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Effect of manure origin and effective microorganisms on the maturation time and nutritional quality of banana residue bokashi

Trabajo de integración curricular presentado como requisito para la obtención del título de Petroquímico

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Ivan Rogelio Goottman Jadan

DEDICATION

I dedicate this thesis project to my parents. I am the reflection of you. To my brother because it will be your turn soon. To my uncle Jorge who loves me like my father. To everyone who knows me and who does not.

Ivan Rogelio Goottman Jadan

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RESUMEN

En 2018, Ecuador ocupó el quinto lugar en la producción mundial de banano. De esa producción se estima 2.5 millones de toneladas métricas de residuos de frutos rechazados y racimos de frutos vacíos (raquis). La mayoría de estos residuos se transfieren al relleno sanitario o se apilan junto a las plantas a la espera de una lenta degradación. Por tanto, si no hay un tratamiento de residuos agrícolas, existirá contaminación del medio ambiente. Una de las muchas alternativas existentes para solucionar este problema es proporcionar un tratamiento de residuos agrícolas. La fermentación en estado sólido transforma los desechos orgánicos, obteniéndo como producto fertilizantes orgánicos. Además, la aplicación de fertilizantes orgánicos resuelve los problemas presentes en la agricultura convencional, revitalizando suelos afectados por el uso excesivo de agroquímicos.

Bokashi es un fertilizante orgánico procesado de origen japonés. Este es producto de una descomposición aeróbica de materia orgánica, acelerada por la influencia de microorganismos efectivos.

En este proyecto de tesis, en búsqueda de mejorar las propiedades químicas y biológicas del bokashi, se considera la novedosa adición de *Azolla carolinaina*, los residuos de la industria bananera y una mezcla de otros aditivos para producir un fertilizante orgánico enfocado al cultivo del banano, donde el nitrógeno y el potasio son las bases para una buena producción.

Palabras clave: raquis de banano, *Azolla*, bokashi, organismos, nitrógeno, potasio.

ABSTRACT

In 2018, Ecuador ranked fifth in world banana production. This production corresponds to an estimate of 2.5 million metric tonnes of residues from rejected fruit and empty fruit bunches (rachis). The majority of these residues are transferred to landfill or piled up besides plants waiting for a slow degradation. Therefore, if there is not an agricultural residues treatment, there will be environmental pollution.

One of many existing alternatives to solve this problem is applying a management of agricultural residues. The solid-state fermentation transforms organic wastes, producing value-added products such as organic fertilizer. Furthermore, application of organic fertilizer solves agricultural problems that are present in conventional farming, revitalizing soils with limited microbiota by the excessive use of agrochemicals.

Bokashi is a processed biofertilizer with a Japanese origin. It is a product of an aerobic decomposition of organic material, accelerated by the influence of effective microorganisms (EM).

In this thesis project, as a way to improve chemical and biological properties of bokashi, the novel addition of *Azolla caroliniana* Willd, the residues of banana industry, and a mixture of other additives are considered to yield an organic fertilizer focused on banana crop, where nitrogen and potassium are the bases for a good production.

Keywords: banana rachis, *Azolla*, bokashi, microorganisms, nitrogen, potassium.

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LIST OF ABBREVIATIONS

ALOF	Liquid organic fertilizer
AP	Azolla treated with phosphate fertilizer solution
C	Carbon
C:N	Carbon to Nitrogen ratio
Ca	Calcium
CPH	Cocoa pod shells/husks
EM	Effective Microorganisms
F: B	Fungi to bacteria ratio
H	Hydrogen
K	Potassium
LAB	Lactic Acid Bacteria
MEM	Mountain Effective Microorganisms
Mg	Magnesium
MOL	Local Microorganisms
N	Nitrogen
N:K	Nitrogen to Potassium ratio
O	Oxygen
P	Phosphor
pH	Potential of Hydrogen
PNSB	Purple Non-sulfur Bacteria
PPFB	Plug-flow bio-digester
RHA	Rice Husk Ash
SOM	Soil Organic Mater
SynComs	Synthetic Microbial Communities
T	Temperature
UM-OM	Uncharacterized Organic Matter
VFA	Volatile Fatty Acids

CHAPTER 1. INTRODUCTION AND FIELD AREA

1.1 General Introduction

Ecuador is a tropical agricultural country which is located in Latin America. This country is very known for the exportation of dessert bananas and cocoa beans. For example, in 2019, Ecuador reported 190,381 hectares of dessert banana [1, 2]; and the previous year, Ecuadorian production was 6,505,635 metric tonnes of bananas, placing the county in 5th place in banana world production [3, 4]. This production corresponds to an estimate of 2.5 million metric tonnes of residues of rejected fruit "*rechazo*"[5] and banana rachis [6, 7]. Also, it occurs in cocoa beans harvesting, which produces at least 2.8 million metric tonnes of cocoa pod shells (CPH).

The majority of the Ecuadorian residues are transferred to the landfill or piled up beside plants waiting for slow aerobic degradation. Also, it occurs to the other Ecuadorian agricultural wastes such as animal manure and rice husks. The improper handling of wastes contributes to the pollution of water. Therefore, if there is non-agricultural residues treatment, there is an affectation in environmental pollution [8].

One of many existing alternatives to solve this environmental problem is applying biotechnology processes to organic wastes. The solid-state fermentation of agricultural residues to produce organic fertilizers is an alternative. Furthermore, the application of organic fertilizer solves agriculture problems that are present in conventional farming [9], revitalizing soils with limited microbiota by the excessive use of agrochemicals [10, 11].

