the purpose to grow the probiotic strain then, incorporate into aquaculture feed to improve the health and growth of *Heteropneustes fossilis*, a species of freshwater catfish. Besides, an abundant number of bacteria from phylum *Firmicutes* (30%) and *Actinobacteria* (3%) was sampled from the gut and casts of earthworm *Lumbricus terrestris* by Sun, et al. (2020). Adnan and Joshi (2013) also reported that *Streptomyces limanii* was present in the gut of earthworm and possess potential probiotic activity. In another study by Szmigiel, et al. (2021), *Bacillus subtilis* strain 87Y isolated from the gut of *Eisenia fetida* was observed to promote probiotic *Lactococcus* spp. growth and inhibit *Salmonella* spp. and *Staphylococcus aureus* pathogenicity.

2.3.1 Desiccation and Thermal Tolerance of Lactic Acid Bacteria

Li, et al. (2020) reported that lactic acid bacteria (LAB) have a relatively interminable history of being utilized as probiotics. Miglani, et al. (2023) mentioned that LAB belong to the *Firmicutes* phylum that is found abundant in the earthworm gut as reported by Sun, et al. (2020) and these bacteria are Gram-positive and is related to the Bacilli class. According to Perez-Chabela, et al. (2007), two *Lactobacillus* and two *Pediococcus* strains of lactic acid bacteria

isolated from processed sausages were able to survive thermal treatment at 70°C for an hour. These strains were also reported to remain viable during storage at 8°C for 12 days. Thus, certain LAB notably has intrinsic thermal tolerance. Besides that, Kang, et al. (2015) also reported that *Lactococcus lactis* sourced from the stock culture preserved in Korea was able to withstand heat shock for 20 minutes at 50°C when it was treated with heat adaptation for 10 minutes at 42°C. Subsequently, Kang, et al. (2015) mentioned that since heat treatment is required in dairy industry during processing of products such as, yoghurt, there is no doubt that *Lactococcus* sp. is able to survive heat treatments and remain viable to be able to produce the yoghurt that is sold commercially in the market.

Next, Kimelman and Shemesh (2019) reported that *Bacillus subtilis*, a strain of LAB probiotic can tolerate desiccation by producing a bio-coat known as protective extracellular matrix (ECM). In addition to that, Kimelman and Shemesh (2019) mentioned that the production of ECM by this strain is also able to accommodate to those LAB which are desiccant-sensitive through extensive production of ECM. In addition, Moretti, et al. (2023) reported that LAB produces exopolysaccharides that makes up the ECM to withstand stresses including heat and drying. Consequently, Moretti, et al. (2023) mentioned that drying is one of the ideal methods in obtaining the best possible viable count of LAB when preserving foods. It is noteworthy that LAB does not require oxygen to carry out its enzymatic metabolism to convert carbohydrates into lactic acid hence, LAB thrives anaerobically. In addition, Maresca, et al. (2018) reported that LAB tend

to produce oxygen by-products such as hydrogen peroxide in aerobic conditions which, can be highly toxic to LAB when accumulated in high amount and eventually lead to programmed cell death or apoptosis.

2.3.2 Method of Screening for Lactic Acid Bacteria

There are several preliminary standard screening methods to determine the presence of LAB. Namely, selective de Man, Rogosa, and Sharpe (MRS) agar for growth, catalase test with hydrogen peroxide, Gram staining to determine Gram-positive bacilli or cocci under light microscope and most importantly acid and bile tolerance test to simulate the gastrointestinal tract environment where LAB is known to deploy their beneficial effects to the host (Leandro, et al., 2021).

Zhang, et al. (2022) cultured the isolated LAB in MRS broth and incubate for 24 to 72 hours under aerobic condition at 37°C then, subject the cultured LAB to acid and bile tolerance test by altering the solution pH with 1M HCl and adding bile salts to the solution, respectively. Consecutively, the isolates incubated in acid and bile were grown on MRS agar for incubations at 0-hour, 3 hour and 7 hour. Loh, et al. (2009) reported to have performed a 10-fold serial dilution before growing the LAB on MRS agar for colony forming unit (CFU) count. Furthermore, Ismail, et al. (2018) performed serial dilution as well as growing each diluted LAB sample on MRS agar for CFU count before subjecting the pure culture to biochemical tests such as, Gram staining, catalase test, growth

on different salt concentrations in MRS broth, growth in 14°C and 37°C for 7 daysand motility test.

Furthermore, molecular identification provides a definite and detailed LAB identification to the species level. Abdullah, et al. (2021) reported that after culturing LAB in MRS broth for 24 hours at 37°C, the isolates were subjected to DNA extraction by Promega KIT followed by polymerase chain reaction (PCR) using a universal primer, 16S rRNA of ~1.5 KB gene fragment prior to gel electrophoresis. The DNA was then extracted from agarose gel using FastGene® Gel/PCR Extraction Kit for purification prior to running BLAST then, alignment of sequence to finally construct the phylogenetic tree for analysis. Olatunde, et al. (2018) reported to have done the same molecular identification protocol using the same universal primer subsequent to preliminary biochemical screening methods.

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

This research was conducted in Universiti Tunku Abdul Rahman, Faculty of Science laboratory from January 2023 to July 2023. The African Nightcrawler (ANC), *Eudrilus eugeniae* was sourced from a home-based vermicompost farm using cattle manure and kitchen waste as feedstock in Batu Gajah, Perak. The overview of this research methodology is as shown in Figure 3.1.

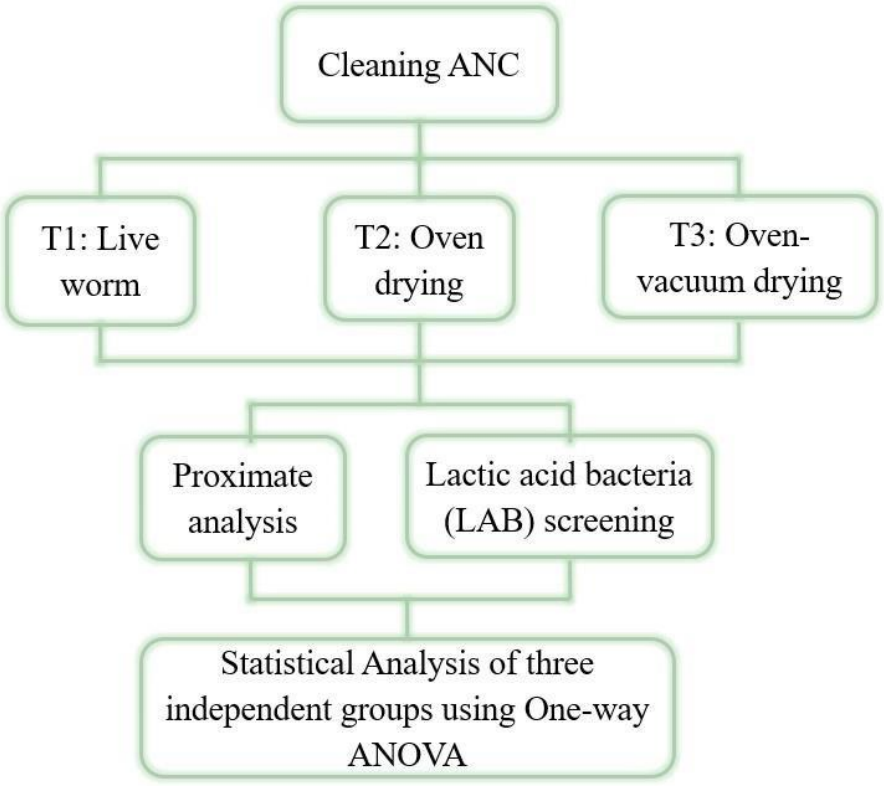


Figure 3.1: Overview of research methodology.

3.2 Preparation of Live African Nightcrawler

The method of live ANC preparation was adapted and slightly modified from research done by Gunya, et al. (2016) and Bou-Maroun, et al. (2013). About 60 g of ANC was hand-picked and cleaned for each sample treatment. Altogether there were three treatments, and each treatment was performed in triplicates. Hence, the total weight of ANC needed for this research was 540 g. The feedstock residue attached on the hand-picked ANC was thoroughly rinsed with distilled water using a wash bottle and sieve (Figure 3.2). After washing, the ANC was immediately dabbed dry gently using paper towels. The cleaned ANC was then transferred onto a makeshift aluminium weighing boat for weight measurement using the Mettler-Toledo ML304T electronic analytical balance (Figure 3.3). The ANC was then sacrificed with thermal drying in the conventional oven (BINDER^{TM} FED 115-UL) for 5 minutes before proceeding with the drying treatments using either the conventional oven (BINDER^{TM} FED 115-UL) or vacuum oven (BINDER^{TM} VD 53).

Figure 3.2: ANC in a sieve during washing process.

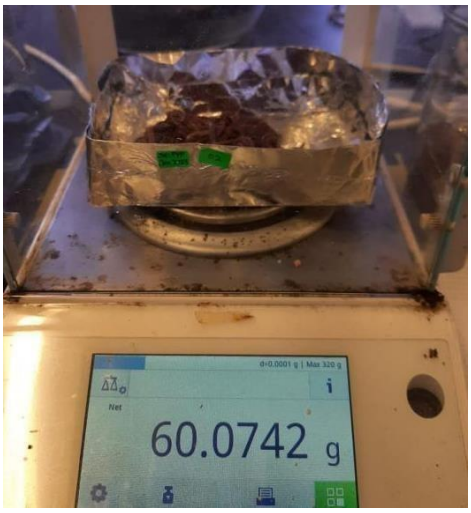


Figure 3.3: Weight taking of ANC using an electronic balance.

3.3 Drying Treatments of African Nightcrawler

The first drying treatment of ANC was done according to Bou-Maroun, et al. (2013) using a conventional oven at 60°C for 4 hours. The conventional oven was pre-heated to 60°C for 15 minutes prior spreading the 60 g of ANC onto a metal mesh attached above a metal tray (Figure 3.4). The ANC was dried in the conventional oven for 4 hours. After 4 hours, the dried ANC was then transferred onto the same makeshift aluminium weighing boat to cool down in the desiccator. After cooling down the ANC was blended into powder form using a blender. The blended ANC was then transferred into a 50 ml centrifuge tube, sealed with thermoplastic, Parafilm M® self-sealing film and kept in the desiccator for proximate analysis and lactic acid bacteria testing. The procedure was done for another two replicates for conventional oven treatment.

The second drying treatment of ANC was adapted from the research done by Kröncke, et al. (2018) with modification using vacuum oven to dry meal worms at 60°C. However, for this research ANC was in lieu of meal worms and was dried for 3 hours at 60°C. The vacuum oven was pre-heated to 60°C. The same metal tray was used for the drying process. The ANC were evenly spread out on the metal mesh. After ANC were dried, the cooling down process, blending and storing procedure like the conventional oven sample processing were conducted. The procedure was done for another two replicates for vacuum oven. The duration of drying for vacuum oven was an hour shorter than that of the conventional oven because vacuum oven was reported by Yao, et al. (2019) to have a shorter duration of drying compared to conventional oven due to the rapid moisture evaporation assisted by the vacuum pump.

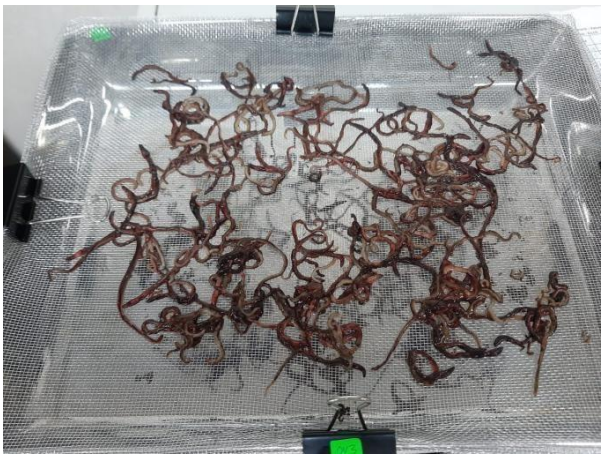


Figure 3.4: Metal mesh secured on top of metal tray with ANC.

3.4 Proximate Analysis

The proximate analysis for live worm, conventional oven-dried and vacuum oven-dried ANC treatments were done according to AOAC (2005), official methods of analysis for dry matter, crude protein, crude fat, and ash determination.

3.4.1 Dry Matter and Moisture Determination

Dry matter determination was conducted by weighing the empty, clean crucible which was recorded as (A) g. The weight of the crucible was tare and approximately 2 g of sample was weight using the same crucible and recorded as (B) g. The same procedure was performed for all the treatment triplicates before drying in the conventional oven for 2 hours at 135°C. After drying, the crucibles were allowed to cool down in the desiccator before weighing it as (C) g. The recorded weights were used in the calculation (Formula 3.1) to calculate the dry matter percentage and moisture percentage.

Formula 3.1:

Crucible = (A) g
Sample weight = (B) g
Crucible + dried sample = (C) g
Dried sample (D) = (C) - (A)
Dry matter percentage (DM%) = (D) ÷ (B) × 100
Moisture percentage = 100 - DM%

3.4.2 Crude Protein Determination

Crude protein determination involves three processes. The first being digestion process, to digest the sample. The second being distillation process, to extract the nitrogen from the digested sample. Lastly, titration process which is to determine the amount of nitrogen extracted or present in the digested sample. The digestion block (BUCHI^{TM} Speed Digester K-425) was pre-heated to 420°C with a magnitude of 8. The catalyst was prepared with a mixture of 7 g of potassium sulphate and 0.8 g of copper sulphate added into a Kjeldahl tubes. This was done for 10 Kjeldahl tubes. About 1 g of sample was weight for each treatment replicate and added into the Kjeldahl tubes, respectively. Next, 20 ml of concentrated sulphuric acid was added to each of the Kjeldahl tube. One Kjeldahl tube was set as a blank with only the catalyst and concentrated sulphuric acid. After the digester block has been pre-heated for about 10 minutes, the prepared Kjeldahl tubes were then secured into the block to be digested for 1.5 hours. After digestion the solution in the tube would turn bluish green (Figure 3.6) in colour indicating the protein digestion has completed. The tubes were allowed to cool down for 1 hour.

During that 1-hour cool down period, 50 ml of boric acid was added into a conical flask with 10 drops of methyl red indicator. The same procedure was repeated for another 9 conical flasks to prepare for distillation process. The distillation unit (BUCHI^{TM} Distillation Unit K-355) was cleaned by using sodium hydroxide and distilled water. After an hour of cooling down the tubes, the Kjeldahl tube with blank was secured into the distillation unit then,

30 ml of distilled water was added, and sodium hydroxide was added until the solution in the tube turned black. The distillation program was selected to run for 2 minutes with the sample outlet pipe inserted into the prepared conical flask. The same procedure goes for the following tubes with digested samples. After each distillation, the conical flask solution would change from red to clear green.

After completing distillation for all the tubes, each conical flask that contains each distilled blank and samples were subjected to titration using 0.1N hydrochloric acid (HCl). During titration, the turning point of titration was noted when the solution in the conical flask changed from clear green to bluish grey (Figure 3.7) and the burette tap was shut. At this point, a drop of HCl is sufficient to turn the bluish-grey solution to reddish pink (Figure 3.7) which marks the end of titration and the volume of HCl used was recorded as V1. The procedure was repeated for the rest of the conical flask with distilled samples and blank. The HCl volume used for blank was recorded as V2. The recorded volumes were used in the calculation (Formula 3.2) to calculate the protein percentage.

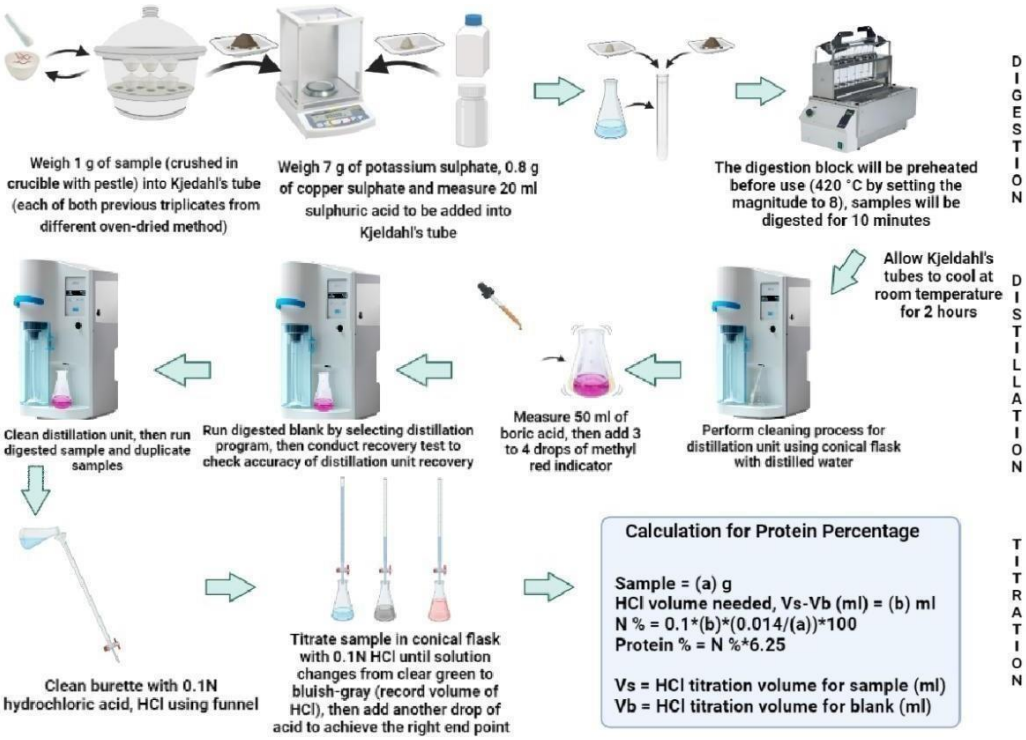
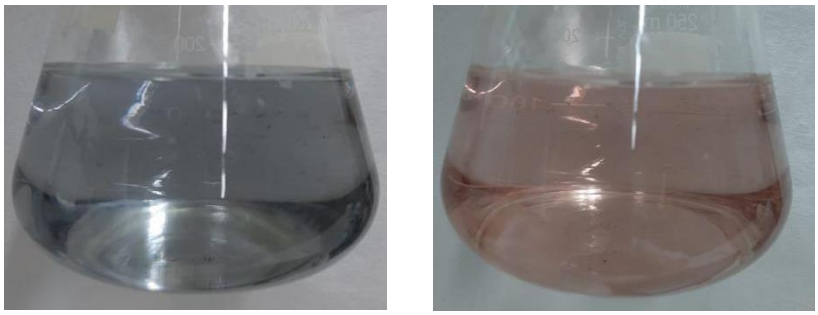


Figure 3.5: Crude protein determination methodology.