Bokashi, compost and, vermicompost are processed organic fertilizers. Bokashi production takes at most 28 days [12]; compost and vermicompost take at least three months [13] and fourteen weeks [14], respectively. Bokashi could be the best option because bokashi maturation time is less time-consuming. It is because bokashi fermentation is accelerated by the influence of effective microorganisms (EM) [11]. Ecuador has one of the biggest biodiversity in the world in its virgin forest soils and mulch. There are high amounts of native microorganisms that could be effective microorganisms (EM) in Ecuadorian soils. For that reason, the Ecuadorian microbial consortium could enhance the fermentation process of bokashi and revitalize soils affected by intensive farming.

Focusing on the fact that banana farming is highly demanding in nitrogen and potassium for good production [15], some organic fertilizers are considered not good

options due to their low content of nutrients. Therefore, it is essential the optimal raw materials for the bokashi preparation. In this research, to reach the proposal of improving the chemical and biological properties of bokashi, Ecuadorian agroindustry wastes are evaluated, considering their capacity for organic matter transformation. Also, the novel addition of *Azolla caroliniana Willd* blended with native microorganisms from Ecuadorian virgin forest soils and mulch was contemplated. The bokashi preparation includes parameters such as temperature, pH level, and moisture (%) changes that are recorded. The chemical characterization of K and N of the samples allowed the analysis of the bokashi quality.

1.2 Problem Statement

The Ecuadorian agroindustry produces high amounts of wastes, and the improper handling of the wastes contributes to water pollution. Manage of wastes to obtain organic fertilizers could be an alternative. However, compost and vermicompost applications could be unviable due to the low amounts of nutrients they have, and they are very time-consuming. The bokashi technology could be a solution. The technology reduces composting time, promotes a circular economy, and its preparation requires effective microorganisms, which also stimulate plants yields. This research aims to evaluate the effect of the kind of agricultural waste, *Azolla caroliniana* Willd, and effective microorganisms (EM) on the maturation time and nutritional quality of the bokashi cake by using parameters such as organic matter transformations, temperature, pH level, moisture percentage, potassium, and nitrogen content.

1.3 General and Specific Objectives

1.3.1 General Objective

- To create a bokashi cake with agricultural residues.

1.3.2 Specific Objectives

- To make bokashi from Ecuadorian agroindustry wastes.
- To obtain a bokashi with high organic matter (OM) transformation.
- To make bokashi by using Ecuadorian native microorganisms.
- To get bokashi in the shortest time possible.

- To increase the nitrogen content of bokashi by using *Azolla caroliniana* Willd substrate.
- To get bokashi with high nutritional contents of nitrogen and potassium.

1.4 Motivation

Ecuador is a fertilizer importer due to the non-existent infrastructure necessary for its production. Thus, Ecuador has a dependence on the international market of fertilizers. Between 2016 and 2020 the fertilizers importation was an average of 87 times above the exportations [16]. Fertilizer importation negatively affects to Ecuadorian economy because the country has foreign currency (dollarized), and the importation activity promotes the exit of currencies. In 2014, the fertilizer importation cost $ 397 million dollars (USD) for Ecuador [17].

Ecuador is the largest world exporter of bananas (**Figure 1**). The giant amounts of biomass generated by harvesting activities promote applying novel technologies to prepare organic fertilizers. The recycling and reuse of agricultural wastes are relevant aspects of a circular economy. It could create new job places and eco-friendly management for the production of commodities [18]. This research promotes changing the Ecuadorian productive matrix because it develops a value-added product from agricultural wastes to the primary agribusiness.

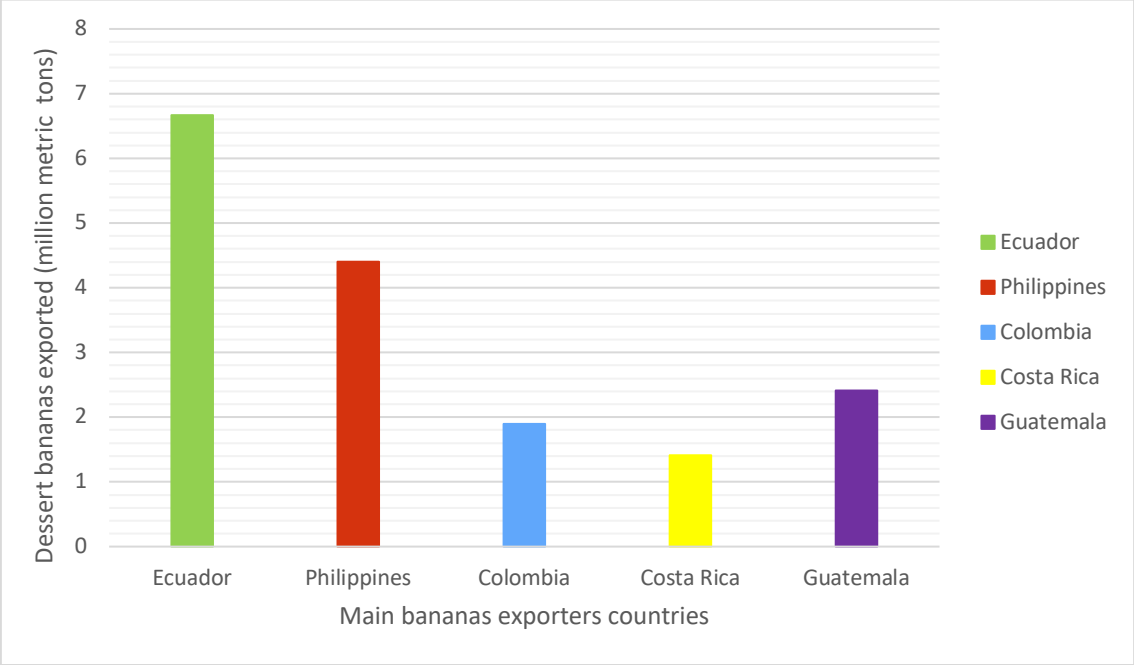

Figure 1. *Main exporters of dessert bananas in the world, in 2018.*

1.5 Field Area

Machala city is the capital of El Oro province, and it is located in Ecuador (DMS location is 3º 16’ 0’’ S. 79º 58’ 0’’W). Machala is known as banana capital of the world. Machala geographically to the north borders with the El Guabo city, to the south with the Santa Rosa city, to the east with Pasaje city, to the west with Jambelí Archipelago (from Santa Rosa city) and the Pacific Ocean [19]. El Oro province is located in a tropical region that have the perfect climatological conditions for banana cultivation (**Figure 2**).

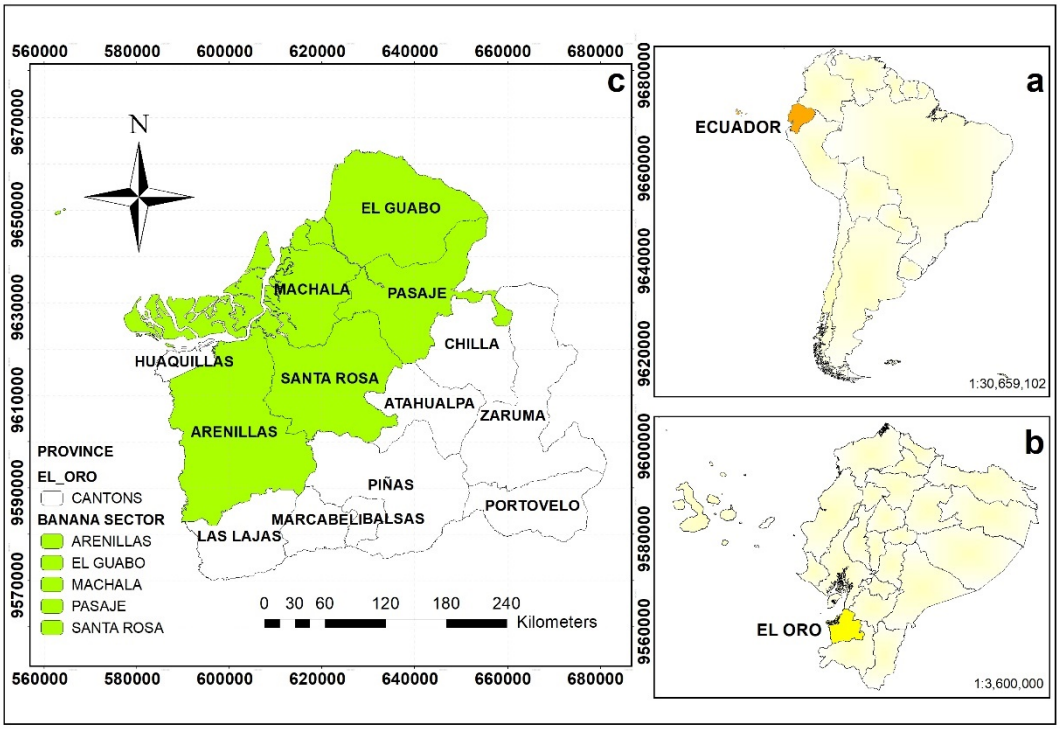

Figure 2. *Ecuadorian Agro-industry banana sector. a Geographical ubication of Ecuador in Latin America. b Geographical ubication of El Oro Province in Ecuador. c Banana productive sector in El Oro province.*

CHAPTER 2. BACKGROUND INFORMATION

2.1 Ecuadorian Agricultural Wastes

2.1.1 Banana rachis

Empty banana bunches, banana floral stalks, banana bunch stalks or banana rachis is one of the main agroindustry wastes in banana farming. In 2018, Ecuadorian banana farming produced an estimate of 2.1 million metric tonnes of banana rachis [6, 7]. Banana rachis composition is divided in water, minerals, carbohydrates and lignocellulose contents [20]. Also, it is possible to have different banana rachis composition according to banana variety. Banana rachis from Grande naine variety has lignocellulosic fractions of 36% cellulose, 18% hemicellulose content, 13%w/w in water retention capacity and 43%w/w of potassium content in ash [21]. This information shows the relevance of banana rachis as a rich nutrient source, that's why banana waste transformation into biofertilizers by solid-state fermentation is a good nutrient to banana farming; moreover, this kind of biofertilizer increases fruit production [22].