Figure 3.6: End of protein digestion by BUCHI^{TM} Speed Digester.



(a) (b)

Figure 3.7: Titration. (a) Turning point of titration; (b) End point of titration.

Formula 3.2:

Sample = (a) g

HCl volume needed, V1 – V2 (ml) = (b) ml

Nitrogen percentage (N%) = 0.1 × (b) × [0.014 ÷ (a)] × 100

Protein percentage = N% × 6.25

Vs = HCl titration volume needed for sample (ml)

Vb = HCl titration volume needed for blank (ml)

3.4.3 Crude Fat Determination

Crude fat was determined by using fat analyser (Gerhardt™ SOXTHERM® SOX 416). Three boiling stones were added into each extraction beakers prior to drying in the oven for 1 hour at 105°C. After drying, the extraction beakers were cooled down in the desiccator for 1 hour followed by weighing the extraction beakers with boiling stone and recorded as M1. During the 1 hour cooling down of extraction beakers, sample preparations were done. The filter paper was folded to create a pouch. The filter paper pouch weight was tare and about 5 g of sample was weighed and recorded as M0. The opening of the

pouch was then folded to close the opening and the entire pouch was inserted into the thimble and secured with a cotton ball inserted into the thimble opening. The same procedure of weighing the sample to inserting into the thimble was done for all treatment samples and live ANC. After both the M1 and M0 has been recorded, each thimble was then inserted into the holder in the extraction beakers. About 90 ml of petroleum ether (40-60°C) was added to each beaker in the fume hood. The extraction beakers with samples were then secured onto the extraction beaker holders. The program for SOXTHERM® was selected to run for 2.5 hours. At the end of extraction (Figure 3.8), the extraction beakers were then placed into the holder and dried for 1 hour at 105°C in the oven. After drying, the extraction was left to cool in the desiccator for 1 hour prior to weighing and recorded as M2. The recorded weights were used in the calculation (Formula 3.3) to calculate the fat percentage.

Figure 3.8: End of fat extraction by SOXTHERM®.

Formula 3.3:

Fat percentage = [(M2 - M1) ÷ M0] × 100

3.4.4 Crude Ash Determination

The crude ash determination was performed by using furnace (Nabertherm™ LT-14). The crucible was weighed and recorded as (a) g. Then, about 3 g of samples was weighed and recorded as (b) g using the tare crucible earlier. The same was done for all treatment samples and live ANC. The crucibles were then placed in the furnace (Figure 3.9). The furnace was programmed to ash dry the samples at 550°C for 5 hours and left to cool overnight. The crucible with ashed samples were weighed and recorded as (c) g. The recorded weights were used in the calculation (Formula 3.4) to calculate the ash percentage.

Figure 3.9: Crucibles with samples in furnace.

Formula 3.4:

Crucible = (a) g
Sample = (b) g
Crucible and ashed sample = (c) g
Ashed sample (d) = (c) - (a)
Ash percentage = (d) ÷ (b) × 100

3.5 Lactic Acid Bacteria Isolation and Biochemical Test

The lactic acid bacteria presence in the sample treatments and live ANC were evaluated by culturing the sample on selective media for lactic acid bacteria which is the de Man, Rogosa and Sharpe (MRS) agar. The colonies were enumerated and subculture, followed by biochemical tests including Gram staining, catalase test, acid and bile tolerance test. The methods were a combination of adaptation and slight modification from studies performed by Zhang, et al. (2022), Leandro, et al. (2021), Ismail, et al. (2018) and Loh, et al. (2009). All the procedures were done under aseptic condition in the horizontal laminar flow cabinet (Esco^{TM} AHC-4D1) using autoclaved materials and apparatus, working close to the fire from Bunsen burner and ensured that there was little to no traffic with frequent sanitisation using 70% ethanol.

3.5.1 Serial Dilution

About 5 g of sample was weighed and diluted in 45 ml of 0.1% peptone water as a stock solution. The stock solution was homogenized by using vortex. The stock solution was subjected to a 10-fold serial dilution up until dilution factor 10^6. The micropipette was used to aspirate 1 ml of stock solution into 9 ml of 0.1% peptone water for dilution factor 10^1 then vortex, followed by aspirating 1 ml of 10^1 solution into 9 ml of 0.1% peptone water for dilution factor 10^2. This procedure was repeated up until dilution factor 10^6 as shown in Figure 3.10.

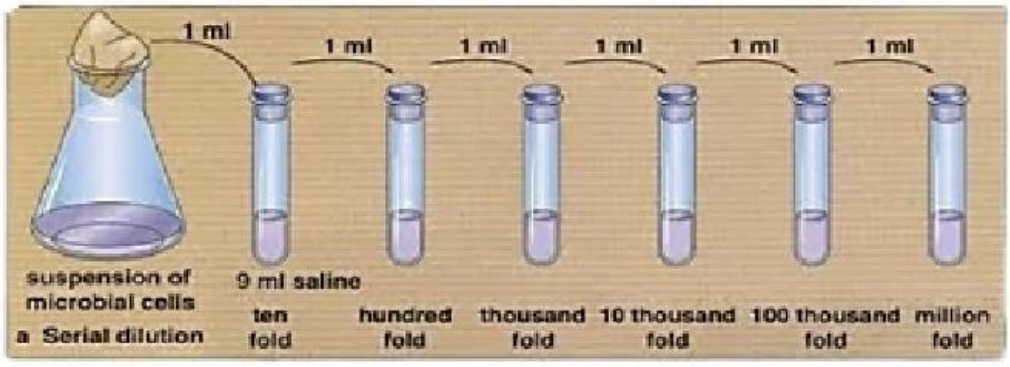


Figure 3.10: Serial dilution.

3.5.2 Pour Plating

After serial dilution, 1 ml of each dilution factor solution was aspirated into clean Petri plates, and this was done in triplicates for each dilution factor. The Petri plates were then filled with liquefied MRS agar until half the height of the plate. Each time the plate was filled, the liquid agar was mixed thoroughly with the

sample solution in the direction of the figure 8. A control was done with 0.1% peptone water and liquefied MRS agar only. The Petri plates were allowed to solidify with Bunsen burner close by in the laminar flow. After cooling down, The Petri plates were sealed with Parafilm M® self-sealing film and incubated invertedly in the incubator at 37°C for 24 hours.

3.5.3 Colony Forming Unit Count

After 24 hours of incubation, colony forming unit (CFU) was counted for plate with countable number of colonies range from 30 to 300 colonies according to O'Toole (2016). The CFU count was recorded and calculated in CFU/g unit according to Formula 3.5.

Formula 3.5:

CFU/g = [total number of colonies counted ÷ (amount of ANC in mg in 0.1 ml aliquoted from the 10^{-3} serial dilution tube onto the culture plate which the colonies were counted)] × 1000

3.5.4 Subculture

From the Petri plate with countable CFU, one plate from each sample, two colonies with different colony morphology were transferred using an inoculation

loop into an autoclaved universal bottle containing 10 ml of MRS broth, respectively. Then the labelled Day 1 universal bottles were closed with the caps and incubated at 37°C for 24 hours.

After the first 24-hour incubation, 1 ml of the activated culture was aspirated from Day 1 universal bottles into the Day 2 universal bottles containing 9 ml of MRS broth, respectively. The universal bottles were closed with the caps and incubated at 37°C for the next 24 hours. The same procedure was repeated with cultures incubated for Day 3. The activated culture was then used for the subsequent acid and bile tolerance test.

3.5.5 Gram Stain

The Gram staining method was adapted from research done by Tripathi and Sapra (2023) The Gram stain was done with Day 2 activated culture. A drop of distilled water was added onto a clean microscope glass slide and the activated culture was transferred and mixed onto the drop with an inoculation loop. The glass slide was gently and swiftly flamed using a Bunsen burner by passing it over the flame twice. Crystal violet stain was then added onto the droplet with activate culture and left for 60 seconds before gently rinsing with distilled water. Next, iodine was added and left for 60 seconds and rinsed gently with distilled water followed by 95% ethanol. Lastly, safranin stain is added and left for 60 seconds then, rinsed gently with distilled water. The glass slide was gently and swiftly flamed again before viewing under the light microscope using objective

lens magnification of 4×, 10× and 40× to observe the presence of Gram-positive bacilli or cocci.

3.5.6 Catalase Test

Catalase test was performed using Day 2 activated culture as well. Three drops of 3% hydrogen peroxide were dropped onto a clean glass slide individually. The first drop from the left was inoculated with a positive control, followed by the activated culture of the treatment sample then, a negative control with distilled water (Figure 3.11).

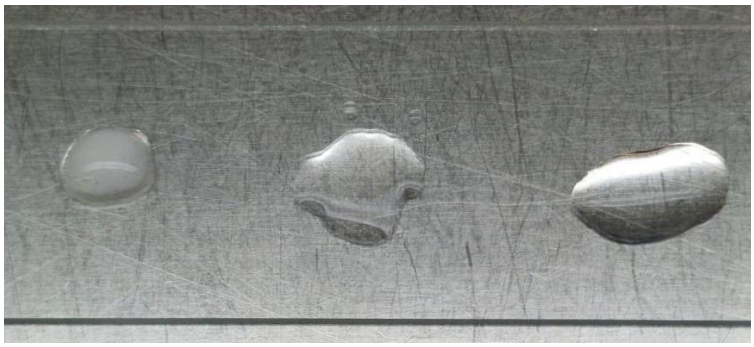


Figure 3.11: Catalase test (From the left: Positive control, treatment, negative control).

3.5.7 Bile Tolerance Test

The bile tolerance test was prepared by adding 8 ml of MRS broth and 1 ml of 3% bile into the universal bottle to achieve 0.3% bile tolerance test when 1 ml of Day 3 activated culture was added. After the bile tolerance test was prepared, 1 ml of each activated culture was aspirated into each bottle of 0.3% bile. A blank was prepared with only 3% bile and 9 ml of MRS broth. The optical density (OD) was read at 600 nm wavelength for 0 hour before incubation for those inoculated with activated culture and blank using a spectrophotometer (Thermo Scientific™ GENESYS™ 10S UV-VIS). The OD reading was taken for 1, 2 and 3 hours of incubation for those inoculated with activated culture. The OD readings were recorded in triplicates. The calculation of growth increment (Formula 3.6) was done using the mean of OD readings.

3.5.8 Acid Tolerance Test

The method used in acid tolerance test was adapted with modification from the research done by Ismail, et al. (2018). The acid tolerance test was prepared by adding 8 ml of MRS broth, 1 ml of 10% pepsin and pH of the media was decreased by adding 1M of HCl in a universal bottle for each Day 3 activated culture. There were three different pH prepared. About 2.5 ml of 1M HCl was added to achieve pH 1, about 1.5 ml for pH 2 and about 0.5 ml with 10 drops for pH 3. After the acid tolerance test was prepared, 1 ml of each activated culture was aspirated into each bottle of pH 1, pH 2 and pH 3. Blank was prepared for each pH without

activated culture and 9 ml of MRS broth instead of 8 ml. The optical density (OD) was read at 600 nm wavelength for 0 hour before incubation for those inoculated with activated culture and blank using a spectrophotometer. The OD reading was taken for 1, 2 and 3 hours of incubation for those inoculated with activated culture. The OD readings were recorded in triplicates. The calculation of growth increment (Formula 3.6) was done using the mean of OD readings.

Formula 3.6:

OD_0 = mean of OD reading at 0 h

OD_3 = mean of OD reading at 3 h

Growth increment (%) = $[(OD_3 - OD_0) \div OD_0] \times 100$

3.6 Statistical Analysis

All the measurements recorded in this research were performed in triplicates. The statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) software, IBM SPSS Statistics Version 21. The analysis of mean comparison of the nutritional value and lactic acid bacteria CFU/g and OD readings between the control (live worm), conventional oven-dried (T1) and vacuum oven-dried (T2) ANC was conducted using One-way analysis of variance (ANOVA), followed by Duncan's multiple comparisons and Tukey's honestly significant difference (HSD) at the significance level of $\alpha = 0.05$. All the data were tabulated and expressed as mean ± standard deviation based on Duncan's multiple comparisons.

CHAPTER 4

RESULTS

4.1 External Morphology and Proximate Analysis of African Nightcrawler (ANC) With and Without Drying Treatments

The morphology of ANC samples for each treatment, live ANC (T1), conventional oven-dried ANC (T2) and vacuum oven-dried (T3) are shown in Figure 4.1. The vacuum oven-dried ANC (T3) was observed to have a relatively preserved form in terms of the colour and structure similar to the live ANC (T1), while the ANC sample from conventional oven-dried (T2) looks darker in colour and compactly deformed in structure.

(a)

(b)

(c)

Figure 4.1: ANC treatments. (a) Live ANC (T1); (b) conventional oven-dried (T2); (c) vacuum oven-dried (T3).

4.1.1 Dry Matter, Moisture, Crude Protein, Crude Fat and Crude Ash Percentage of Different African Nightcrawler (ANC) Treatments

The proximate analysis for live ANC (T1), conventional oven-dried ANC (T2) and vacuum oven-dried ANC (T3) were performed to determine the percentage of dry matter, moisture, crude protein, crude fat and crude ash. The results are shown in Table 4.1 and Figure 4.2.

The samples of ANC for all the 3 treatments were significantly different ($p < 0.05$) with samples from T3 showing the highest dry matter percentage (99.75 ± 0.03), followed by T2 (99.42 ± 0.05) and control T1 the lowest (22.14 ± 0.00). In contrast to the percentage of dry matter, the moisture percentage of the control was significantly the highest ($p < 0.05$) compared to T2 and T3.

In terms of the crude protein content, ANC dried using vacuum oven (T3) showed significantly highest ($p < 0.05$) crude protein percentage (54.46 ± 0.53%) compared to T2 (47.14 ± 0.86%). and control T1 (9.82 ± 0.06%). Interestingly, the percentage of crude fat for T2 (5.18 ± 0.20%) samples and T3 (5.44 ± 0.22%) does not have any significant difference ($p \geq 0.05$). While for the live worms (T1) the percentage of crude fat determined was 0.00%. The results for the crude ash showed that ANC dried using vacuum oven (T3) (45.55 ± 0.86%) have significantly higher ($p < 0.05$) percentage compared to T2 and T1 with 42.30±0.80% and 9.09 ± 0.02%, respectively.

Table 4.2: Nutritional Composition of African Nightcrawler (ANC) between Different Treatments.

Nutritional Content	T1	T2	T3
Dry Matter (%)	22.14 ± 0.00[a]	99.42 ± 0.05[b]	99.75 ± 0.03[c]
Moisture (%)	77.86 ± 0.00[c]	0.58 ± 0.05[b]	0.25 ± 0.03[a]
Crude Protein (%)	9.82 ± 0.06[a]	47.14 ± 0.86[b]	54.46 ± 0.53[c]
Crude Fat (%)	0.00 ± 0.00[a]	5.18 ± 0.20[b]	5.44 ± 0.22[b]
Crude Ash (%)	9.09 ± 0.02[a]	42.30 ± 0.80[b]	45.55 ± 0.86[c]

Key: T1 live worm, T2 conventional oven dried, T3 vacuum oven dried.

[a, b, c] means with different superscripts in a row were significantly different ($p < 0.05$).

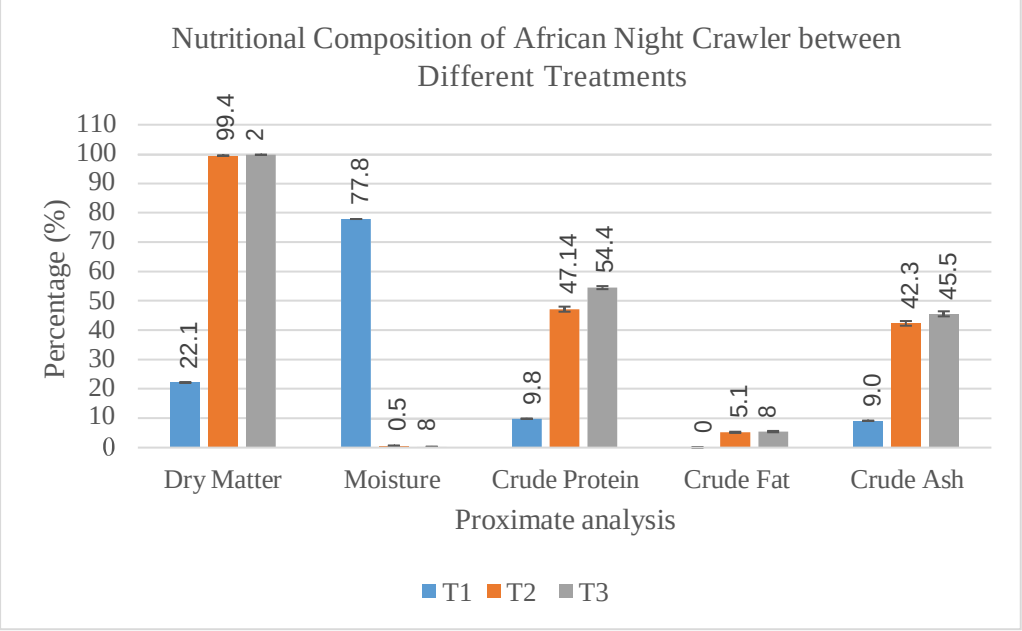


Figure 4.2: Nutritional Composition of African Nightcrawler (ANC) between Different Treatments

4.2 Potential Lactic Acid Bacteria Isolates

The cultured potential lactic acid bacteria (LAB) isolates were subjected to preliminary standard screening for LAB. This involved colony morphology identification, catalase test, Gram staining, colony count reported as colony forming unit (CFU) per gram, acid tolerance and bile tolerance test. Two colonies were isolated from each treatment culture for the preliminary standard screening.

4.2.1 Characteristics of Potential Lactic Acid Bacteria Isolates

There are two distinct colony morphologies as shown in Figure 4.4 was observed to have similar biochemical characteristics namely between the first group of isolates C1, C3, C5 each isolates derived from T1, T2 and T3, respectively and the second group of isolates C2, C4, C6 derived from T1, T2 and T3, respectively. The differences between these two colonies morphologies are the colour and elevation of the colony. C1, C3, C5 colonies were observed to be glistening, yellowish-white colonies with convex elevation on MRS agar, while C2, C4, C6 were whitish translucent colonies that are embedded in the MRS agar. The similarities observed between these two colonies were circular form, entire margin, catalase-negative and are Gram-positive bacilli and cocci (Figure 4.3).

Table 4.3: Characteristics of Potential Lactic Acid Bacteria Isolates.

Isolates		Colour	Form	Margin	Elevation	Catalyst Test	Gram Stain
T1	**C1**	GYW	Circular	Entire	Convex	Negative	+ bacilli
	C2	WT	Circular	Entire	Embedded	Negative	+ bacilli
T2	**C3**	GYW	Circular	Entire	Convex	Negative	+ bacilli
	C4	WT	Circular	Entire	Embedded	Negative	+ bacilli
T3	**C5**	GYW	Circular	Entire	Convex	Negative	+ bacilli
	C6	WT	Circular	Entire	Embedded	Negative	+ bacilli

Key: T1 live worm, T2 conventional oven dried, T3 vacuum oven dried, C1 colony 1, C2 colony 2, C3 colony 3, C4 colony 4, C5 colony 5, C6 colony 6, GYW glistening yellowish-white, WT whitish translucent, + Gram-positive.

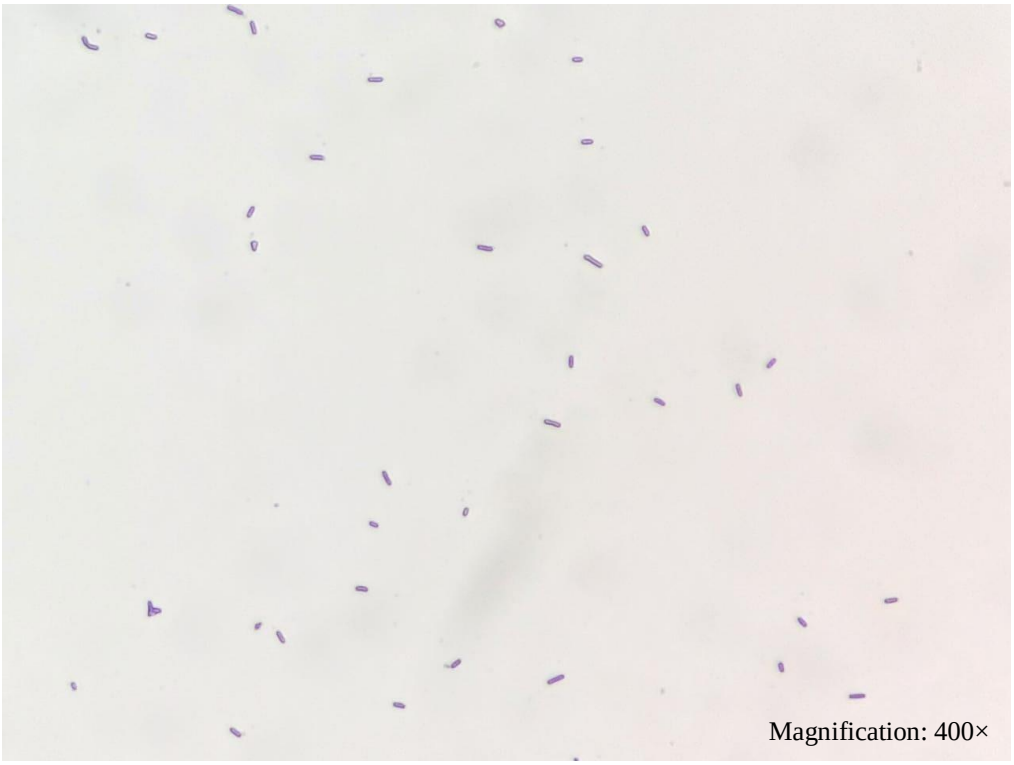
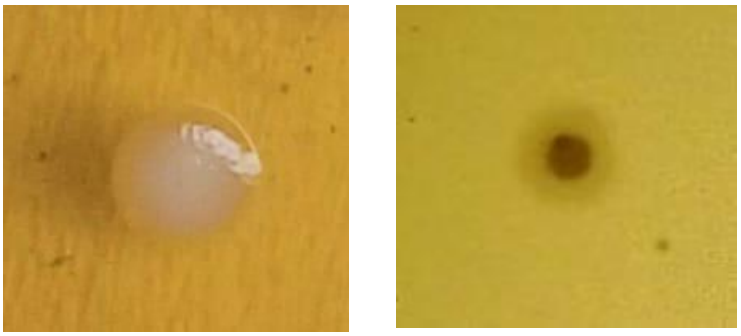


Figure 4.3: Gram staining (Gram-positive bacilli and cocci).



(a) (b)

Figure 4.4: Elevation of colony. (a) Convex elevated; (b) Embedded.

4.2.2 Colony Forming Unit Per Gram of Potential Lactic Acid Bacteria Isolates

The colony forming unit (CFU) count was done for three culture plates with dilution factor 10^3 from each treatment as it was within the countable range of 30 to 100 colonies after 24-hour incubation at 37°C. The colony forming unit per gram (CFU/g) was calculated with respect to the initial weight of sample diluted for stock solution and the amount of aliquot cultured in the MRS agar from dilution factor 10^3. The results of the colony count are shown in Table 4.3. The CFU/g of potential lactic acid bacteria isolates from live ANC sample, T1 (11.4×109 ± 0.35) was significantly higher ($p < 0.05$) compared to than T2 and T35.87×109 ± 0.15, 4.90×109 ± 0.60) CFU/g, respectively. The CFU/g of potential lactic acid bacteria between samples from T2 and T3 does not differ significantly ($p \geq 0.05$).

Table 4.4: Average Colony Forming Unit (CFU) Per Gram of Potential Lactic Acid Bacteria Isolates

Colony	CFU/g
T1	$11.4\times10^{9} \pm 0.35^{b}$
T2	$5.87\times10^{9} \pm 0.15^{a}$
T3	$4.90\times10^{9} \pm 0.60^{a}$

Key: T1 live worm, T2 conventional oven dried, T3 vacuum oven dried.

$^{a, b}$ means with different superscripts in the column were significantly different ($p < 0.05$).

4.2.3 Optical Density (OD) Reading of Growth in Bile Tolerance Test of Potential Lactic Acid Bacteria Isolates

There is several research proving the presence of lactic acid bacteria (LAB) isolated from the gut of earthworms (Das, et al., 2021; Szmigiel, et al., 2021; Sun, et al., 2020; Adnan and Joshi, 2013). In this study, potential LAB isolated from T1, T2 and T3 was subjected to bile tolerance test. There was no significant difference ($p \geq 0.05$) observed from the 0 hour to the 3rd hour for all the potential LAB isolates, except for isolate C5 that showed a significant decrease in growth from the 0th hour to the 1st hour (0.39 ± 0.04 to 0.20 ± 0.03). Although most of the tabulated growth values was observed to have no significant difference ($p \geq 0.05$), there is a noticeable decrease in growth for isolates C1, C3, and C5, and an increase in growth for isolates C2, C4, and C6 as shown in Figure 4.5.

Table 4.5: Treatment 1, 2 and 3 Optical Density 600 nm Reading of Growth at Each Hour for Bile Tolerance Test.

Isolates		0 h	1 h	2 h	3 h
T1	**C1**	0.38 ± 0.06[a]	0.36 ± 0.03[a]	0.35 ± 0.03[a]	0.35 ± 0.11[a]
	C2	0.30 ± 0.05[a]	0.27 ± 0.07[a]	0.31 ± 0.05[a]	0.42 ± 0.25[a]
T2	**C3**	0.32 ± 0.03[a]	0.28 ± 0.12[a]	0.30 ± 0.09[a]	0.28 ± 0.04[a]
	C4	0.33 ± 0.03[a]	0.31 ± 0.11[a]	0.33 ± 0.01[a]	0.35 ± 0.10[a]
T3	**C5**	0.39 ± 0.04[b]	0.20 ±0.03[a]	0.25 ± 0.04[a]	0.23 ± 0.03[a]
	C6	0.27 ± 0.12[a]	0.27 ± 0.05[a]	0.30 ± 0.00[a]	0.32 ± 0.06[a]

Key: T1 live worm, T2 conventional oven dried, T3 vacuum oven dried, C1 colony 1, C2 colony 2, C3 colony 3, C4 colony 4, C5 colony 5, C6 colony 6.

[a, b] means with different superscripts in a row were significantly different ($p < 0.05$).

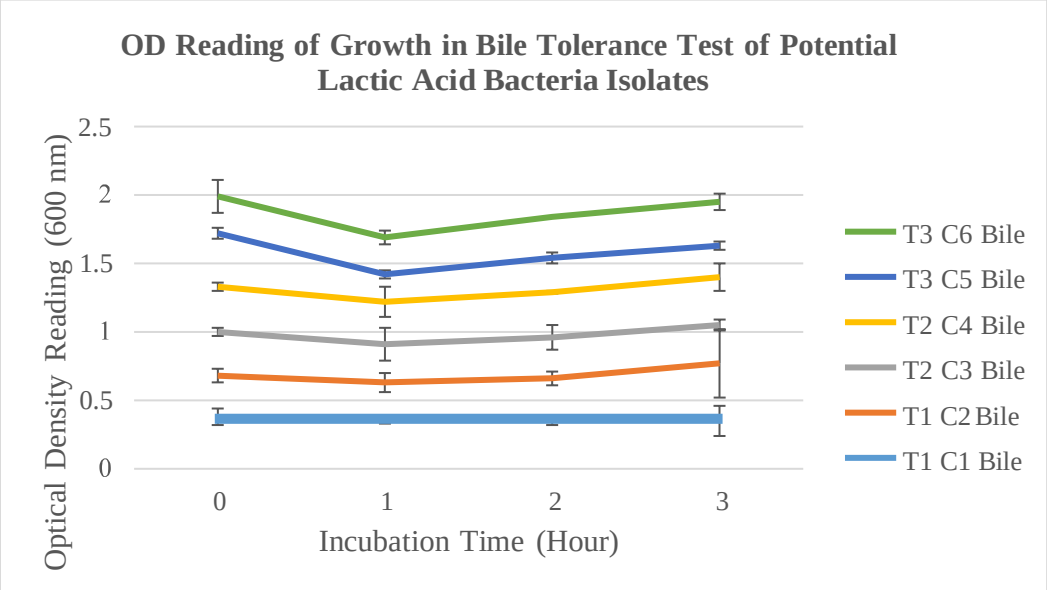


Figure 4.5: OD Reading of Growth in Bile Tolerance Test of Potential Lactic Acid Bacteria Isolates

4.2.4 Optical Density (OD) Reading of Growth in Acid Tolerance Test of Potential Lactic Acid Bacteria Isolates

The same potential LAB isolated from T1, T2, and T3 was also tested for acid tolerance at three distinct pH levels, namely pH 1, pH 2, and pH 3. All potential LAB isolates showed no significant difference ($p \geq 0.05$) in growth from the 0^{th} hour to the 3^{rd} hour, except for isolate C5, which showed a significant decrease ($p < 0.05$) in growth from the 0^{th} hour to the 1^{st} hour in pH 1 (1.31 ± 0.17 to 0.93 ± 0.20), pH 2 (0.61 ± 0.03 to 0.41 ± 0.03), and pH 3 (0.45 ± 0.03 to 0.32 ± 0.03) according to Table 4.7, followed by a slight increase before marginally decrease again, closing in on constant as depicted perspicuously in Figure 4.10.

Although most of the tabulated growth values was observed to have no significant difference ($p \geq 0.05$), there is an obvious decreasing growth trend for both isolate C1 and C5 by the 3^{rd} hour as illustrated in Figure 4.6 and Figure 4.10, respectively. Notably, isolate C2 was able to grow slightly in pH 2 and 3 but decreased in growth in pH 1 as shown in Figure 4.7.

Whereas isolate C6 was observed to have a stable increase in pH 1, 2 and 3 up until the 3^{rd} hour (Figure 4.11), while isolate C3 and C4 only showed an increase by the end of 3^{rd} hour incubation represented by Figure 4.8 and Figure 4.9, respectively.

Table 4.6: Treatment 1 Optical Density 600 nm Reading of Growth at Each Hour for Acid Tolerance Test.

Isolates		Tolerance Test	0 h	1 h	2 h	3 h
T1	**C1**	**pH 1**	1.00 ± 0.10[a]	1.04 ± 0.26[a]	0.87 ± 0.24[a]	0.63 ± 0.19[a]
		pH 2	0.99 ± 0.11[a]	1.05 ± 0.25[a]	0.88 ± 0.24[a]	0.66 ± 0.19[a]
		pH 3	0.58 ± 0.04[b]	0.57 ± 0.15[ab]	0.48 ± 0.13[ab]	0.35 ± 0.11[a]
	C2	**pH 1**	0.78 ± 0.17[a]	0.83 ± 0.36[a]	0.85 ± 0.39[a]	0.54 ± 0.04[a]
		pH 2	0.79 ± 0.15[a]	0.88 ± 0.41[a]	0.89 ± 0.43[a]	0.82 ± 0.57[a]
		pH 3	0.44 ± 0.10[a]	0.42 ± 0.25[a]	0.46 ± 0.18[a]	0.47 ± 0.19[a]

Key: T1 live worm, T2 conventional oven dried, T3 vacuum oven dried, C1 colony 1, C2 colony 2, C3 colony 3, C4 colony 4, C5 colony 5, C6 colony 6.

[a, b] means with different superscripts in a row were significantly different ($p < 0.05$).

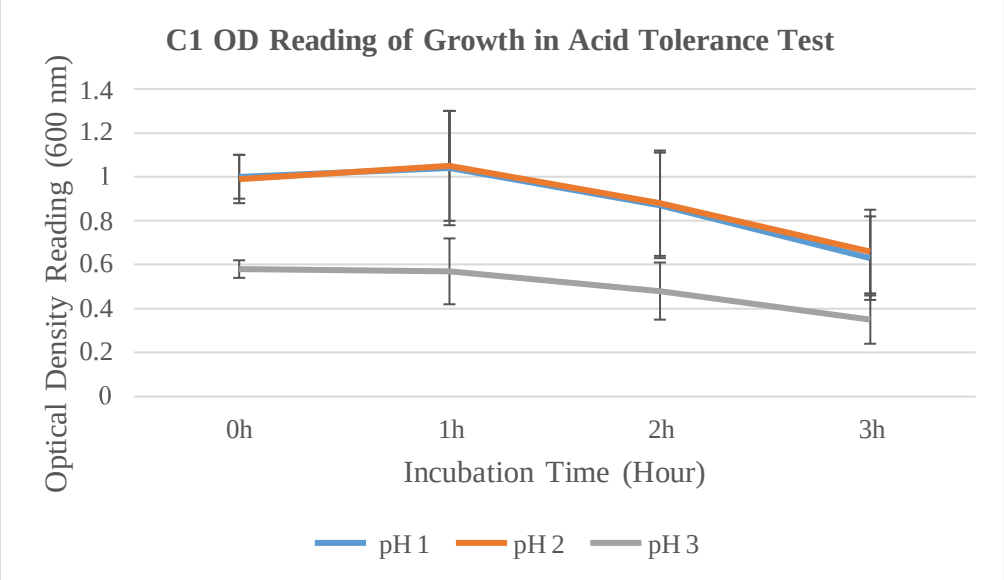


Figure 4.6: C1 isolate OD Reading of Growth in Acid Tolerance Test

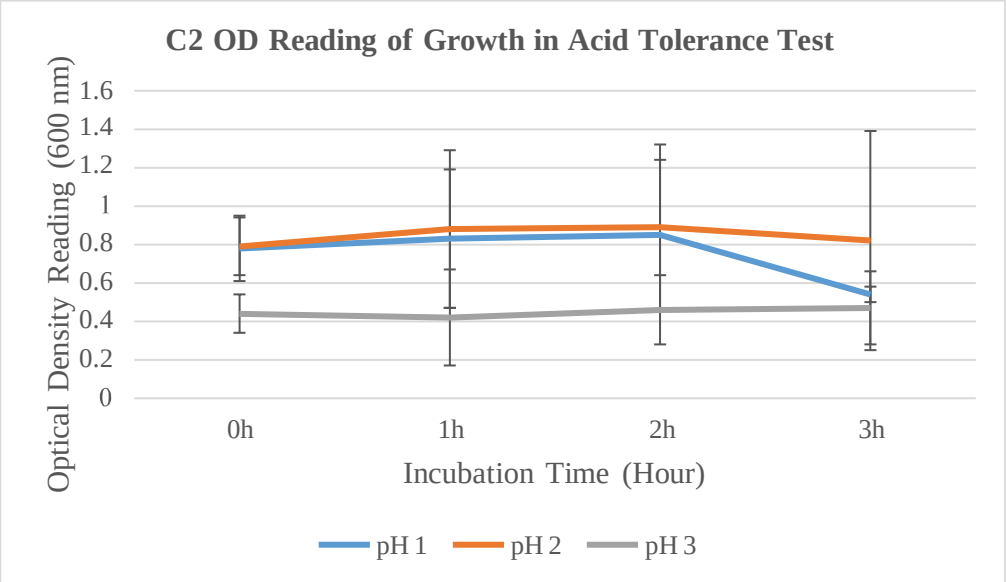


Figure 4.7: C2 isolate OD Reading of Growth in Acid Tolerance Test

Table 4.7: Treatment 2 Optical Density 600 nm Reading of Growth at Each Hour for Acid Tolerance Test.

Isolates		**Tolerance Test**	**0 h**	**1 h**	**2 h**	**3 h**
T2	**C3**	**pH 1**	0.32± 0.01^{a}	0.46± 0.28^{a}	0.47 ± 0.03^{a}	0.40 ± 0.05^{a}
		pH 2	0.30 ± 0.01^{a}	0.60 ± 0.27^{b}	0.49 ± 0.01^{ab}	0.41 ± 0.04^{ab}
		pH 3	0.30 ± 0.01^{a}	0.43 ± 0.20^{a}	0.42 ± 0.11^{a}	0.34 ± 0.06^{a}
	C4	**pH 1**	0.30 ± 0.01^{a}	0.46 ± 0.14^{a}	0.38 ± 0.07^{a}	0.41 ± 0.07^{a}
		pH 2	0.33 ± 0.02^{a}	0.53 ± 0.14^{b}	0.46 ± 0.06^{ab}	0.46 ± 0.04^{ab}
		pH 3	0.37 ± 0.05^{a}	0.35 ± 0.01^{a}	0.36 ± 0.04^{a}	0.37 ± 0.02^{a}

Key: T1 live worm, T2 conventional oven dried, T3 vacuum oven dried, C1 colony 1, C2 colony 2, C3 colony 3, C4 colony 4, C5 colony 5, C6 colony 6.

$^{a, b}$ means with different superscripts in a row were significantly different ($p <$ 0.05).