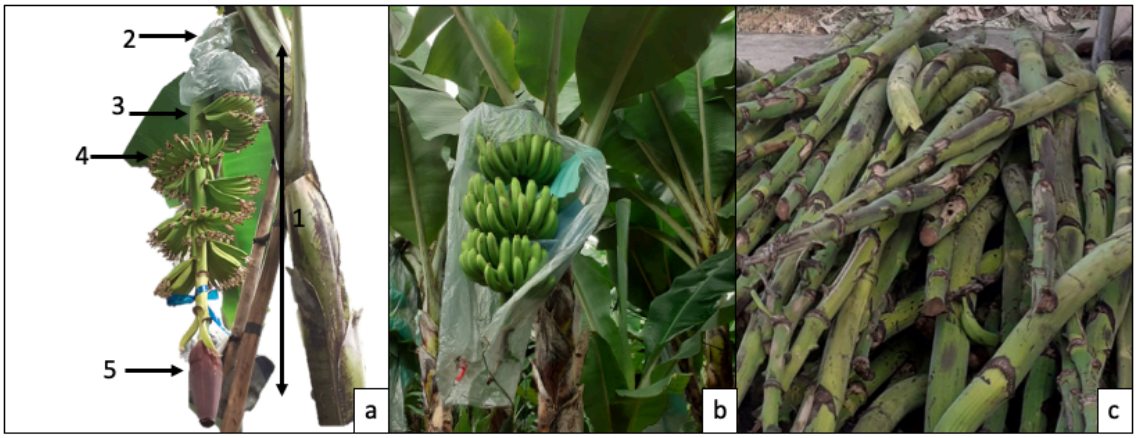

***Figure 3**. a Morphology of banana bunch, 1 banana bunch, 2 banana peduncle, 3 banana rachis, 4 female flowers, 5 male bud. b Female flowers developed into banana fingers. c Empty banana bunches/banana rachis as waste from banana harvesting station.*

2.1.2 Cocoa pod shells

Cocoa pod shell/husk (CPH) is a waste coproduct from cocoa farming. It constitutes 76% of the fruit weight, and as a consequence, for one ton of cocoa beans harvested, it is produced 10 tonnes of cocoa pod shells [23, 24]. In 2019, Ecuadorian cocoa farming produces an estimated 2.8 million metric tonnes of cocoa pod husks [25].

CPH is a source of fibre, minerals, pectin, and theobromine. The morphology of the cocoa pod husk consists of three layers, they are endocarp, mesocarp and epicarp (inner, middle, and outer epicarp, respectively). CPH turns black after a rot process with the sun-dry. [24]. After a cocoa bean harvesting, cocoa farmers usually leave cocoa pod shells on the soil. This practice should return CPH nutrients to the soil, however, this practice promotes cocoa black pod disease. For that reason, farmers are encouraged to retire CPH from their farms. A pellet formed by a mixture of starch and CPH ash could be a rich potash fertilizer, because CPH ash contains 40% of potassium [23].

Figure 4. *a Ecuadorian National Cocoa. b Cocoa pod shell recollected.*

2.1.3 Manure

Manure, dung, or muck is the name of the animal excretes. Manure is a by-product of domestic animal production, and it is considered waste if not managed properly. Animal manure is categorized according to its moisture content into solid manure, mixed manure, liquid manure (slurry). Solid manure contains more than 25% solids, mixed manure between 5 and 25% in solids, and slurry up to 5% of solids [26, 27].

Slurry and solid animal dung have a highly plant nutrient value for its macro and micronutrient contents. There is water-soluble potassium ranging from 75 to 97% present in animal manure and urine, and micronutrients such as copper, zinc, and iron in values of parts per million. Manure is a N source of easy mineralized nitrogenous compounds,

uric acid, and ammonium [28]. If exists conditions of high temperatures in storage and high pH level in manure is possible to have N losses by volatilization [27, 29].

The chemical composition and physical structure of manure for crop production varies with animal species and among the same species. Also, the parameters of climate, location [26], animal size and feeding influence in muck quality. Some domestic animal manures are poultry, livestock, goat, horse and swine dung. Poultry liquid and solid manures contains a higher nitrogen content than cattle and pig dung [28]. Nevertheless, livestock manure could be better for C/N values for plants that requires phosphorus in clayey soils [30].

There are three manure storage: Anaerobic liquid treatment, aerobic liquid treatment, and store in piles. Further, in every dung storage system there is N losses. For example, in aerobic digestion nitrogen losses by volatilized ammonia. In spite of pilled manure treatment being the best at reducing nitrogen losses, responses in crops are better for anaerobic and fermented liquid forms [28]. Moreover, during storage there is decomposition of manure, it causes N losses related with dry matter manure contents [29].

Manure is a source of dangerous microorganisms (bacteria, virus and parasite), such as *Escherichia coli*, salmonella, hepatitis A, rotavirus. For this reason, some strategies in manure management are required to reduce risks. They are treated with alum, anaerobic digestion, drying and composting [26]. Drying treatment is advantageous because it is cheap and facilitates manure transportation, but it also increases N losses and may carry pathogens [27]. The fermentation composting process with microorganisms is efficient at killing pathogens in manure [26]. Animal manure may be applied like soil amendment [28], but exist better nutrient crop assimilation for manure made products such as compost, vermicompost, and bokashi, because these technologies in mix with some additives satisfy crop requirements in fertilization [12].

2.1.4 Rice bran and rice husk ash.

Paddy or rough rice is the name of the rice which has been harvested. Morphology of the paddy conforms to three parts. They are rice husk/hull, bran, and kernel [31]. Rice husk is the rice grain covering. During the milling process rice grains are separated from their husks. Therefore, it produces the largest quantities of waste material. Rice husks constitute 20% of the rice weight and is constituted by cellulose, lignin, moisture, and silica. Rice husk is completely burned to produce rice husk ash (RHA). The chemical

composition of RHA contains more than 80% of silica content and other minerals [32]. Also, RHA serves as a corrective of soil acidity, helping in the development of crops [33]. The milling process consists of removing rice husks from the grain, then the grain needs further milling to remove rice bran from white rice [32] . Rice bran constitutes 10% of the rice weight [31] and it contains 11% proteins, 12% oil, 15% moisture, 6% fibres [34]. It is rich in minerals, such as silica, potassium, and phosphorus content of 0.6, and 1.9, 2.1g per 100 grams, respectively. These values depend on the rice variety [35]. For nutritional qualities rice bran is considered as a mineral and carbon source [36].

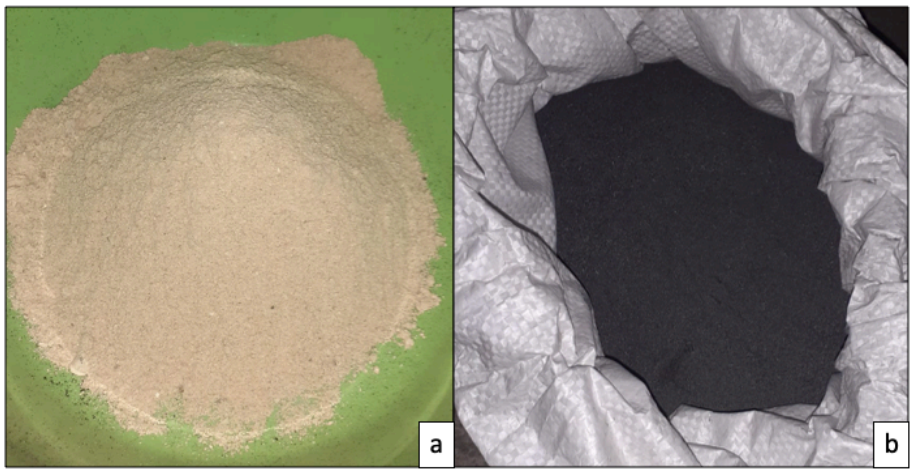

Figure 5. *a Rice bran. b Rice husk ash.*

2.1.5 Sugarcane molasses

Molasses or dark treacle is the common name for the concentrated solution from sugarcane factories. Sugarcane/cane or blackstrap molasses is a dark brown viscid liquid byproduct from raw sugar refinery or sugarcane factory. It is estimated that for 100 tonnes of cane processed, it produces 4 tonnes of molasses [37]. Blackstrap molasses has acid pH between 5 and 7, a sugars constitution of approximately 50% [38], 4% of trace elements (potassium, calcium, magnesium) [39], and 80º Brix; considering that physicochemical properties of cane molasses depend on its composition. Molasses composition pivots on a kind of refining process and source [40]. Many applications for molasses exist through fermentations. This is because cane molasses has 50% of fermentable sugars content, making it a fundamental feedstock for fermentation process [41]. Sugarcane molasses are used as an organic fertilizer supplement [42] and soil fertilizer [37]. Cane molasses are a good carbon source of easy assimilation and help in the reduction of N-NH3 losses during composting of harvesting wastes; moreover, it is

an essential subtract (energy) for lactic acid bacteria to obtain lactic acid [42]. It is also known that molasses promotes microbial activity serving into organic matter degradation process and humification composting [43].

Figure 6. *Sugarcane molasses.*

2.2 Microorganism's sources

2.2.1 Azolla caroliniana Willd

Azolla belongs to Salviniaceae, which is the family of aquatic ferns [44]. According to reproductive structures and morphology there are two sub-sections, *Tetrasporocarpia* and *Azolla*. Also, *Azolla* is divided into two sub-genera, Euazolla and Rhizosperma. There are six known Euzolla and they are *A. caroliniana, A. microphylla, A. mexicana, A. rubra, A. filiculoides and A. pinnata. Azolla caroliniana* Willd is a small free-floating fern which is particular from the others species because it has a rounded shape of dorsal leaf lobe [45]. This plant growths exponentially and it is able to duplicate its mass in 10 days [46]. *Azolla* lives with a symbiotic N_2-fixing blue-green alga. The symbiont is known as *Anabaena azollae*, and it is present in all growing stages of the fern [44]. The cyanobacteria coexist in the stomata of the *Azolla caroliniana* Willd [47]. The cyanobiont has a filamentous form with heterocystous, and it can supply the total nitrogen requirement to the host. Besides, when the cyanobacteria is removed from the host, it is able to fix N_2 and CO_2, but releases more than the nitrogen it fixes in ammonia [44]. *Azolla caroliniana* is valuable for its nitrogen fixing capacity, it is used as a biofertilizer in the east and south of Asia [47] for rice culture [44].

Azolla growth is promoted by a phosphorus supplementation [44]. Phosphorus increases the growth rate of the free-floating fern because it acts catalysing its biochemical reactions. The optimum phosphorus concentration is 30mg l^{-1}. The lack of phosphorus and calcium decrease nitrogen content and growth of *Azolla*. Nevertheless, the absence of potassium and magnesium has no relevant effect in the fern growth. Abiotic parameters influencing *Azolla* growth rate are temperature, photoperiod, light intensity, salinity, moisture and pH. The temperature range of 18 to 28ºC is the best for the plant growing. It is considered 20 hours of photoperiod and between 15-18 klx as the optimal light intensity for the plant development. *Azolla* is a green plant that changes its colour to red-brown with higher light exposition. Although Azolla can tolerate NaCl, salinity acts like a growth inhibitor. Moisture higher than 75% and less of 70% influences negatively in *Azolla* growth, because in the first it reduces the nutrient uptake and transpiration, and in the second, the fern gets dry and consequently fragile. The optimal pH medium for a good growth rate should be between 4.5 and 7.5 [48].