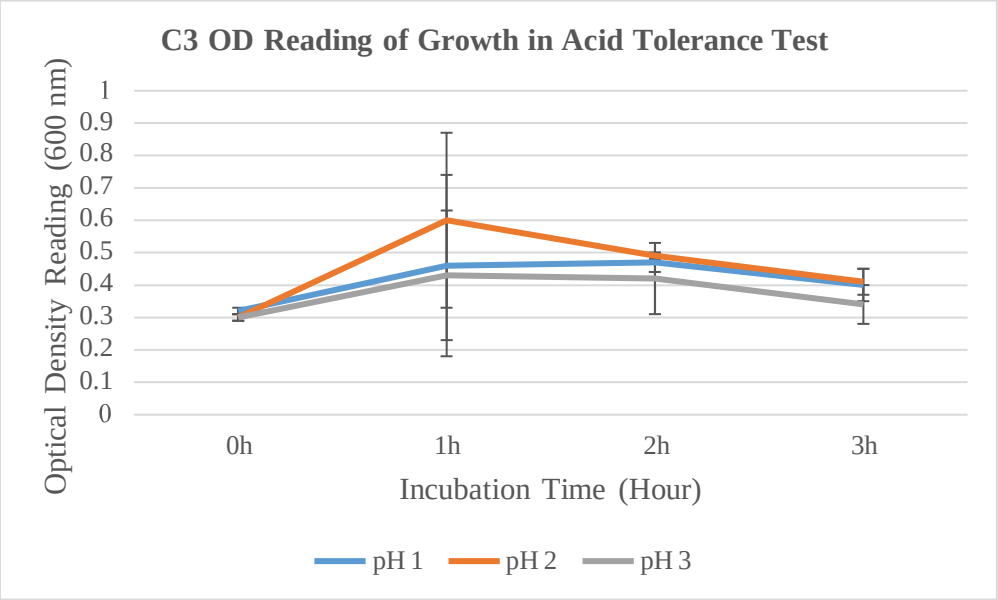


Figure 4.8: C3 isolate OD Reading of Growth in Acid Tolerance Test

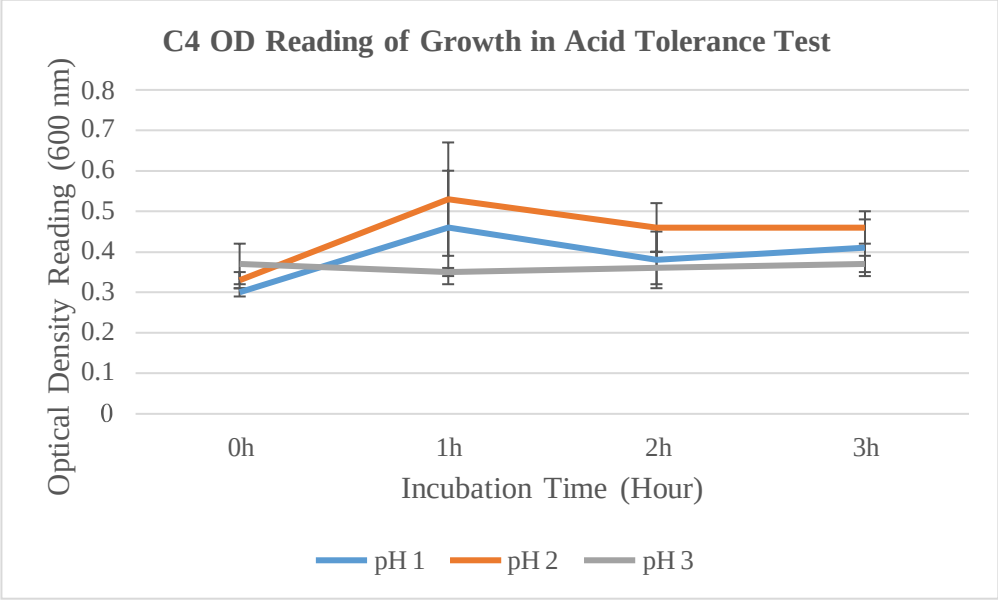


Figure 4.9: C4 isolate OD Reading of Growth in Acid Tolerance Test

Table 4.8: Treatment 3 Optical Density 600 nm Reading of Growth at Each Hour for Acid Tolerance Test.

Isolates		Tolerance Test	0 h	1 h	2 h	3 h
T3	**C5**	**pH 1**	1.31 ± 0.17^{b}	0.93 ± 0.20^{a}	1.07 ± 0.21^{ab}	1.06 ± 0.17^{ab}
		pH 2	0.61 ± 0.03^{b}	0.41 ± 0.03^{a}	0.47 ± 0.03^{a}	0.46 ± 0.02^{a}
		pH 3	0.45 ± 0.03^{b}	0.32 ± 0.03^{a}	0.36 ± 0.06^{ab}	0.36 ± 0.06^{ab}
	C6	**pH 1**	1.12 ± 0.29^{a}	1.19 ± 0.12^{a}	1.41 ± 0.30^{a}	1.42 ± 0.53^{a}
		pH 2	0.45 ± 0.08^{a}	0.47 ± 0.08^{a}	0.59 ± 0.13^{a}	0.57 ± 0.25^{a}
		pH 3	0.39 ± 0.07^{a}	0.38 ± 0.03^{a}	0.46 ± 0.07^{a}	0.46 ± 0.15^{a}

Key: T1 live worm, T2 conventional oven dried, T3 vacuum oven dried, C1 colony 1, C2 colony 2, C3 colony 3, C4 colony 4, C5 colony 5, C6 colony 6.

$^{a, b}$ means with different superscripts in a row were significantly different ($p < 0.05$).

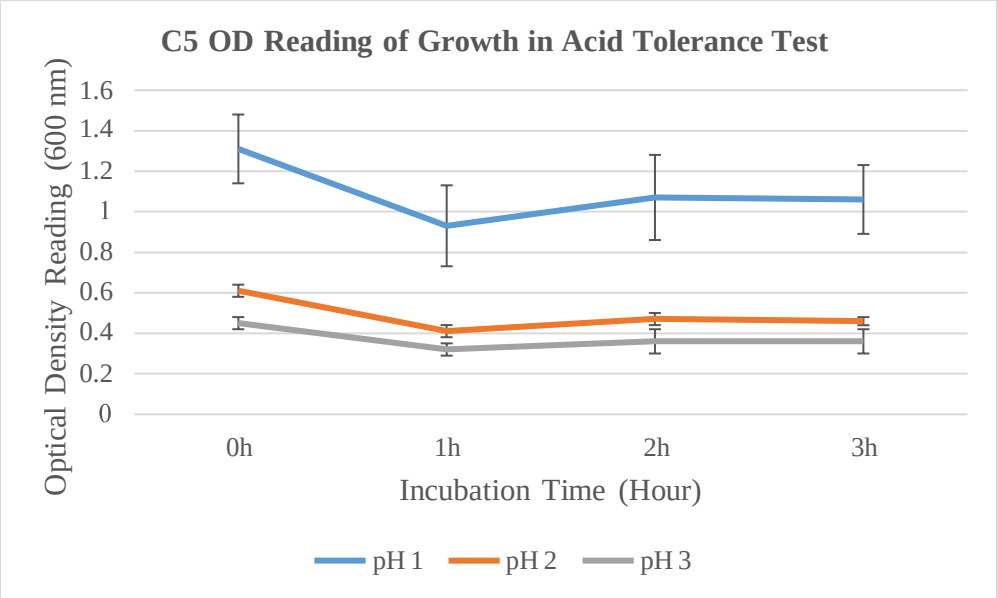


Figure 4.10: C5 isolate OD Reading of Growth in Acid Tolerance Test

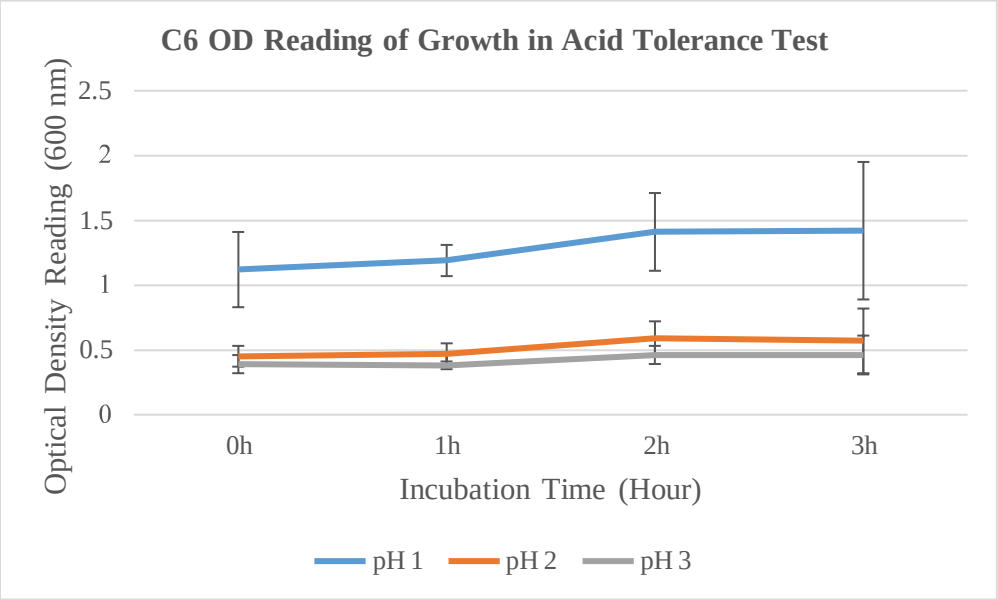


Figure 4.11: C6 isolate OD Reading of Growth in Acid Tolerance Test

4.2.5 Growth Increment Percentage (%) in Bile and Acid Tolerance of Potential Lactic Acid Bacteria Isolates

The growth increment percentage was calculated from the mean of OD reading recorded for bile and acid tolerance test. Interestingly, isolate C4 that was incubated in pH 3 increase to 0.37 ± 0.02 by 3rd hour similar to its 0-hour value which means a constant growth with no increment in growth as shown in Figure 4.12. It is evident by Figure 4.12 that isolate C1 and C5 had a negative growth increment by the end of 3rd hour of incubation for bile and acid tolerance test in contrast with isolate C4 and C6 which had growth increment. Contrasting between isolate C2 and C3 was the negative growth in pH 1 and bile, respectively while both isolates had similarity in growth increased for pH 2 and pH 3.

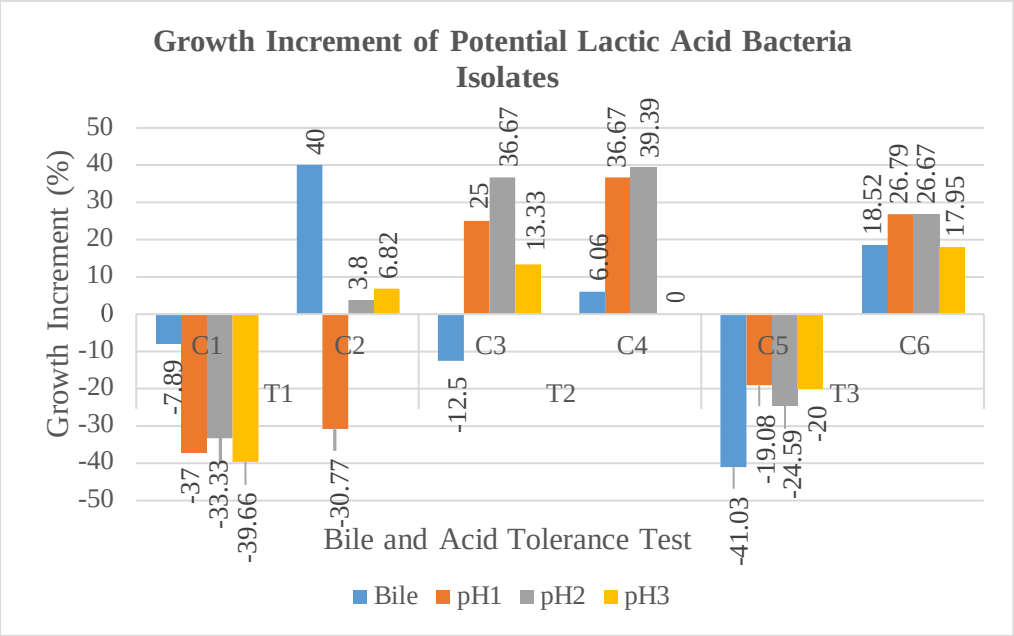


Figure 4.12: Growth Increment Percentage of Potential Lactic Acid Bacteria Isolates

CHAPTER 5

DISCUSSION

5.1 External Morphology and Proximate Analysis of African Night Crawler (ANC) With or Without Drying Treatments

The vacuum oven-dried ANC (T3) was observed to have a relatively better-preserved form in terms of morphology and colour similar to the live ANC (T1) when compared to conventional oven-dried ANC (T2). This is because vacuum oven drying requires a shorter duration of drying in the absence of oxygen. Hence, this reduces the time of exposure to heat and protects oxygen-sensitive organic compounds such as, protein and fat lipids from denaturation and degradation. Besides that, the colour of the sample can be preserved (Monteiro et al., 2015; Gunasekaran 1999).

In addition, Ngamwonglumlert and Devahastin (2018) reported that vacuum oven-dried products were observed to have high porosity thus, the product was observed to have a lesser degree of shrinkage compared to conventionally dried products similarly observed in Figure 4.1 for comparison between T2 and T3.

5.1.1 Dry Matter and Moisture Content of African Night Crawler (ANC) With or Without Drying Treatments

The significantly lower ($p < 0.05$) moisture content was observed in T3 ANC (0.25%) compared to T2 ANC (0.58%). The rapid evaporation due to vacuum-incorporated oven drying draws moisture out of samples (Chong, et al., 2019) efficiently leaving behind significantly higher ($p < 0.05$) dry matter content observed in T3 ANC (99.75%) compared to T2 ANC (99.42%). Feed sample with lower moisture content is beneficial as Rożen, et al. (2015) it reduces the perishability rate and simultaneously increases shelf life (Rożen, et al., 2015).

Interestingly, when compared to research done by Gunya, et al. (2016) where *Eisenia fetida* that was dried in the conventional oven at 90°C for 4 hours had a higher moisture content (10.5%). This could be the result of different drying protocol in terms of the batch size subjected to drying and the technique of laying out the earthworms for drying. Tarigan et al. (2020) state that the larger the surface area, the faster the moisture evaporates during drying. In addition, Gunya, et al. (2016) had covered the tray of *Eisenia fetida* with foil, which has obstructed the moisture from evaporating efficiently. Next, a more comparable research that was done by Bou-Maroun, et al. (2013) subjecting *Eisenia fetida* 60°C for 4 hours of drying in the conventional oven also has a higher moisture content (1.14%). This might be due to the method of moisture determination that was used by Bou-Maroun, et al. (2013), Karl Fisher titration which is a process of iodine titrant reacting with water to reach an endpoint indicated by colour change.

Thomas and Simmons (2019) stated that, while Karl Fisher titration may be highly accurate in detecting presence of water due to iodine titrant sensitivity, but it all lies in the operator's input manually to minimize systematic error.

Due to lack of studies in utilizing vacuum oven to dry earthworms, the research done by Kröncke, et al. (2018) using vacuum oven to dry *Tenebrio molitor*, meal worms at 60°C for 24 hours were employed as a close comparative to the vacuum oven-dried ANC (T3) in this research. Kröncke, et al. (2018) reported that the moisture content of meal worms was 1.65% which is higher than T3. The obvious reason is that meal worms are genetically and biologically different from ANC, thus they utilize water from their feed differently resulting indifferent moisture content.

5.1.2 Crude Protein, Crude Fat and Crude Ash Content of African Night Crawler (ANC) With or Without Drying Treatments

Various studies have shown that different drying methods will affect the nutritional content of the samples in terms of crude protein, crude fat and crude ash (Dada, et al., 2023; Kröncke, et al., 2018; Gunya, et al., 2016; Suarez-Hernandez, et al., 2016; Rożen, et al., 2015; Bou-Maroun, et al., 2013). In this study, the ANC dried using vacuum oven drying methods showed significantly higher ($p < 0.05$) crude protein (54.46%) compared to T2 ANC (47.14%). The T2 ANC crude protein content is lower due to protein oxidation while drying

conventionally in the presence of oxygen. Protein oxidation is induced by reactions with hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$) and the superoxide anion ($O_2^{\bullet-}$), which are primary species of reactive oxygen species (ROS) found commonly in aerobic conditions (Izdebska, 2016). Therefore, vacuum oven dried (T3) method of drawing water molecules out of the earthworm helps retain more protein content in the absence of oxygen preventing protein oxidation and denaturation. The live ANC (T1) crude protein content (9.82%) was significantly lower ($p < 0.05$) than T2 and T3. Since, moisture was not drawn out of the live ANC, the protein concentration is much lower per gram of sample.

The relatively higher ($p \geq 0.05$) crude fat content was observed in T3 (5.44%) compared to T2 (5.18%). The crude fat content was lower in T2 ANC that was dried by conventional oven in the presence of oxygen, which initiates peroxidation of lipids (Ayala, et al., 2014). It is the process of fatty acid decomposition into free radicals such as, ketones and aldehydes reducing the lipid and fat content (Li, et al., 2021). In addition, the process of lipid peroxidation disintegrates cell membranes which, are made up of phospholipid bilayers (Li, et al., 2021; Ayala, et al., 2014). The continuous free radical chain reactions cause oxidative stress that triggers programmed cell death or apoptosis (Li, et al., 2021; Ayala, et al., 2014). Thus, proteins in the cell disintegrate under oxidative stress and this contributes to further protein disintegration (Ayala, et al., 2014). Thus, T3 vacuum dried ANC in the absence of oxygen reduces the incidence of lipid peroxidation and retains relatively higher crude fat content.

Aykın-Dinçer, et al. (2019) reported that protein and fat content was higher in vacuum oven-dried (TABRS 5.77 μmol MA/kg) meat compared to oven-dried (TABRS 6.04 μmol MA/kg). TABRS is the abbreviation for thiobarbituric acid reactive substance assay used in lipid peroxidation determination to detect the amount of the by-product, thiobarbituric acid that is produced during lipid peroxidation. The higher the TABRS value, indicates high lipid peroxidation, which simultaneously triggers protein oxidation (Ayala, et al., 2014). Next, a significant ($p < 0.05$) crude ash content was observed in T3 (45.55%) compared to T2 (42.30%) due to lower moisture content which, increases the concentration of crude ash content per gram of sample (Mahirah, et al., 2018), while T1 has comparatively the highest moisture content (77.86%), thus the lowest ash content (9.09%).

In view of the research done by Gunya, et al. (2016) and Bou-Maroun, et al. (2013), where *Eisenia fetida* that was dried in the conventional oven at 90°C and 60°C, respectively for 4 hours had a higher crude protein, 59.7% and 67.31%, respectively. This could be due to the different feedstock the *Eisenia fetida* was grown in as the type of feedstock affects the crucial C/N ratio that is important for earthworm growth as mentioned by Biruntha, et al. (2020) and Rostami (2011). Similar rationale applies to crude fat (9.5%) content that was only reported by Gunya, et al. (2016), was higher than treatment ANC in this research. However, as a close comparison to T3 ANC due to lack of studies, Kröncke, et

al. (2018) reported that the crude protein and crude fat content of meal worms were 54.80% and 31.40%, respectively which is higher than T3. The obvious reason is that meal worms are genetically hence, biologically different from ANC, thus they procure and metabolize nutrients from the feed differently resulting in different crude protein and crude fat content.

5.2 Potential Lactic Acid Bacteria Isolates

Lactic acid bacteria (LAB) are a kind of bacterium that is often utilized in food fermentation processes such as making yoghurt, cheese, and sauerkraut. These bacteria are in charge of turning carbohydrates into lactic acid, which gives these fermented products their distinctive sour flavour. The positive features of LAB to gut health piqued the interest of researchers, who sought to cultivate and incorporate them into feed for animal well-being.

5.2.1 Characteristics of Potential Lactic Acid Bacteria Isolates

There are two distinct colony morphologies observed to have similar biochemical characteristics namely between C1, C3, C5 and C2, C4, C6 each from T1, T2 and T3, respectively. The colony morphology of C1, C3 and C5 is similar to that of lactic acid bacteria (LAB) reported by Escobar, et al. (2020) and Ary, et al. (2018) to be yellowish-white colonies with convex elevation on MRS agar. While the colony morphology of C2, C4 and C6 is similar to that of

LAB reported by Neogen (2019) to have whitish colonies that grows within the MRS agar. Khushboo, et al. (2023) and Ismail, et al. (2019) reported that LAB strains were observed to be catalase negative and is Gram-positive similar to that of the isolated colonies in this study. To explain, LAB are facultative anaerobes, which means they prefer anaerobic environments for growth but may live in microaerophilic settings provided bythe typical incubator (Amelia, et al., 2020). Hence, two types of colonies were observed in this study one that is embedded preferring to grow away from direct oxygen while another able to survive above the MRS agar in a microaerophilic condition. Instead of incubating under microaerophilic condition similar to this study, researchers might opt for incubation under anaerobic conditions by supplying carbon dioxide to the incubator or utilizing an anaerobe jar in order to specifically isolate strict-anaerobe LAB (Santillan, et al., 2015; Mulaw, et al., 2019). In addition, according to de Man, Rogosa and Sharpe (1960) and ThermoFisher Scientific (2023), MRS agar may be selective towards *Lactobacillus* strains but *Streptococcus, Pediococcus* and *Leuconostoc* under the probiotic community are still able to thrive. In this study, the colony morphology of isolates was similar to that of *Lactobacillus* strains unlike the *Streptococcus*, *Pediococcus* and *Leuconostoc* strains (Khushboo, et al., 2023; Ismail, et al., 2019; Neogen, 2019).

5.2.2 Colony Forming Unit Per Gram of Potential Lactic Acid Bacteria Isolates

The relatively higher ($p \geq 0.05$) CFU count was observed in T2 ANC (5.87×10^{8} CFU/g) compared to T3 ANC (4.90×10^{8} CFU/g). The concentration CFU/g of LAB in both T2 and T3 conforms to the minimum requirement (10^{6} CFU/g) needed to provide beneficial effects to the host (Gallardo-Rivera, et al., 2021; Jha, et al., 2020; Zommiti, et al., 2020). Gallardo-Rivera, et al. (2021) and Banu, et al. (2010) also reported that vacuum-oven drying LAB count is lower (3.5 Log-CFU/mL) than that of conventional oven (7.3 to 7.4 Log-CFU/mL) due to rapid moisture evaporation and air outlet by the vacuum system, micro-organisms tend to degrade thermally. LAB, as opposed to thermophilic microorganisms, are typically mesophiles that live in the temperature range of 20°C to 45°C, with optimal growth occurring between 30°C and 39°C (Schiraldi and De Rosa, 2015). As a result, most LAB enzymes denature when exposed to a rapid heating environment, as these enzymes are not like thermophilic enzymes that can withstand severe heat stress (Schiraldi and De Rosa, 2015). Zommiti, et al. (2020) and Vieco-Saiz, et al. (2019) also mentioned that a minimum supplementation range of $10^{6\text{-}11}$ CFU/g depending on LAB strain and type of animal. However, live ANC (11.4×10^{9}) has a lower CFU/g when compared to live *Eisenia fetida* (4.6×10^{10}) (Tedesco, et al., 2020) as the feedstock used was different and evidently both earthworms are genetically different.

5.2.3 Optical Density Reading of Growth and Growth Increment in Bile and Acid Tolerance Test of Potential Lactic Acid Bacteria Isolates

Isolate C2 was observed to tolerate bile salts in selective media, pH2 and pH3 but unable to grow under pH1 similar to certain *Lactobacillus* strains that do not survive well in pH1 after the third hour of incubation (Hassanzadazar, et al., 2012). According to Papadimitriou, et al. (2016), free acids created by LAB during the fermentation process might collect in the growth media and create an acidic environment, which can hinder the development of LAB and other microorganisms. When the acidity of the environment surpasses a particular threshold, LAB growth is impeded, and cell death may occur. This is because an overly acidic environment might affect bacterial biological activities, such as their capacity to absorb and digest nutrients. Free acids can also lower the pH of the growth medium, which can impair LAB growth. LAB can normally grow in a small pH range, and if the pH goes too low, they cannot develop or live. As a result, the formation of free acids can limit their growth and survival in some settings. Isolate C4 and C6 growth was observed to have an increasing trend in acid and bile tolerance test similar to that of the common probiotic *Lactobacillus* strains (Khushboo, et al., 2023; Escobar, et al., 2020). Isolate C1 and C5 were observed to decline in growth by the end of 3rd hour of incubation for acid and bile tolerance test. This might be a unique strain of lactic acid bacteria discovered in the gut of *Eudrilus eugeniae* (ANC) which needs further validation in future study. Since, C1 and C5 isolates could not tolerate the bile and acid test, it is likely that these 2 isolates could not be classified as probiotics since probiotic strains should exhibit tolerance to acid and bile salts (Samson, et al., 2020).

Isolate C3 growth was observed to slightly decline in 0.3% bile selective media but able to grow under pH 1, pH 2 and pH 3 similar to that of *Lactobacillus strain* reported by Hassanzadazar, et al. (2012), which recorded a slow decline in growth by the end of 3rd hour incubation of 0.3% bile. In addition, Govindarajan and Prabaharan (2015) reported to have successfully isolated lactic acid bacteria strains such as, *Streptococcus* sp., *Pseudomonas*, *Bacillus cereus* and *Bacillus subtilus* from the gut tissue of *Eudrilus eugeniae*.

5.3 General Discussion

The ANC from T3 has higher nutritional values compared to T2 ANC. In addition to that, T3 has a lower moisture content which helps in extending the shelf life, reducing perishability rate and has a higher dry matter content. Thus, T3 ANC requires lesser amount on dry matter basis to be supplemented as animal feed to meet the nutritional requirements. Other than that, the potential lactic acid bacteria concentration is sufficient to provide beneficial probiotic effects for the host. ANC versus meal worm (MW) and black soldier fly larvae (BSFL), ANC has an advantage over these two commonly researched animal feeds as it has a lack of chitin layer which, does not require an additional processing step to remove chitin for better digestibilityof nutrients especially protein (Siddiqui, et al., 2022). In addition, BSFL and MW are terrestrial organisms similar to ANC, hence the fats determined lack EPA and DHA that are beneficial lipids derived from fish oil. Thus, the high fat content in BSFL and MW lack beneficial impact to host. English, et al. (2021) reported that since BSFL is rich in fat content, another additional processing step to de-fat

BSFL is essential in ensuring longer storage of produced feed in order to prevent the fat from going rancid. Moreover, BSFL and MW has a higher feed conversion ratio (FCR) of 2.3 to 5.5 and 1.98, respectively (Gligorescu, et al., 2020; Thévenot, et al., 2018) compared to ANC that has a minimum FCR of 0.5 (Lacap and Dantis, 2020).

In comparison with the current trending feed made from meal worm (MW) and black soldier fly larvae (BSFL), where MW was dried with vacuum oven at 60°C for 24 hours (Kröncke, et al., 2018) and BSFL was dried with spray drying technique (Zulkifli, et al., 2022), vacuum oven-dried ANC from this research has lower moisture content (0.25%) and higher crude ash (45.55%) compared to MW (1.73% moisture, ash not reported) and BSFL (7.10% moisture, 8.27% ash). In consideration of comparison made with BSFL of different drying technique, as a leverage Grattepanche and Lacroix (2013) reported that spray drying technique dries materials faster than vacuum drier. Nevertheless, the spray-dried BSFL moisture and ash content could not top the vacuum oven-dried ANC in this research. In addition, BSFL crude protein (48.20%) is lower than that of the vacuum oven-dried ANC (54.46%), while MW crude protein (54.80%) is slightly higher. However, BSFL (25.69%) and MW (31.40%) crude fat is higher than that of ANC (5.44%). Nevertheless, vacuum oven-dried ANC meets the minimum crude protein and crude fat required by poultry (19-21%, 2-5%) (Akinmutimi, et al., 2018; Ravindran, et al., 2016) and ruminants (≥ 7%, 5-6%) (Harty and Olson, 2020; Bionaz, et al., 2020). When we compare the nutritional

composition of ANC discovered in our study to earlier research, we find significant variation. This might be attributed to changes in methodological setup, ambient conditions, feedstock variation, and processing methods in this research such as, enhanced drying method by utilizing metal mesh and tray to allow more surface area of ANC to be exposed to heat and drying (Dada, et al., 2023; Gunya, et al., 2016; Suarez-Hernandez, et al., 2016; Bou-Maroun, et al., 2013; Kröncke, et al., 2018; Rożen, et al., 2015). Nonetheless, we found the disparities between our findings and those of Gunya, et al. (2016) and Bou-Maroun, et al. (2013) perplexing, given that these authorsevaluated earthworms using relatively similar processing methods. As a result, we believe that genetic variability of the common red worm (*Eisenia fetida*) and ANC (*Eudrilus eugeniae*) is an additional aspect that should be examined and investigated.

5.3.1 Suggestion for Future Study

The current research has laid a foundation on the feasibility of producing ANC meals using both conventional and vacuum oven drying methods with the latter showed a better retention in nutritional value. However, the characterisation of the lactic acid bacteria that were isolated needs further validation using molecular methods in future studies. By determining the specific LAB species, research can detect any possible novel LAB found in ANC gut. Furthermore, screening for pathogens on the ANC meals can be conducted to evaluate the safety of using the ANC meal as animal feed. Other than that, to refine the drying

method for vacuum oven in order to load larger batch of ANC for efficient drying in the interest of livestock commercial feed production. This way, producers can process ANC in bulk to market and sell for ease of logistics management.

CHAPTER 6

CONCLUSIONS

The vacuum oven drying method (T3) for processed ANC meals were able to retain higher nutritional values, with a crude protein (CP) of 55.03%, crude fat (CF) of 5.44%, crude ash (CA) of 45.55% and dry matter (DM) of 22.79%, compared to conventional oven drying method (T2). In terms of reducing the drying duration and amount of DM required for feed formulation, T3 fulfils the criteria. The processing methods, vacuum oven and conventional oven method did not have adverse effects on the LAB count with 3 isolates out of 4 derived from the conventional and vacuum oven-dried ANC were found able to tolerate bile and acid medium in this study. Thus, vacuum oven drying method is more effective in the process of drying ANC to be use as worm meals production as it retains higher nutrient content and beneficial amount of LAB probiotics.

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APPENDICES

Appendix: Appendix for Chapter 4 (Results)

Appendix (Table 1): One-Way ANOVA results for proximate analysis of live ANC (T1), conventional oven-dried ANC (T2) and vacuum oven-dried ANC (T3)

One-Way ANOVA

Descriptive

Proximate analysis

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Dry_matter_content	T1	3	22.139300	.0015875	.0009165	22.135357	22.143243	22.1381	22.1411
	T2	3	99.420000	.0458258	.0264575	99.306163	99.533837	99.3700	99.4600
	T3	3	99.750000	.0300000	.0173205	99.675476	99.824524	99.7200	99.7800
	Total	9	73.769767	38.7231233	12.9077078	44.004539	103.534994	22.1381	99.7800
Moisture_content	T1	3	77.860667	.0016442	.0009493	77.856582	77.864751	77.8588	77.8619
	T2	3	.580000	.0458258	.0264575	.466163	.693837	.5400	.6300
	T3	3	.250000	.0300000	.0173205	.175476	.324524	.2200	.2800
	Total	9	26.230222	38.7231067	12.9077022	-3.534992	55.995437	.2200	77.8619
Crude_protein	T1	3	9.820000	.0600000	.0346410	9.670952	9.969048	9.7600	9.8800
	T2	3	47.136667	.8580404	.4953899	45.005176	49.268157	46.2400	47.9500

	T3	3	54.463333	.5300314	.3060138	53.146662	55.780004	53.9300	54.9900
	Total	9	37.140000	20.7403062	6.9134354	21.197589	53.082411	9.7600	54.9900
Crude_fat	T1	3	.000000	.0000000	.0000000	.000000	.000000	.0000	.0000
	T2	3	5.183333	.1960442	.1131862	4.696333	5.670334	5.0000	5.3900
	T3	3	5.436667	.2236813	.1291425	4.881011	5.992322	5.1800	5.5900
	Total	9	3.540000	2.6614235	.8871412	1.494249	5.585751	.0000	5.5900
Crude_ash	T1	3	9.091833	.0163711	.0094519	9.051165	9.132501	9.0791	9.1103
	T2	3	42.299000	.1760341	.1016333	41.861707	42.736293	42.1350	42.4850
	T3	3	45.550000	.8574964	.4950758	43.419861	47.680139	45.0400	46.5400
	Total	9	32.313611	17.4786153	5.8262051	18.878358	45.748864	9.0791	46.5400

ANOVA

Proximate analysis

		Sum of Squares	df	Mean Square	F	Sig.
Dry_matter_content	Between Groups	11995.836	2	5997.918	5992884.101	.000
	Within Groups	.006	6	.001		
	Total	11995.842	8			
Moisture_content	Between Groups	11995.826	2	5997.913	5992513.040	.000
	Within Groups	.006	6	.001		
	Total	11995.832	8			
Crude_protein	Between Groups	3439.241	2	1719.620	5053.908	.000
	Within Groups	2.042	6	.340		
	Total	3441.282	8			
Crude_fat	Between Groups	56.488	2	28.244	957.792	.000
	Within Groups	.177	6	.029		
	Total	56.665	8			
Crude_ash	Between Groups	2442.483	2	1221.241	4779.461	.000
	Within Groups	1.533	6	.256		
	Total	2444.016	8			

Post Hoc Tests

Multiple Comparisons

Proximate analysis of live ANC (T1), conventional oven-dried ANC (T2) and vacuum oven-dried ANC (T3)

Dependent Variable		(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Dry_matter_content	Tukey HSD	T1	T2	-77.2807000*	.0258307	.000	-77.359956	-77.201444
			T3	-77.6107000*	.0258307	.000	-77.689956	-77.531444
		T2	T1	77.2807000*	.0258307	.000	77.201444	77.359956
			T3	-.3300000*	.0258307	.000	-.409256	-.250744
		T3	T1	77.6107000*	.0258307	.000	77.531444	77.689956
			T2	.3300000*	.0258307	.000	.250744	.409256
Moisture_content	Tukey HSD	T1	T2	77.2806667*	.0258315	.000	77.201408	77.359925
			T3	77.6106667*	.0258315	.000	77.531408	77.689925
		T2	T1	-77.2806667*	.0258315	.000	-77.359925	-77.201408
			T3	.3300000*	.0258315	.000	.250742	.409258
		T3	T1	-77.6106667*	.0258315	.000	-77.689925	-77.531408
			T2	-.3300000*	.0258315	.000	-.409258	-.250742
Crude_protein	Tukey HSD	T1	T2	-37.3166667*	.4762741	.000	-38.778006	-35.855327

			T3	-44.6433333*	.4762741	.000	-46.104673	-43.181994
		T2	T1	37.3166667*	.4762741	.000	35.855327	38.778006
			T3	-7.3266667*	.4762741	.000	-8.788006	-5.865327
		T3	T1	44.6433333*	.4762741	.000	43.181994	46.104673
			T2	7.3266667*	.4762741	.000	5.865327	8.788006
Crude_fat	Tukey HSD	T1	T2	-5.1833333*	.1402115	.000	-5.613541	-4.753126
			T3	-5.4366667*	.1402115	.000	-5.866874	-5.006459
		T2	T1	5.1833333*	.1402115	.000	4.753126	5.613541
			T3	-.2533333	.1402115	.246	-.683541	.176874
		T3	T1	5.4366667*	.1402115	.000	5.006459	5.866874
			T2	.2533333	.1402115	.246	-.176874	.683541
Crude_ash	Tukey HSD	T1	T2	-33.2071667*	.4127297	.000	-34.473535	-31.940799
			T3	-36.4581667*	.4127297	.000	-37.724535	-35.191799
		T2	T1	33.2071667*	.4127297	.000	31.940799	34.473535
			T3	-3.2510000*	.4127297	.001	-4.517368	-1.984632
		T3	T1	36.4581667*	.4127297	.000	35.191799	37.724535
			T2	3.2510000*	.4127297	.001	1.984632	4.517368

*. The mean difference is significant at the 0.05 level.