Figure 7. *Azolla caroliniana* Willd recently harvested

2.2.2 Virgin rainforest soil and mulch

Virgin rainforest soil and mulch is the recovered superficial area of soil and mulch from native soil which are richest in effective microorganisms (EM). This soil is particular because they have not been affected by anthropogenic activities. The Ecuadorian rainforests are known for their rich microorganism biodiversity. There are high bacteria amounts in this kind of terrestrial ecosystem. It is important for the

correlation between communities of microorganisms and organic matter in soils [49]. Vegetable biomass is a renewable resource which is composed of cellulose, hemicellulose, and lignin. Soil microorganisms transform cellulose in organic matter by cellulose-degrading process. The main microorganisms into this process are bacteria and fungi because they have extracellular enzymes. *Bacillus* bacteria, *Trichoderma* (sp. or spp.), and *Actinomycete* are the most representative organisms present in Ecuadorian soils. They have high levels of degradation capacity by their enzymatic hydrolysis of cellulose [50]. Moreover, these effective microorganisms have properties that make them special for biological control. They have a high capacity for survival and reproduction, rapid growth, highly competitive ability, be free of natural antagonists, and adaptability to the treated plant. EMs are used in biofertilizers because some of them have symbiotic activity with plants, showing positive results in crop nutrition and biological control within integrated pest management [54, 55].

2.3 Kinds of Bio Fertilizers

2.3.1 Compost and Vermicompost

Composting is a process of blending solid heterogeneous organic materials, which accomplish a thermophilic phase due to high microbiota activity. The microbiological process releases minerals, water, carbon dioxide, phytotoxins, and organic matter [53]. Compost comes from the word composite, meaning materials which have been mixed and transformed in a bulk of organic matter. Heterogeneous substrates and living microorganisms are necessary to obtain composting reactions. It refers to blend rich carbon and nitrogen sources such as lignocellulosic and animal wastes, respectively. Then living microorganisms are which consume nutrient wastes by chemical and physical parameters that support their physiology. Heterotrophic microorganisms use carbon sources like energy source that requires oxidation of two carbon atoms for each carbon assimilated. Nitrogen is a base component of enzymes, coenzymes, amino acids, nucleic acids which are fundamental for cells [53]. Also, composting process considering moisture, C/N ratio, pH, composting method and duration could help introducing technologies and optimal conditions to control to reduces carbon and nitrogen losses [54]. Animal residues such as manure, are good nitrogen sources, that means, low C/N ratio; in contrast, lignocellulosic matter is a carbon source of highest C/N ratio. The blending of these sources allows to obtain compost around 30 C/N ratio by compost pile method

process. C/N ratio is important in compost because it is an indicator of soil quality and fertility. Also, this factor is related with microorganism communities, and the terrestrial nitrogen and carbon cycle [55].

Banana wastes are lignocellulosic matter; in that way, it is a rich carbon source to made compost. Composting process of banana wastes takes three months. The product of this process in its application improves nutrients assimilations such as N, P, and K by crops [13]. Further, compost could go through pelleting process to enhance its soil amendments characteristics and also to reduces production costs in storage and transportation [56].

Vermicompost is very similar to compost, the distinguished factor between composting and vermicomposting processes is worms [13], which accelerate agricultural wastes feedstock decomposition at less temperature [57]. Vermicomposting process is a biological oxidative process [58] where agricultural residues are degraded by earthworms such as *Eisenia fetida, Lumbricus rubellus, Eisenia andrei*, etc. Earthworms consume lignocellulosic matter thank to they have in their gut microgflora enzymes such as cellulase, amylase, lipase, protease, etc [14]. The final product is rich in crop nutrients like potassium and nitrates, auxins and cytokinins. Also, it has hormones which promotes plant growth, hydrolytic enzymes [59]. There some researches about management of biomass by producing vermicompost where feed substrates such as potato waste biomass [58], mixtures of banana and manure, and some vegetable wastes show interesting results. For example, banana leaves are a rich source of lignocellulosic content and plant nutrients. Mixing banana leaves with cattle manure and adding *Eusenia fetida* to produces reducing pH, NPK rich vermicompost with C/N ratio from 8.9 to 24.3. Also, this process takes at least fourteen weeks to conclude earthworm activity, and varies with substrate mixing quantities [14]. Some parameters in vermicompost are C/N ratio, humification index, growth of earthworm, nutrients and microorganism profiles [58].

2.3.2 Bokashi

Bokashi is a Japanese term to describe a fermented organic solid, liquid, or semi-liquid fertilizer. It is a mix of agricultural residues (vegetal and animal wastes) and efficient microorganisms (EM) [60]. Due to EM are into the bokashi recipe, bokashi takes 7, 15, 18, and 21 days [12] to be prepared. Also, bokashi could use local microorganisms (MOL). MOL are very similar to EM, the difference is MOL are produced from vegetable or animal sources. For example, banana humps are rich sources of *Azospirillum sp.,*

Bacillus sp., Aspergillus niger, and Azotobacter sp. In cattle manure at least 60 beneficial species of microorganisms has been found, most of them pectin-, hemicellulose, and cellulose-degrading bacteria [61]. Another alternative for EM to make bokashi is biofresh. Biological agents biofresh is constituted by endophytic rizhobacterial strains (*Serratia sp., Bacillus cereus*, and *B. subtilis*.) in a medium that secrets enzymes (proteinase, cellulase and chitinase), capable to fixating nitrogen from the air and dissolving phosphates [65, 66]. Further, the bokashi formula can change by adding minerals like rock phosphate [64] or biochar [65] promoting higher nutrient concentration and obtaining a competitive product in fertilizers market. Bokashi preparation could be by aerobic or anaerobic treatment.