Post Hoc Tests

Homogeneous Subsets

Dry matter content of live ANC (T1), conventional oven-dried ANC (T2) and vacuum oven-dried ANC (T3)

Tukey HSD and Duncan

	Treatment	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	T1	3	22.139300		
	T2	3		99.420000	
	T3	3			99.750000
	Sig.		1.000	1.000	1.000
Duncan[a]	T1	3	22.139300		
	T2	3		99.420000	
	T3	3			99.750000
	Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Post Hoc Tests

Homogeneous Subsets

Moisture content of live ANC (T1), conventional oven-dried ANC (T2) and vacuum oven-dried ANC (T3)

Tukey HSD and Duncan

	Treatment	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	T3	3	.250000		
	T2	3		.580000	
	T1	3			77.860667
	Sig.		1.000	1.000	1.000
Duncan[a]	T3	3	.250000		
	T2	3		.580000	
	T1	3			77.860667
	Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Post Hoc Tests

Homogeneous Subsets

Crude protein content of live ANC (T1), conventional oven-dried ANC (T2) and vacuum oven-dried ANC (T3)

Tukey HSD and Duncan

	Treatment	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	T1	3	9.820000		
	T2	3		47.136667	
	T3	3			54.463333
	Sig.		1.000	1.000	1.000
Duncan[a]	T1	3	9.820000		
	T2	3		47.136667	
	T3	3			54.463333
	Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Post Hoc Tests

Homogeneous Subsets

Crude fat content of live ANC (T1), conventional oven-dried ANC (T2) and vacuum oven-dried ANC (T3)

Tukey HSD and Duncan

	Treatment	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	T1	3	.000000	
	T2	3		5.183333
	T3	3		5.436667
	Sig.		1.000	.246
Duncan[a]	T1	3	.000000	
	T2	3		5.183333
	T3	3		5.436667
	Sig.		1.000	.121

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Post Hoc Tests

Homogeneous Subsets

Crude ash content of live ANC (T1), conventional oven-dried ANC (T2) and vacuum oven-dried ANC (T3)

Tukey HSD and Duncan

	Treatment	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	T1	3	9.091833		
	T2	3		42.299000	
	T3	3			45.550000
	Sig.		1.000	1.000	1.000
Duncan[a]	T1	3	9.091833		
	T2	3		42.299000	
	T3	3			45.550000
	Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix (Table 2): One-Way ANOVA results for CFU per gram of live ANC (T1), conventional oven-dried ANC (T2) and vacuum oven-dried ANC (T3)

One-Way ANOVA

Descriptive

CFU per gram

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
T1	3	11.3667	.35119	.20276	10.4943	12.2391	11.00	11.70
T2	3	5.8667	.15275	.08819	5.4872	6.2461	5.70	6.00
T3	3	4.9000	.60000	.34641	3.4095	6.3905	4.30	5.50
Total	9	7.3778	3.04170	1.01390	5.0397	9.7158	4.30	11.70

ANOVA

CFU per gram

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	73.002	2	36.501	216.125	.000
Within Groups	1.013	6	.169		
Total	74.016	8			

Post Hoc Tests

Multiple Comparisons

CFU per gram of live ANC (T1), conventional oven-dried ANC (T2) and vacuum oven-dried ANC (T3)

	(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	T1	T2	5.50000*	.33555	.000	4.4704	6.5296
		T3	6.46667*	.33555	.000	5.4371	7.4962
	T2	T1	-5.50000*	.33555	.000	-6.5296	-4.4704
		T3	.96667	.33555	.063	-.0629	1.9962
	T3	T1	-6.46667*	.33555	.000	-7.4962	-5.4371
		T2	-.96667	.33555	.063	-1.9962	.0629

*. The mean difference is significant at the 0.05 level.

Post Hoc Tests

Homogeneous Subsets

CFU per gram of live ANC (T1), conventional oven-dried ANC (T2) and vacuum oven-dried ANC (T3)

Tukey HSD and Duncan

	Sample	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	T3	3	4.9000		
	T2	3	5.8667		
	T1	3		11.3667	
	Sig.		.063	1.000	
Duncan[a]	T3	3	4.9000		
	T2	3		5.8667	
	T1	3			11.3667
	Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix (Table 3): One-Way ANOVA results for Treatment 1, C1 Optical Density 600 nm Reading of Growth at Each Hour for Bile and Acid Tolerance Test

One-Way ANOVA

Descriptive

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Bile	C1 0h	3	.383333	.0602771	.0348010	.233597	.533070	.3200	.4400
	C1 1h	3	.356667	.0305505	.0176383	.280775	.432558	.3300	.3900
	C1 2h	3	.346667	.0305505	.0176383	.270775	.422558	.3200	.3800
	C1 3h	3	.350000	.1058301	.0611010	.087104	.612896	.2700	.4700
	Total	12	.359167	.0571216	.0164896	.322873	.395460	.2700	.4700
pH1	C1 0h	3	1.000000	.0953939	.0550757	.763028	1.236972	.9400	1.1100
	C1 1h	3	1.036667	.2610236	.1507021	.388248	1.685085	.7900	1.3100
	C1 2h	3	.866667	.2371357	.1369104	.277589	1.455744	.6700	1.1300
	C1 3h	3	.626667	.1913984	.1105039	.151207	1.102127	.4700	.8400
	Total	12	.882500	.2430909	.0701743	.728047	1.036953	.4700	1.3100
pH2	C1 0h	3	.993333	.1115049	.0643774	.716340	1.270327	.9100	1.1200
	C1 1h	3	1.046667	.2500667	.1443761	.425467	1.667867	.8000	1.3000
	C1 2h	3	.883333	.2444040	.1411067	.276200	1.490467	.6700	1.1500
	C1 3h	3	.663333	.1929594	.1114052	.183996	1.142671	.5100	.8800

	Total	12	.896667	.2341846	.0676033	.747873	1.045460	.5100	1.3000
pH3	C1 0h	3	.583333	.0404145	.0233333	.482938	.683729	.5400	.6200
	C1 1h	3	.570000	.1539480	.0888819	.187572	.952428	.4400	.7400
	C1 2h	3	.483333	.1305118	.0753510	.159124	.807543	.3800	.6300
	C1 3h	3	.350000	.1058301	.0611010	.087104	.612896	.2700	.4700
	Total	12	.496667	.1384547	.0399684	.408697	.584637	.2700	.7400

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Bile	Between Groups	.002	3	.001	.199	.894
	Within Groups	.033	8	.004		
	Total	.036	11			
pH1	Between Groups	.310	3	.103	2.429	.140
	Within Groups	.340	8	.043		
	Total	.650	11			
pH2	Between Groups	.259	3	.086	2.012	.191
	Within Groups	.344	8	.043		
	Total	.603	11			
pH3	Between Groups	.104	3	.035	2.582	.126
	Within Groups	.107	8	.013		
	Total	.211	11			

Post Hoc Tests

Multiple Comparisons

Treatment 1, C1 Optical Density 600 nm Reading of Growth at Each Hour for Bile and Acid Tolerance Test

Dependent Variable		(I) Treatment_1	(J) Treatment_1	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Bile	Tukey HSD	C1 0h	C1 1h	.0266667	.0527573	.956	-.142281	.195614
			C1 2h	.0366667	.0527573	.896	-.132281	.205614
			C1 3h	.0333333	.0527573	.919	-.135614	.202281
		C1 1h	C1 0h	-.0266667	.0527573	.956	-.195614	.142281
			C1 2h	.0100000	.0527573	.997	-.158947	.178947
			C1 3h	.0066667	.0527573	.999	-.162281	.175614
		C1 2h	C1 0h	-.0366667	.0527573	.896	-.205614	.132281
			C1 1h	-.0100000	.0527573	.997	-.178947	.158947
			C1 3h	-.0033333	.0527573	1.000	-.172281	.165614
		C1 3h	C1 0h	-.0333333	.0527573	.919	-.202281	.135614
			C1 1h	-.0066667	.0527573	.999	-.175614	.162281
			C1 2h	.0033333	.0527573	1.000	-.165614	.172281
pH1	Tukey HSD	C1 0h	C1 1h	-.0366667	.1683746	.996	-.575861	.502528

			C1 2h	.1333333	.1683746	.856	-.405861	.672528
			C1 3h	.3733333	.1683746	.198	-.165861	.912528
		C1 1h	C1 0h	.0366667	.1683746	.996	-.502528	.575861
			C1 2h	.1700000	.1683746	.749	-.369195	.709195
			C1 3h	.4100000	.1683746	.147	-.129195	.949195
		C1 2h	C1 0h	-.1333333	.1683746	.856	-.672528	.405861
			C1 1h	-.1700000	.1683746	.749	-.709195	.369195
			C1 3h	.2400000	.1683746	.519	-.299195	.779195
		C1 3h	C1 0h	-.3733333	.1683746	.198	-.912528	.165861
			C1 1h	-.4100000	.1683746	.147	-.949195	.129195
			C1 2h	-.2400000	.1683746	.519	-.779195	.299195
pH2	Tukey HSD	C1 0h	C1 1h	-.0533333	.1692795	.988	-.595426	.488759
			C1 2h	.1100000	.1692795	.913	-.432093	.652093
			C1 3h	.3300000	.1692795	.282	-.212093	.872093
		C1 1h	C1 0h	.0533333	.1692795	.988	-.488759	.595426
			C1 2h	.1633333	.1692795	.772	-.378759	.705426
			C1 3h	.3833333	.1692795	.186	-.158759	.925426
		C1 2h	C1 0h	-.1100000	.1692795	.913	-.652093	.432093
			C1 1h	-.1633333	.1692795	.772	-.705426	.378759
			C1 3h	.2200000	.1692795	.588	-.322093	.762093
		C1 3h	C1 0h	-.3300000	.1692795	.282	-.872093	.212093
			C1 1h	-.3833333	.1692795	.186	-.925426	.158759
			C1 2h	-.2200000	.1692795	.588	-.762093	.322093

pH3	Tukey HSD	C1 0h	C1 1h	.0133333	.0944869	.999	-.289247	.315914
			C1 2h	.1000000	.0944869	.722	-.202580	.402580
			C1 3h	.2333333	.0944869	.140	-.069247	.535914
		C1 1h	C1 0h	-.0133333	.0944869	.999	-.315914	.289247
			C1 2h	.0866667	.0944869	.797	-.215914	.389247
			C1 3h	.2200000	.0944869	.170	-.082580	.522580
		C1 2h	C1 0h	-.1000000	.0944869	.722	-.402580	.202580
			C1 1h	-.0866667	.0944869	.797	-.389247	.215914
			C1 3h	.1333333	.0944869	.527	-.169247	.435914
		C1 3h	C1 0h	-.2333333	.0944869	.140	-.535914	.069247
			C1 1h	-.2200000	.0944869	.170	-.522580	.082580
			C1 2h	-.1333333	.0944869	.527	-.435914	.169247

Post Hoc Tests

Homogeneous Subsets

Treatment 1, C1 Optical Density 600 nm Reading of Growth at Each Hour for Bile

Tukey HSD and Duncan

	Treatment_1	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	C1 2h	3	.346667
	C1 3h	3	.350000
	C1 1h	3	.356667
	C1 0h	3	.383333
	Sig.		.896
Duncan[a]	C1 2h	3	.346667
	C1 3h	3	.350000
	C1 1h	3	.356667
	C1 0h	3	.383333
	Sig.		.530

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Post Hoc Tests

Homogeneous Subsets

Treatment 1, C1 Optical Density 600 nm Reading of Growth at Each Hour for pH 1

Tukey HSD and Duncan

	Treatment_1	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	C1 3h	3	.626667
	C1 2h	3	.866667
	C1 0h	3	1.000000
	C1 1h	3	1.036667
	Sig.		.147
Duncan[a]	C1 3h	3	.626667
	C1 2h	3	.866667
	C1 0h	3	1.000000
	C1 1h	3	1.036667
	Sig.		.052

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Post Hoc Tests

Homogeneous Subsets

Treatment 1, C1 Optical Density 600 nm Reading of Growth at Each Hour for pH 2

Tukey HSD and Duncan

	Treatment_1	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	C1 3h	3	.663333
	C1 2h	3	.883333
	C1 0h	3	.993333
	C1 1h	3	1.046667
	Sig.		.186
Duncan[a]	C1 3h	3	.663333
	C1 2h	3	.883333
	C1 0h	3	.993333
	C1 1h	3	1.046667
	Sig.		.066

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Post Hoc Tests

Homogeneous Subsets

Treatment 1, C1 Optical Density 600 nm Reading of Growth at Each Hour for pH 3

Tukey HSD and Duncan

	Treatment_1	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	C1 3h	3	.350000	
	C1 2h	3	.483333	
	C1 1h	3	.570000	
	C1 0h	3	.583333	
	Sig.		.140	
Duncan[a]	C1 3h	3	.350000	
	C1 2h	3	.483333	.483333
	C1 1h	3	.570000	.570000
	C1 0h	3		.583333
	Sig.		.056	.340

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix (Table 4): One-Way ANOVA results for Treatment 1, C2 Optical Density 600 nm Reading of Growth at Each Hour for Bile and Acid Tolerance Test

One-Way ANOVA

Descriptive

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
BIle	C2 0h	3	.306667	.0503322	.0290593	.181634	.431699	.2600	.3600
	C2 1h	3	.270000	.0700000	.0404145	.096110	.443890	.1900	.3200
	C2 2h	3	.313333	.0450925	.0260342	.201317	.425349	.2700	.3600
	C2 3h	3	.416667	.2454248	.1416961	-.193002	1.026336	.2700	.7000
	Total	12	.326667	.1261553	.0364179	.246511	.406822	.1900	.7000
pH1	C2 0h	3	.776667	.1700980	.0982061	.354120	1.199214	.6500	.9700
	C2 1h	3	.833333	.3601851	.2079530	-.061416	1.728083	.4800	1.2000
	C2 2h	3	.850000	.3923009	.2264950	-.124529	1.824529	.5800	1.3000
	C2 3h	3	.763333	.5427093	.3133333	-.584831	2.111498	.4500	1.3900
	Total	12	.805833	.3344319	.0965422	.593345	1.018321	.4500	1.3900
pH2	C2 0h	3	.790000	.1473092	.0850490	.424064	1.155936	.7000	.9600
	C2 1h	3	.876667	.4106499	.2370888	-.143444	1.896778	.4800	1.3000
	C2 2h	3	.890000	.4250882	.2454248	-.165978	1.945978	.6200	1.3800
	C2 3h	3	.816667	.5744853	.3316792	-.610434	2.243767	.4800	1.4800

	Total	12	.843333	.3596294	.1038161	.614836	1.071831	.4800	1.4800
pH3	C2 0h	3	.440000	.0953939	.0550757	.203028	.676972	.3500	.5400
	C2 1h	3	.416667	.2454248	.1416961	-.193002	1.026336	.2700	.7000
	C2 2h	3	.456667	.1803700	.1041367	.008603	.904731	.2700	.6300
	C2 3h	3	.466667	.1858315	.1072898	.005036	.928298	.3400	.6800
	Total	12	.445000	.1587164	.0458175	.344156	.545844	.2700	.7000

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
BIle	Between Groups	.036	3	.012	.682	.587
	Within Groups	.139	8	.017		
	Total	.175	11			
pH1	Between Groups	.016	3	.005	.035	.990
	Within Groups	1.214	8	.152		
	Total	1.230	11			
pH2	Between Groups	.021	3	.007	.039	.989
	Within Groups	1.402	8	.175		
	Total	1.423	11			
pH3	Between Groups	.004	3	.001	.042	.988
	Within Groups	.273	8	.034		
	Total	.277	11			

Post Hoc Tests

Multiple Comparisons

Treatment 1, C2 Optical Density 600 nm Reading of Growth at Each Hour for Bile and Acid Tolerance Test

Dependent Variable		(I) Treatment_1	(J) Treatment_1	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
BIle	Tukey HSD	C2 0h	C2 1h	.0366667	.1077806	.985	-.308485	.381818
			C2 2h	-.0066667	.1077806	1.000	-.351818	.338485
			C2 3h	-.1100000	.1077806	.743	-.455152	.235152
		C2 1h	C2 0h	-.0366667	.1077806	.985	-.381818	.308485
			C2 2h	-.0433333	.1077806	.977	-.388485	.301818
			C2 3h	-.1466667	.1077806	.554	-.491818	.198485
		C2 2h	C2 0h	.0066667	.1077806	1.000	-.338485	.351818
			C2 1h	.0433333	.1077806	.977	-.301818	.388485
			C2 3h	-.1033333	.1077806	.776	-.448485	.241818
		C2 3h	C2 0h	.1100000	.1077806	.743	-.235152	.455152
			C2 1h	.1466667	.1077806	.554	-.198485	.491818
			C2 2h	.1033333	.1077806	.776	-.241818	.448485
pH1	Tukey HSD	C2 0h	C2 1h	-.0566667	.3180933	.998	-1.075313	.961980
			C2 2h	-.0733333	.3180933	.995	-1.091980	.945313

			C2 3h	.0133333	.3180933	1.000	-1.005313	1.031980
		C2 1h	C2 0h	.0566667	.3180933	.998	-.961980	1.075313
			C2 2h	-.0166667	.3180933	1.000	-1.035313	1.001980
			C2 3h	.0700000	.3180933	.996	-.948647	1.088647
		C2 2h	C2 0h	.0733333	.3180933	.995	-.945313	1.091980
			C2 1h	.0166667	.3180933	1.000	-1.001980	1.035313
			C2 3h	.0866667	.3180933	.992	-.931980	1.105313
		C2 3h	C2 0h	-.0133333	.3180933	1.000	-1.031980	1.005313
			C2 1h	-.0700000	.3180933	.996	-1.088647	.948647
			C2 2h	-.0866667	.3180933	.992	-1.105313	.931980
pH2	Tukey HSD	C2 0h	C2 1h	-.0866667	.3418252	.994	-1.181311	1.007978
			C2 2h	-.1000000	.3418252	.991	-1.194645	.994645
			C2 3h	-.0266667	.3418252	1.000	-1.121311	1.067978
		C2 1h	C2 0h	.0866667	.3418252	.994	-1.007978	1.181311
			C2 2h	-.0133333	.3418252	1.000	-1.107978	1.081311
			C2 3h	.0600000	.3418252	.998	-1.034645	1.154645
		C2 2h	C2 0h	.1000000	.3418252	.991	-.994645	1.194645
			C2 1h	.0133333	.3418252	1.000	-1.081311	1.107978
			C2 3h	.0733333	.3418252	.996	-1.021311	1.167978
		C2 3h	C2 0h	.0266667	.3418252	1.000	-1.067978	1.121311
			C2 1h	-.0600000	.3418252	.998	-1.154645	1.034645
			C2 2h	-.0733333	.3418252	.996	-1.167978	1.021311
pH3	Tukey HSD	C2 0h	C2 1h	.0233333	.1507758	.999	-.459504	.506170

			C2 2h	-.0166667	.1507758	.999	-.499504	.466170
			C2 3h	-.0266667	.1507758	.998	-.509504	.456170
		C2 1h	C2 0h	-.0233333	.1507758	.999	-.506170	.459504
			C2 2h	-.0400000	.1507758	.993	-.522837	.442837
			C2 3h	-.0500000	.1507758	.986	-.532837	.432837
		C2 2h	C2 0h	.0166667	.1507758	.999	-.466170	.499504
			C2 1h	.0400000	.1507758	.993	-.442837	.522837
			C2 3h	-.0100000	.1507758	1.000	-.492837	.472837
		C2 3h	C2 0h	.0266667	.1507758	.998	-.456170	.509504
			C2 1h	.0500000	.1507758	.986	-.432837	.532837
			C2 2h	.0100000	.1507758	1.000	-.472837	.492837

Post Hoc Tests

Homogeneous Subsets

Treatment 1, C2 Optical Density 600 nm Reading of Growth at Each Hour for Bile

Tukey HSD and Duncan

	Treatment_1	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	C2 1h	3	.270000
	C2 0h	3	.306667
	C2 2h	3	.313333
	C2 3h	3	.416667
	Sig.		.554
Duncan[a]	C2 1h	3	.270000
	C2 0h	3	.306667
	C2 2h	3	.313333
	C2 3h	3	.416667
	Sig.		.236

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Post Hoc Tests

Homogeneous Subsets

Treatment 1, C2 Optical Density 600 nm Reading of Growth at Each Hour for pH 1

Tukey HSD and Duncan

	Treatment_1	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	C2 3h	3	.763333
	C2 0h	3	.776667
	C2 1h	3	.833333
	C2 2h	3	.850000
	Sig.		.992
Duncan[a]	C2 3h	3	.763333
	C2 0h	3	.776667
	C2 1h	3	.833333
	C2 2h	3	.850000
	Sig.		.803

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Post Hoc Tests

Homogeneous Subsets

Treatment 1, C2 Optical Density 600 nm Reading of Growth at Each Hour for pH 2

Tukey HSD and Duncan

	Treatment_1	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	C2 0h	3	.790000
	C2 3h	3	.816667
	C2 1h	3	.876667
	C2 2h	3	.890000
	Sig.		.991
Duncan[a]	C2 0h	3	.790000
	C2 3h	3	.816667
	C2 1h	3	.876667
	C2 2h	3	.890000
	Sig.		.789

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Post Hoc Tests

Homogeneous Subsets

Treatment 1, C2 Optical Density 600 nm Reading of Growth at Each Hour for pH 3

Tukey HSD and Duncan

	Treatment_1	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	C2 1h	3	.416667
	C2 0h	3	.440000
	C2 2h	3	.456667
	C2 3h	3	.466667
	Sig.		.986
Duncan[a]	C2 1h	3	.416667
	C2 0h	3	.440000
	C2 2h	3	.456667
	C2 3h	3	.466667
	Sig.		.762

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix (Table 5): One-Way ANOVA results for Treatment 2, C3 Optical Density 600 nm Reading of Growth at Each Hour for Bile and Acid Tolerance Test

One-Way ANOVA

Descriptive

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Bile	C3 0h	3	.323333	.0251661	.0145297	.260817	.385849	.3000	.3500
	C3 1h	3	.280000	.1153256	.0665833	-.006485	.566485	.1900	.4100
	C3 2h	3	.300000	.0866025	.0500000	.084867	.515133	.2500	.4000
	C3 3h	3	.276667	.0404145	.0233333	.176271	.377062	.2400	.3200
	Total	12	.295000	.0676219	.0195208	.252035	.337965	.1900	.4100
pH1	C3 0h	3	.320000	.0100000	.0057735	.295159	.344841	.3100	.3300
	C3 1h	3	.463333	.2753785	.1589899	-.220745	1.147412	.1800	.7300
	C3 2h	3	.466667	.0288675	.0166667	.394956	.538378	.4500	.5000
	C3 3h	3	.403333	.0450925	.0260342	.291317	.515349	.3600	.4500
	Total	12	.413333	.1348624	.0389314	.327646	.499021	.1800	.7300
pH2	C3 0h	3	.303333	.0057735	.0033333	.288991	.317676	.3000	.3100
	C3 1h	3	.603333	.2702468	.1560271	-.067997	1.274664	.3400	.8800
	C3 2h	3	.486667	.0115470	.0066667	.457982	.515351	.4800	.5000
	C3 3h	3	.410000	.0435890	.0251661	.301719	.518281	.3600	.4400

	Total	12	.450833	.1635103	.0472013	.346944	.554723	.3000	.8800
pH3	C3 0h	3	.303333	.0115470	.0066667	.274649	.332018	.2900	.3100
	C3 1h	3	.426667	.2013289	.1162373	-.073462	.926795	.2400	.6400
	C3 2h	3	.423333	.1069268	.0617342	.157713	.688954	.3000	.4900
	C3 3h	3	.343333	.0550757	.0317980	.206518	.480149	.2900	.4000
	Total	12	.374167	.1142930	.0329935	.301548	.446785	.2400	.6400

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Bile	Between Groups	.004	3	.001	.241	.866
	Within Groups	.046	8	.006		
	Total	.050	11			
pH1	Between Groups	.042	3	.014	.719	.568
	Within Groups	.158	8	.020		
	Total	.200	11			
pH2	Between Groups	.144	3	.048	2.555	.128
	Within Groups	.150	8	.019		
	Total	.294	11			
pH3	Between Groups	.033	3	.011	.808	.524
	Within Groups	.110	8	.014		
	Total	.144	11			

Post Hoc Tests

Multiple Comparisons

Treatment 2, C3 Optical Density 600 nm Reading of Growth at Each Hour for Bile and Acid Tolerance Test

Dependent Variable		(I) Treatment_2	(J) Treatment_2	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Bile	Tukey HSD	C3 0h	C3 1h	.0433333	.0620036	.895	-.155224	.241891
			C3 2h	.0233333	.0620036	.981	-.175224	.221891
			C3 3h	.0466667	.0620036	.873	-.151891	.245224
		C3 1h	C3 0h	-.0433333	.0620036	.895	-.241891	.155224
			C3 2h	-.0200000	.0620036	.988	-.218557	.178557
			C3 3h	.0033333	.0620036	1.000	-.195224	.201891
		C3 2h	C3 0h	-.0233333	.0620036	.981	-.221891	.175224
			C3 1h	.0200000	.0620036	.988	-.178557	.218557
			C3 3h	.0233333	.0620036	.981	-.175224	.221891
		C3 3h	C3 0h	-.0466667	.0620036	.873	-.245224	.151891
			C3 1h	-.0033333	.0620036	1.000	-.201891	.195224
			C3 2h	-.0233333	.0620036	.981	-.221891	.175224
pH1	Tukey HSD	C3 0h	C3 1h	-.1433333	.1146008	.615	-.510325	.223659
			C3 2h	-.1466667	.1146008	.599	-.513659	.220325

			C3 3h	-.0833333	.1146008	.884	-.450325	.283659
		C3 1h	C3 0h	.1433333	.1146008	.615	-.223659	.510325
			C3 2h	-.0033333	.1146008	1.000	-.370325	.363659
			C3 3h	.0600000	.1146008	.951	-.306992	.426992
		C3 2h	C3 0h	.1466667	.1146008	.599	-.220325	.513659
			C3 1h	.0033333	.1146008	1.000	-.363659	.370325
			C3 3h	.0633333	.1146008	.943	-.303659	.430325
		C3 3h	C3 0h	.0833333	.1146008	.884	-.283659	.450325
			C3 1h	-.0600000	.1146008	.951	-.426992	.306992
			C3 2h	-.0633333	.1146008	.943	-.430325	.303659
pH2	Tukey HSD	C3 0h	C3 1h	-.3000000	.1118779	.104	-.658272	.058272
			C3 2h	-.1833333	.1118779	.411	-.541606	.174939
			C3 3h	-.1066667	.1118779	.778	-.464939	.251606
		C3 1h	C3 0h	.3000000	.1118779	.104	-.058272	.658272
			C3 2h	.1166667	.1118779	.731	-.241606	.474939
			C3 3h	.1933333	.1118779	.371	-.164939	.551606
		C3 2h	C3 0h	.1833333	.1118779	.411	-.174939	.541606
			C3 1h	-.1166667	.1118779	.731	-.474939	.241606
			C3 3h	.0766667	.1118779	.900	-.281606	.434939
		C3 3h	C3 0h	.1066667	.1118779	.778	-.251606	.464939
			C3 1h	-.1933333	.1118779	.371	-.551606	.164939
			C3 2h	-.0766667	.1118779	.900	-.434939	.281606
pH3	Tukey HSD	C3 0h	C3 1h	-.1233333	.0958587	.595	-.430307	.183640

			C3 2h	-.1200000	.0958587	.615	-.426973	.186973
			C3 3h	-.0400000	.0958587	.974	-.346973	.266973
		C3 1h	C3 0h	.1233333	.0958587	.595	-.183640	.430307
			C3 2h	.0033333	.0958587	1.000	-.303640	.310307
			C3 3h	.0833333	.0958587	.820	-.223640	.390307
		C3 2h	C3 0h	.1200000	.0958587	.615	-.186973	.426973
			C3 1h	-.0033333	.0958587	1.000	-.310307	.303640
			C3 3h	.0800000	.0958587	.837	-.226973	.386973
		C3 3h	C3 0h	.0400000	.0958587	.974	-.266973	.346973
			C3 1h	-.0833333	.0958587	.820	-.390307	.223640
			C3 2h	-.0800000	.0958587	.837	-.386973	.226973

Post Hoc Tests

Homogeneous Subsets

Treatment 2, C3 Optical Density 600 nm Reading of Growth at Each Hour for Bile

Tukey HSD and Duncan

	Treatment_2	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	C3 3h	3	.276667
	C3 1h	3	.280000
	C3 2h	3	.300000
	C3 0h	3	.323333
	Sig.		.873
Duncan[a]	C3 3h	3	.276667
	C3 1h	3	.280000
	C3 2h	3	.300000
	C3 0h	3	.323333
	Sig.		.498

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Post Hoc Tests

Homogeneous Subsets

Treatment 2, C3 Optical Density 600 nm Reading of Growth at Each Hour for pH 1

Tukey HSD and Duncan

	Treatment_2	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	C3 0h	3	.320000
	C3 3h	3	.403333
	C3 1h	3	.463333
	C3 2h	3	.466667
	Sig.		.599
Duncan[a]	C3 0h	3	.320000
	C3 3h	3	.403333
	C3 1h	3	.463333
	C3 2h	3	.466667
	Sig.		.263

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Post Hoc Tests

Homogeneous Subsets

Treatment 2, C3 Optical Density 600 nm Reading of Growth at Each Hour for pH 2

Tukey HSD and Duncan

	Treatment_2	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	C3 0h	3	.303333	
	C3 3h	3	.410000	
	C3 2h	3	.486667	
	C3 1h	3	.603333	
	Sig.		.104	
Duncan[a]	C3 0h	3	.303333	
	C3 3h	3	.410000	.410000
	C3 2h	3	.486667	.486667
	C3 1h	3		.603333
	Sig.		.155	.136

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Post Hoc Tests

Homogeneous Subsets

Treatment 2, C3 Optical Density 600 nm Reading of Growth at Each Hour for pH 3

Tukey HSD and Duncan

	Treatment_2	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	C3 0h	3	.303333
	C3 3h	3	.343333
	C3 2h	3	.423333
	C3 1h	3	.426667
	Sig.		.595
Duncan[a]	C3 0h	3	.303333
	C3 3h	3	.343333
	C3 2h	3	.423333
	C3 1h	3	.426667
	Sig.		.260

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix (Table 6): One-Way ANOVA results for Treatment 2, C4 Optical Density 600 nm Reading of Growth at Each Hour for Bile and Acid Tolerance Test

One-Way ANOVA

Descriptive

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
BIle	C4 0h	3	.3300	.03000	.01732	.2555	.4045	.30	.36
	C4 1h	3	.3067	.11015	.06360	.0330	.5803	.18	.38
	C4 2h	3	.3333	.01155	.00667	.3046	.3620	.32	.34
	C4 3h	3	.3467	.10017	.05783	.0978	.5955	.25	.45
	Total	12	.3292	.06667	.01925	.2868	.3715	.18	.45
pH1	C4 0h	3	.3000	.01000	.00577	.2752	.3248	.29	.31
	C4 1h	3	.4600	.13892	.08021	.1149	.8051	.30	.55
	C4 2h	3	.3833	.06807	.03930	.2142	.5524	.33	.46
	C4 3h	3	.4067	.07024	.04055	.2322	.5811	.34	.48
	Total	12	.3875	.09430	.02722	.3276	.4474	.29	.55
pH2	C4 0h	3	.3267	.02309	.01333	.2693	.3840	.30	.34
	C4 1h	3	.5300	.13892	.08021	.1849	.8751	.37	.62
	C4 2h	3	.4600	.05568	.03215	.3217	.5983	.40	.51
	C4 3h	3	.4567	.03786	.02186	.3626	.5507	.43	.50
	Total	12	.4433	.10156	.02932	.3788	.5079	.30	.62

pH3	C4 0h	3	.3667	.05132	.02963	.2392	.4941	.31	.41
	C4 1h	3	.3500	.01000	.00577	.3252	.3748	.34	.36
	C4 2h	3	.3600	.04000	.02309	.2606	.4594	.32	.40
	C4 3h	3	.3667	.02082	.01202	.3150	.4184	.35	.39
	Total	12	.3608	.03029	.00874	.3416	.3801	.31	.41

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
BIle	Between Groups	.002	3	.001	.143	.931
	Within Groups	.046	8	.006		
	Total	.049	11			
pH1	Between Groups	.040	3	.013	1.836	.219
	Within Groups	.058	8	.007		
	Total	.098	11			
pH2	Between Groups	.065	3	.022	3.542	.068
	Within Groups	.049	8	.006		
	Total	.113	11			
pH3	Between Groups	.001	3	.000	.156	.923
	Within Groups	.010	8	.001		
	Total	.010	11			

Post Hoc Tests

Multiple Comparisons

Treatment 2, C4 Optical Density 600 nm Reading of Growth at Each Hour for Bile and Acid Tolerance Test

Dependent Variable		(I) Treatment_2	(J) Treatment_2	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
BIle	Tukey HSD	C4 0h	C4 1h	.02333	.06218	.981	-.1758	.2225
			C4 2h	-.00333	.06218	1.000	-.2025	.1958
			C4 3h	-.01667	.06218	.993	-.2158	.1825
		C4 1h	C4 0h	-.02333	.06218	.981	-.2225	.1758
			C4 2h	-.02667	.06218	.972	-.2258	.1725
			C4 3h	-.04000	.06218	.915	-.2391	.1591
		C4 2h	C4 0h	.00333	.06218	1.000	-.1958	.2025
			C4 1h	.02667	.06218	.972	-.1725	.2258
			C4 3h	-.01333	.06218	.996	-.2125	.1858
		C4 3h	C4 0h	.01667	.06218	.993	-.1825	.2158
			C4 1h	.04000	.06218	.915	-.1591	.2391
			C4 2h	.01333	.06218	.996	-.1858	.2125
pH1	Tukey HSD	C4 0h	C4 1h	-.16000	.06948	.176	-.3825	.0625
			C4 2h	-.08333	.06948	.644	-.3058	.1392

			C4 3h	-.10667	.06948	.462	-.3292	.1158
		C4 1h	C4 0h	.16000	.06948	.176	-.0625	.3825
			C4 2h	.07667	.06948	.698	-.1458	.2992
			C4 3h	.05333	.06948	.867	-.1692	.2758
		C4 2h	C4 0h	.08333	.06948	.644	-.1392	.3058
			C4 1h	-.07667	.06948	.698	-.2992	.1458
			C4 3h	-.02333	.06948	.986	-.2458	.1992
		C4 3h	C4 0h	.10667	.06948	.462	-.1158	.3292
			C4 1h	-.05333	.06948	.867	-.2758	.1692
			C4 2h	.02333	.06948	.986	-.1992	.2458
pH2	Tukey HSD	C4 0h	C4 1h	-.20333	.06373	.051	-.4074	.0007
			C4 2h	-.13333	.06373	.234	-.3374	.0707
			C4 3h	-.13000	.06373	.251	-.3341	.0741
		C4 1h	C4 0h	.20333	.06373	.051	-.0007	.4074
			C4 2h	.07000	.06373	.700	-.1341	.2741
			C4 3h	.07333	.06373	.671	-.1307	.2774
		C4 2h	C4 0h	.13333	.06373	.234	-.0707	.3374
			C4 1h	-.07000	.06373	.700	-.2741	.1341
			C4 3h	.00333	.06373	1.000	-.2007	.2074
		C4 3h	C4 0h	.13000	.06373	.251	-.0741	.3341
			C4 1h	-.07333	.06373	.671	-.2774	.1307
			C4 2h	-.00333	.06373	1.000	-.2074	.2007
pH3	Tukey HSD	C4 0h	C4 1h	.01667	.02819	.932	-.0736	.1069

			C4 2h	.00667	.02819	.995	-.0836	.0969
			C4 3h	.00000	.02819	1.000	-.0903	.0903
		C4 1h	C4 0h	-.01667	.02819	.932	-.1069	.0736
			C4 2h	-.01000	.02819	.984	-.1003	.0803
			C4 3h	-.01667	.02819	.932	-.1069	.0736
		C4 2h	C4 0h	-.00667	.02819	.995	-.0969	.0836
			C4 1h	.01000	.02819	.984	-.0803	.1003
			C4 3h	-.00667	.02819	.995	-.0969	.0836
		C4 3h	C4 0h	.00000	.02819	1.000	-.0903	.0903
			C4 1h	.01667	.02819	.932	-.0736	.1069
			C4 2h	.00667	.02819	.995	-.0836	.0969

Post Hoc Tests

Homogeneous Subsets

Treatment 2, C4 Optical Density 600 nm Reading of Growth at Each Hour for Bile

Tukey HSD and Duncan

	Treatment_2	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	C4 1h	3	.3067
	C4 0h	3	.3300
	C4 2h	3	.3333
	C4 3h	3	.3467
	Sig.		.915
Duncan[a]	C4 1h	3	.3067
	C4 0h	3	.3300
	C4 2h	3	.3333
	C4 3h	3	.3467
	Sig.		.560

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Post Hoc Tests

Homogeneous Subsets

Treatment 2, C4 Optical Density 600 nm Reading of Growth at Each Hour for pH 1

Tukey HSD and Duncan

	Treatment_2	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	C4 0h	3	.3000
	C4 2h	3	.3833
	C4 3h	3	.4067
	C4 1h	3	.4600
	Sig.		.176
Duncan[a]	C4 0h	3	.3000
	C4 2h	3	.3833
	C4 3h	3	.4067
	C4 1h	3	.4600
	Sig.		.063