Aerobic treatment refers to partial aerobic conditions befall in the outer layers, whereas in the middle of the bokashi pile is under anaerobic conditions [60]. Thermophilic bacteria increase bokashi temperature, so it is necessary to turn around to refresh it. For that reason, operational parameters are followed in the bokashi process. Some of them are temperature, pH and moisture level. According to bokashi formula, a solid and a liquid (leaches) bokashi fraction can be obtained. A wet-based bokashi usually have transportation problems and add extra cost to farmers. Therefore, drying methods could be a solution, but this methodology promotes carbon and nitrogen volatilization. In that way, granule-shaped bokashi as product of mixing adhesive materials, bokashi, and EM, could be a solution if C/N ratio is maintained [66] as a parameter focused on crop nutrition. On the other hand, humic acids are present in bokashi leaches. Showing bioactive and stimulant effects in the promotion of H+ -ATPase enzymes in plant cells, likewise to auxinic effect (plant growth regulator) [67].

Regarding anaerobic treatment is accomplished in closed bioreactors under lactic acid fermentation (LAF), in which is possible to balancing macronutrients, adjusting C:N ratio, and supply trace elements. Further, this treatment needs moisture regulation for a favourable fermentation, and temperatures above 18 ºC. Depending on bioreactor design solid, liquid, or semiliquid bokashi and methane are produced. The anaerobic treatment is characterized by the acidic environment which quickly rid of pathogens presence [68]. It is supported by Łozicki et. al [69] who found that application of aqueous solution of EM bokashi have beneficial effects in animal growth by the improvement of hygienic conditions, reducing putrefying fungi and bacteria. Besides, it is recommended anaerobic treatment for household organic waste [70].

Efficient microorganisms such as lactic acid bacteria (LAB), purple non-sulfur bacteria (PNSB), actinomycetes, yeast, and filamentous fungi [68] improves beneficial microorganisms' culture by producing harmful microbial inhibiting substances such as chelating agents, low molecular polysaccharides, polyphenols, saponin, inositol, ubiquinone [60]. Some of the EM mixtures are, *Lactobacillus casei, Lactobacillus plantarum, Streptococcus lactis* (LAB), *Rhodopseudomonas* (PNSB), *Streptomyces griseus* and *Streptomyces albus* (actinomycetes), *Candida utilis* and *Saccharomyces cerevisiae* (yeast) [42]. EM enhances productivity in organic farming systems with higher crop yields, mineralization of carbon and nitrogen, greater crop water stress resistance, better soil properties, improved penetration of plant roots [68].

Cattle manure bokashi compost increases organic matter content, which also rises humus levels in soil. Humus is hydrophilic material; this property enhances holding water capacity in soil reducing evaporation and percolation. It has high beneficial effects on dry lands. [71]. Fermented EM bokashi compost enhances solid organic waste degradation, N mineralization and soil fertility [60]. Pacheco, et al claims EM bokashi influences in the stabilization of soil organic matter (SOM). For example, the EM bokashi addition improves chemical attributes for tropical soils like Dystric Cambisol [72]. It suggests that bokashi formulation, application, and yield crop, also will depend on soil mineralogy.

Bokashi application has beneficial effects in crop production. For example, some research show how bokashi influences in crop development, either applied it mixed with other fertilizers or alone. For example, bokashi and inorganic NPK applications shows better results in shallot crop on dry lands [71]. Further, bokashi could replace NPK fertilizer in tomato crops, because it increases tomato weight [73] and diameter [74]. Besides bokashi mixed with compost showed the better plant development rate for passionfruit [75]. Also, the bokashi application demonstrated higher thickness and weight in harvested fruit than conventional banana farming [76]. Bokashi showed better results than vermicompost and thermophilic compost as supply of inorganic nitrogen, nutritional quality of cultivars by increasing potassium, manganese, zinc contents, and yield and growth in spinach crop [12]. It is closely related bokashi has the highest fungal gene abundance and fungi:bacteria ratio (F:B) in comparison with vermicompost, composted cow manure, municipal solid waste [77].

2.3.3 Biochar

Charcoal or char is a dark porous material product of pyrolysis of natural or fossil carbon sources. Char is considered a residue of the destructive distillation of vegetal or animal stuff in a limited supply of air. Char is different from other impure non-crystalline carbon forms such as soot and coke for its microstructure and properties. Besides, char and coke are products of solid phase pyrolysis, but coke has a fluid phase formed in its carbonization. In the case of soot, it is the result of incomplete combustion in the gas phase [78]. When char is not burned as a fuel producing CO_2, and it is used as a material for non-oxidative applications in agriculture, it is renamed as biochar. This pyrogenic carbonaceous material is used for soil remediation because it reduces nutrient losses from fresh manure or compost. Also, biochar has a distinguishing feature based on high carbon content and cation exchange capacity [79]. For its properties it is applied as a slow-release fertilizer. This multifunctional material could be used as ruminants or poultry feed supplements for agricultural purposes. In addition, biochar can be modified or activated with complex organic liquids as a nutrient carrier for soil amendment [80]. According to biochar type and soil composition, with smaller amounts of 0.5% (w/w) biochar application with effective microorganisms [81], serves as growth promoter of soil biota, by increasing activities of biochemical cycling, and organic matter decomposition. Additionally, biochar amendments help increase recalcitrant organic carbon, and reduce nitrogen losses in N_2O emission and ammonia volatilization from soil [82].