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Post Hoc Tests

Homogeneous Subsets

Treatment 2, C4 Optical Density 600 nm Reading of Growth at Each Hour for pH 2

Tukey HSD and Duncan

	Treatment_2	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	C4 0h	3	.3267	
	C4 3h	3	.4567	
	C4 2h	3	.4600	
	C4 1h	3	.5300	
	Sig.		.051	
Duncan[a]	C4 0h	3	.3267	
	C4 3h	3	.4567	.4567
	C4 2h	3	.4600	.4600
	C4 1h	3		.5300
	Sig.		.080	.302

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Post Hoc Tests

Homogeneous Subsets

Treatment 2, C4 Optical Density 600 nm Reading of Growth at Each Hour for pH 3

Tukey HSD and Duncan

	Treatment_2	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	C4 1h	3	.3500
	C4 2h	3	.3600
	C4 0h	3	.3667
	C4 3h	3	.3667
	Sig.		.932
Duncan[a]	C4 1h	3	.3500
	C4 2h	3	.3600
	C4 0h	3	.3667
	C4 3h	3	.3667
	Sig.		.592

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix (Table 7): One-Way ANOVA results for Treatment 3, C5 Optical Density 600 nm Reading of Growth at Each Hour for Bile and Acid Tolerance Test

One-Way ANOVA

Descriptive

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Bile	C5 0h	3	.386667	.0378594	.0218581	.292619	.480715	.3600	.4300
	C5 1h	3	.203333	.0251661	.0145297	.140817	.265849	.1800	.2300
	C5 2h	3	.246667	.0416333	.0240370	.143244	.350090	.2000	.2800
	C5 3h	3	.230000	.0300000	.0173205	.155476	.304524	.2000	.2600
	Total	12	.266667	.0796964	.0230064	.216030	.317303	.1800	.4300
pH1	C5 0h	3	1.313333	.1721434	.0993870	.885706	1.740961	1.1200	1.4500
	C5 1h	3	.926667	.2023199	.1168094	.424076	1.429257	.8000	1.1600
	C5 2h	3	1.066667	.2136196	.1233333	.536006	1.597327	.9100	1.3100
	C5 3h	3	1.060000	.1670329	.0964365	.645067	1.474933	.9100	1.2400
	Total	12	1.091667	.2178754	.0628952	.953235	1.230098	.8000	1.4500
pH2	C5 0h	3	.610000	.0435890	.0251661	.501719	.718281	.5600	.6400
	C5 1h	3	.406667	.0832666	.0480740	.199821	.613512	.3400	.5000
	C5 2h	3	.466667	.0850490	.0491031	.255393	.677940	.3800	.5500

	C5 3h	3	.463333	.0763763	.0440959	.273604	.653062	.3800	.5300
	Total	12	.486667	.1006645	.0290593	.422708	.550626	.3400	.6400
pH3	C5 0h	3	.450000	.0264575	.0152753	.384276	.515724	.4300	.4800
	C5 1h	3	.323333	.0321455	.0185592	.243479	.403187	.3000	.3600
	C5 2h	3	.363333	.0550757	.0317980	.226518	.500149	.3000	.4000
	C5 3h	3	.363333	.0568624	.0328295	.222079	.504587	.3000	.4100
	Total	12	.375000	.0615704	.0177738	.335880	.414120	.3000	.4800

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Bile	Between Groups	.060	3	.020	17.154	.001
	Within Groups	.009	8	.001		
	Total	.070	11			
pH1	Between Groups	.234	3	.078	2.165	.170
	Within Groups	.288	8	.036		
	Total	.522	11			
pH2	Between Groups	.068	3	.023	4.120	.049
	Within Groups	.044	8	.005		
	Total	.111	11			
pH3	Between Groups	.026	3	.009	4.283	.044
	Within Groups	.016	8	.002		
	Total	.042	11			

Post Hoc Tests

Multiple Comparisons

Treatment 3, C5 Optical Density 600 nm Reading of Growth at Each Hour for Bile and Acid Tolerance Test

Dependent Variable		(I) Treatment_3	(J) Treatment_3	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Bile	Tukey HSD	C5 0h	C5 1h	.1833333*	.0279881	.001	.093706	.272961
			C5 2h	.1400000*	.0279881	.005	.050372	.229628
			C5 3h	.1566667*	.0279881	.002	.067039	.246294
		C5 1h	C5 0h	-.1833333*	.0279881	.001	-.272961	-.093706
			C5 2h	-.0433333	.0279881	.455	-.132961	.046294
			C5 3h	-.0266667	.0279881	.779	-.116294	.062961
		C5 2h	C5 0h	-.1400000*	.0279881	.005	-.229628	-.050372
			C5 1h	.0433333	.0279881	.455	-.046294	.132961
			C5 3h	.0166667	.0279881	.931	-.072961	.106294
		C5 3h	C5 0h	-.1566667*	.0279881	.002	-.246294	-.067039
			C5 1h	.0266667	.0279881	.779	-.062961	.116294
			C5 2h	-.0166667	.0279881	.931	-.106294	.072961
pH1	Tukey HSD	C5 0h	C5 1h	.3866667	.1549731	.135	-.109612	.882945

			C5 2h	.2466667	.1549731	.434	-.249612	.742945
			C5 3h	.2533333	.1549731	.413	-.242945	.749612
		C5 1h	C5 0h	-.3866667	.1549731	.135	-.882945	.109612
			C5 2h	-.1400000	.1549731	.804	-.636278	.356278
			C5 3h	-.1333333	.1549731	.825	-.629612	.362945
		C5 2h	C5 0h	-.2466667	.1549731	.434	-.742945	.249612
			C5 1h	.1400000	.1549731	.804	-.356278	.636278
			C5 3h	.0066667	.1549731	1.000	-.489612	.502945
		C5 3h	C5 0h	-.2533333	.1549731	.413	-.749612	.242945
			C5 1h	.1333333	.1549731	.825	-.362945	.629612
			C5 2h	-.0066667	.1549731	1.000	-.502945	.489612
pH2	Tukey HSD	C5 0h	C5 1h	.2033333*	.0604152	.040	.009863	.396804
			C5 2h	.1433333	.0604152	.160	-.050137	.336804
			C5 3h	.1466667	.0604152	.149	-.046804	.340137
		C5 1h	C5 0h	-.2033333*	.0604152	.040	-.396804	-.009863
			C5 2h	-.0600000	.0604152	.757	-.253471	.133471
			C5 3h	-.0566667	.0604152	.786	-.250137	.136804
		C5 2h	C5 0h	-.1433333	.0604152	.160	-.336804	.050137
			C5 1h	.0600000	.0604152	.757	-.133471	.253471
			C5 3h	.0033333	.0604152	1.000	-.190137	.196804
		C5 3h	C5 0h	-.1466667	.0604152	.149	-.340137	.046804
			C5 1h	.0566667	.0604152	.786	-.136804	.250137
			C5 2h	-.0033333	.0604152	1.000	-.196804	.190137

pH3	Tukey HSD	C5 0h	C5 1h	.1266667*	.0365148	.034	.009733	.243600
			C5 2h	.0866667	.0365148	.160	-.030267	.203600
			C5 3h	.0866667	.0365148	.160	-.030267	.203600
		C5 1h	C5 0h	-.1266667*	.0365148	.034	-.243600	-.009733
			C5 2h	-.0400000	.0365148	.702	-.156933	.076933
			C5 3h	-.0400000	.0365148	.702	-.156933	.076933
		C5 2h	C5 0h	-.0866667	.0365148	.160	-.203600	.030267
			C5 1h	.0400000	.0365148	.702	-.076933	.156933
			C5 3h	.0000000	.0365148	1.000	-.116933	.116933
		C5 3h	C5 0h	-.0866667	.0365148	.160	-.203600	.030267
			C5 1h	.0400000	.0365148	.702	-.076933	.156933
			C5 2h	.0000000	.0365148	1.000	-.116933	.116933

*. The mean difference is significant at the 0.05 level.

Post Hoc Tests

Homogeneous Subsets

Treatment 3, C5 Optical Density 600 nm Reading of Growth at Each Hour for Bile

Tukey HSD and Duncan

	Treatment_3	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	C5 1h	3	.203333	
	C5 3h	3	.230000	
	C5 2h	3	.246667	
	C5 0h	3		.386667
	Sig.		.455	1.000
Duncan[a]	C5 1h	3	.203333	
	C5 3h	3	.230000	
	C5 2h	3	.246667	
	C5 0h	3		.386667
	Sig.		.176	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Post Hoc Tests

Homogeneous Subsets

Treatment 3, C5 Optical Density 600 nm Reading of Growth at Each Hour for pH 1

Tukey HSD and Duncan

	Treatment_3	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	C5 1h	3	.926667	
	C5 3h	3	1.060000	
	C5 2h	3	1.066667	
	C5 0h	3	1.313333	
	Sig.		.135	
Duncan[a]	C5 1h	3	.926667	
	C5 3h	3	1.060000	1.060000
	C5 2h	3	1.066667	1.066667
	C5 0h	3		1.313333
	Sig.		.411	.155

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Post Hoc Tests

Homogeneous Subsets

Treatment 3, C5 Optical Density 600 nm Reading of Growth at Each Hour for pH 2

Tukey HSD and Duncan

	Treatment_3	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	C5 1h	3	.406667	
	C5 3h	3	.463333	.463333
	C5 2h	3	.466667	.466667
	C5 0h	3		.610000
	Sig.		.757	.149
Duncan[a]	C5 1h	3	.406667	
	C5 3h	3	.463333	
	C5 2h	3	.466667	
	C5 0h	3		.610000
	Sig.		.369	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Post Hoc Tests

Homogeneous Subsets

Treatment 3, C5 Optical Density 600 nm Reading of Growth at Each Hour for pH 3

Tukey HSD and Duncan

	Treatment_3	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	C5 1h	3	.323333	
	C5 2h	3	.363333	.363333
	C5 3h	3	.363333	.363333
	C5 0h	3		.450000
	Sig.		.702	.160
Duncan[a]	C5 1h	3	.323333	
	C5 2h	3	.363333	.363333
	C5 3h	3	.363333	.363333
	C5 0h	3		.450000
	Sig.		.324	.052

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix (Table 8): One-Way ANOVA results for Treatment 3, C6 Optical Density 600 nm Reading of Growth at Each Hour for Bile and Acid Tolerance Test

One-Way ANOVA

Descriptive

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Bile	C6 0h	3	.273333	.1222020	.0705534	-.030233	.576900	.1400	.3800
	C6 1h	3	.266667	.0513160	.0296273	.139191	.394143	.2100	.3100
	C6 2h	3	.300000	.0000000	.0000000	.300000	.300000	.3000	.3000
	C6 3h	3	.316667	.0635085	.0366667	.158903	.474431	.2800	.3900
	Total	12	.289167	.0661209	.0190875	.247155	.331178	.1400	.3900
pH1	C6 0h	3	1.116667	.2936551	.1695419	.387187	1.846146	.7800	1.3200
	C6 1h	3	1.193333	.1205543	.0696020	.893860	1.492807	1.0800	1.3200
	C6 2h	3	1.410000	.3026549	.1747379	.658164	2.161836	1.1700	1.7500
	C6 3h	3	1.423333	.5340724	.3083468	.096624	2.750043	1.1100	2.0400
	Total	12	1.285833	.3260914	.0941345	1.078645	1.493022	.7800	2.0400
pH2	C6 0h	3	.450000	.0793725	.0458258	.252828	.647172	.3600	.5100
	C6 1h	3	.466667	.0776745	.0448454	.273712	.659621	.3800	.5300
	C6 2h	3	.586667	.1266228	.0731057	.272118	.901215	.4900	.7300
	C6 3h	3	.573333	.2514624	.1451819	-.051334	1.198001	.3900	.8600

	Total	12	.519167	.1440618	.0415870	.427634	.610699	.3600	.8600
pH3	C6 0h	3	.393333	.0723418	.0417665	.213626	.573040	.3100	.4400
	C6 1h	3	.380000	.0346410	.0200000	.293947	.466053	.3600	.4200
	C6 2h	3	.456667	.0737111	.0425572	.273558	.639775	.4000	.5400
	C6 3h	3	.460000	.1473092	.0850490	.094064	.825936	.3700	.6300
	Total	12	.422500	.0867730	.0250492	.367367	.477633	.3100	.6300

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Bile	Between Groups	.005	3	.002	.302	.823
	Within Groups	.043	8	.005		
	Total	.048	11			
pH1	Between Groups	.214	3	.071	.599	.634
	Within Groups	.955	8	.119		
	Total	1.170	11			
pH2	Between Groups	.045	3	.015	.656	.601
	Within Groups	.183	8	.023		
	Total	.228	11			
pH3	Between Groups	.016	3	.005	.623	.620
	Within Groups	.067	8	.008		
	Total	.083	11			

Post Hoc Tests

Multiple Comparisons

Treatment 3, C6 Optical Density 600 nm Reading of Growth at Each Hour for Bile and Acid Tolerance Test

Dependent Variable		(I) Treatment_3	(J) Treatment_3	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Bile	Tukey HSD	C6 0h	C6 1h	.0066667	.0600000	.999	-.185474	.198808
			C6 2h	-.0266667	.0600000	.969	-.218808	.165474
			C6 3h	-.0433333	.0600000	.886	-.235474	.148808
		C6 1h	C6 0h	-.0066667	.0600000	.999	-.198808	.185474
			C6 2h	-.0333333	.0600000	.942	-.225474	.158808
			C6 3h	-.0500000	.0600000	.837	-.242141	.142141
		C6 2h	C6 0h	.0266667	.0600000	.969	-.165474	.218808
			C6 1h	.0333333	.0600000	.942	-.158808	.225474
			C6 3h	-.0166667	.0600000	.992	-.208808	.175474
		C6 3h	C6 0h	.0433333	.0600000	.886	-.148808	.235474
			C6 1h	.0500000	.0600000	.837	-.142141	.242141
			C6 2h	.0166667	.0600000	.992	-.175474	.208808
pH1	Tukey HSD	C6 0h	C6 1h	-.0766667	.2821347	.992	-.980161	.826828

			C6 2h	-.2933333	.2821347	.733	-1.196828	.610161
			C6 3h	-.3066667	.2821347	.707	-1.210161	.596828
		C6 1h	C6 0h	.0766667	.2821347	.992	-.826828	.980161
			C6 2h	-.2166667	.2821347	.867	-1.120161	.686828
			C6 3h	-.2300000	.2821347	.846	-1.133495	.673495
		C6 2h	C6 0h	.2933333	.2821347	.733	-.610161	1.196828
			C6 1h	.2166667	.2821347	.867	-.686828	1.120161
			C6 3h	-.0133333	.2821347	1.000	-.916828	.890161
		C6 3h	C6 0h	.3066667	.2821347	.707	-.596828	1.210161
			C6 1h	.2300000	.2821347	.846	-.673495	1.133495
			C6 2h	.0133333	.2821347	1.000	-.890161	.916828
pH2	Tukey HSD	C6 0h	C6 1h	-.0166667	.1235584	.999	-.412344	.379011
			C6 2h	-.1366667	.1235584	.696	-.532344	.259011
			C6 3h	-.1233333	.1235584	.755	-.519011	.272344
		C6 1h	C6 0h	.0166667	.1235584	.999	-.379011	.412344
			C6 2h	-.1200000	.1235584	.769	-.515677	.275677
			C6 3h	-.1066667	.1235584	.823	-.502344	.289011
		C6 2h	C6 0h	.1366667	.1235584	.696	-.259011	.532344
			C6 1h	.1200000	.1235584	.769	-.275677	.515677
			C6 3h	.0133333	.1235584	1.000	-.382344	.409011
		C6 3h	C6 0h	.1233333	.1235584	.755	-.272344	.519011
			C6 1h	.1066667	.1235584	.823	-.289011	.502344
			C6 2h	-.0133333	.1235584	1.000	-.409011	.382344

pH3	Tukey HSD	C6 0h	C6 1h	.0133333	.0747960	.998	-.226190	.252857
			C6 2h	-.0633333	.0747960	.831	-.302857	.176190
			C6 3h	-.0666667	.0747960	.810	-.306190	.172857
		C6 1h	C6 0h	-.0133333	.0747960	.998	-.252857	.226190
			C6 2h	-.0766667	.0747960	.740	-.316190	.162857
			C6 3h	-.0800000	.0747960	.716	-.319523	.159523
		C6 2h	C6 0h	.0633333	.0747960	.831	-.176190	.302857
			C6 1h	.0766667	.0747960	.740	-.162857	.316190
			C6 3h	-.0033333	.0747960	1.000	-.242857	.236190
		C6 3h	C6 0h	.0666667	.0747960	.810	-.172857	.306190
			C6 1h	.0800000	.0747960	.716	-.159523	.319523
			C6 2h	.0033333	.0747960	1.000	-.236190	.242857

Post Hoc Tests

Homogeneous Subsets

Treatment 3, C6 Optical Density 600 nm Reading of Growth at Each Hour for Bile

Tukey HSD and Duncan

	Treatment_3	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	C6 1h	3	.266667
	C6 0h	3	.273333
	C6 2h	3	.300000
	C6 3h	3	.316667
	Sig.		.837
Duncan[a]	C6 1h	3	.266667
	C6 0h	3	.273333
	C6 2h	3	.300000
	C6 3h	3	.316667
	Sig.		.454

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Post Hoc Tests

Homogeneous Subsets

Treatment 3, C6 Optical Density 600 nm Reading of Growth at Each Hour for pH 1

Tukey HSD and Duncan

	Treatment_3	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	C6 0h	3	1.116667
	C6 1h	3	1.193333
	C6 2h	3	1.410000
	C6 3h	3	1.423333
	Sig.		.707
Duncan[a]	C6 0h	3	1.116667
	C6 1h	3	1.193333
	C6 2h	3	1.410000
	C6 3h	3	1.423333
	Sig.		.336

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Post Hoc Tests

Homogeneous Subsets

Treatment 3, C6 Optical Density 600 nm Reading of Growth at Each Hour for pH 2

Tukey HSD and Duncan

	Treatment_3	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	C6 0h	3	.450000
	C6 1h	3	.466667
	C6 3h	3	.573333
	C6 2h	3	.586667
	Sig.		.696
Duncan[a]	C6 0h	3	.450000
	C6 1h	3	.466667
	C6 3h	3	.573333
	C6 2h	3	.586667
	Sig.		.328

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Post Hoc Tests

Homogeneous Subsets

Treatment 3, C6 Optical Density 600 nm Reading of Growth at Each Hour for pH 3

Tukey HSD and Duncan

	Treatment_3	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	C6 1h	3	.380000
	C6 0h	3	.393333
	C6 2h	3	.456667
	C6 3h	3	.460000
	Sig.		.716
Duncan[a]	C6 1h	3	.380000
	C6 0h	3	.393333
	C6 2h	3	.456667
	C6 3h	3	.460000
	Sig.		.343

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.