Figure 8. *Biochar.*

2.3.4 Bio-ferment (bio-stimulant)

Bio-ferment or activated liquid organic fertilizer (ALOF) [83] is a by-product from methanogenic fermentation, and agricultural residues are decomposed by anaerobic microorganisms in a bio-digester. Flexible membrane bio-digesters or plug-flow bio-digesters (PPFB) are used in some countries around the word since 1973 [84]. Some parameters that present in the bio-digestion process are temperature, pH, carbon to nitrogen ratio (C/N), and they are called monitoring and operating parameters [85]. Exist limitations with the presence of pathogens (*Coliforms*) in the fermentation process if heating systems are not included. However, it is possible to remove pathogens with biological treatments by using antagonistic microorganisms such as lactic acid bacteria (LAB), phototropic bacteria and yeast. LAB microorganisms produce lactic acids, ethanol, formate which decrease pH and inhibits growth of pathogen bacteria. It is very important because coliforms growth is favoured in alkaline mediums. Mountain effective microorganisms (MEM) are considered in biological treatments, because they promote organic materials degradation for a better crop assimilation, and help in the mineralization of elements and formation of organic matter in soils [84]. Besides, mixing composting process with activated liquid organic fertilizer could enhances nitrogen contents and reduces composting time [83].

***Table 1**. Kinds of biofertilizers according to animal and crop wastes*

Nutrient source	Processing rate	Solid organic fertilizer	Liquid organic fertilizer
Animal or crop wastes	Unprocessed organic wastes	Vegetable wastes: -Harvested crop residues (empty fruit bunches) -Post-harvest residues (sugarcane molasses)	Vegetable wastes: -Harvesting wastes (coffee pulp)
		Animal wastes: -Animal manure -Animal bones	Animal wastes: -Animal urine -Animal blood
		Coverages: -Green manure crops -Mulch	
	Processed organic wastes	Blended of animal and crop wastes: -Compost -Vermicompost -Bokashi -Biochar	Blended of animal and crop wastes: -Bio-stimulants -Compost, vermicompost and bokashi leachates

Adapted from Landis & Dumroese [86] and Ramos & Terry [8].

2.4 Banana Crop Nutrition

Banana crops are high nitrogen demandants in their nutrition. For having a good banana production from eight to twelve nitrogen applications are needed during the plant cycle [87]. For example, potassium to phosphorous ratio is influenced by nitrogen content in fertilization, allowing the balanced assimilation of these elements together [88].

The nitrogen fertilizer is applied around the base of the banana pseudostem, to increases nitrogen consumption. Despite this practice, N losses are unavoidable by leaching [87]. Plant probiotics based on endophytic bacteria are a new proposal to promote nitrogen fixation by diazotrophs. Also, the promising technology of synthetic microbial communities (SynComs) which are designed under laboratory conditions, could be a strategy to supply nutritional aspects [89].

Potassium is the main macronutrient that banana cultivars need in their nutrition. Banana intakes soil macronutrients in the following order K > N > Ca > Mg > P. Potassium is a dominant element in the plant because it is present in yield parameters such as bunch and fruit weight, fruit length and diameter [90]. It means, supply K macronutrient increases yield and quality of banana. Compost and manure application are not good options for its low nutrient content. Potassium is vital element in banana nutrition because banana has a high nutrient demand in its growth and fruiting. Feldspar rock is a rich source of macro-elements such as potassium, calcium, magnesium, and phosphorus. Minerals and bacteria can act together to improves soluble K mineral and create a bio fertilizer [91].

Also, potassium is the highest element concentration in the banana plant, it is present in the leaves showing the nitrogen to potassium ratio (N:K). This ratio should have values about 1:1 and 1:1.6 to supply potassium demand to avoid harvesting problems with the fruit. Potassium and nitrogen should apply 45, 90, 135 and 180 days after planting time [90]. In fertilizer application is very important to consider plant needs, planting density, crop variety, and soil mineralogy, texture, and microbial community. Due to this, values in N and K fertilization could change.

Table 2. *Average nutrient assimilation of banana crop and recommended application rates (g/plant)*

Plant nutrients	Plant nutrients removed by plants (g)	Recommended application rates (g)
N	339	190-359
K_2O	1268	454-1270

Adapted from Topcuoglu [90] and Salem et al [91].

CHAPTER 3. METHODOLOGY

Raw material collection was carried out in order to obtain the essential feedstocks (*Azolla*, banana rachis, CPH, dry leaves, goat manure, RHA, biochar, sugarcane molasses, rice bran, M-M Matrix, Bio-ferment (EM), virgin forest soil and mulch). Then, the suitability of every raw material collected promotes the preparation of the *Azolla caroliniana* Willd experiment. The reproduction of the aquatic fern was evaluated to get it as substrate for the bokashi cake repetitions. Subsequently, two preparation of bokashi experiments were made. The first one focused on the effects of choosing different Ecuadorian agroindustry wastes. The last one considered the impact of Azolla addition in the bokashi samples. Monitoring and control of the two bokashi experiments were made with daily measurements of temperature, pH and moisture during 14 days, where were used a laboratory thermometer and a soil pH and moisture meter, respectively. From each bokashi repetition was selected a sample formed by aliquots of the repetitions. Thus, four bokashi sample generalized were characterized in the chemical laboratory of NEMALAB. Then of 14 days, nitrogen content was determined using Kjeldahl method, and potassium content by flame atomic absorption spectrophotometry. In the bokashi production proposal it is presented a block diagram of the bokashi cake process, and also an economic feasibility for supporting the bokashi industrialization idea. In **figure 9** it is the methodology diagram used in this project.

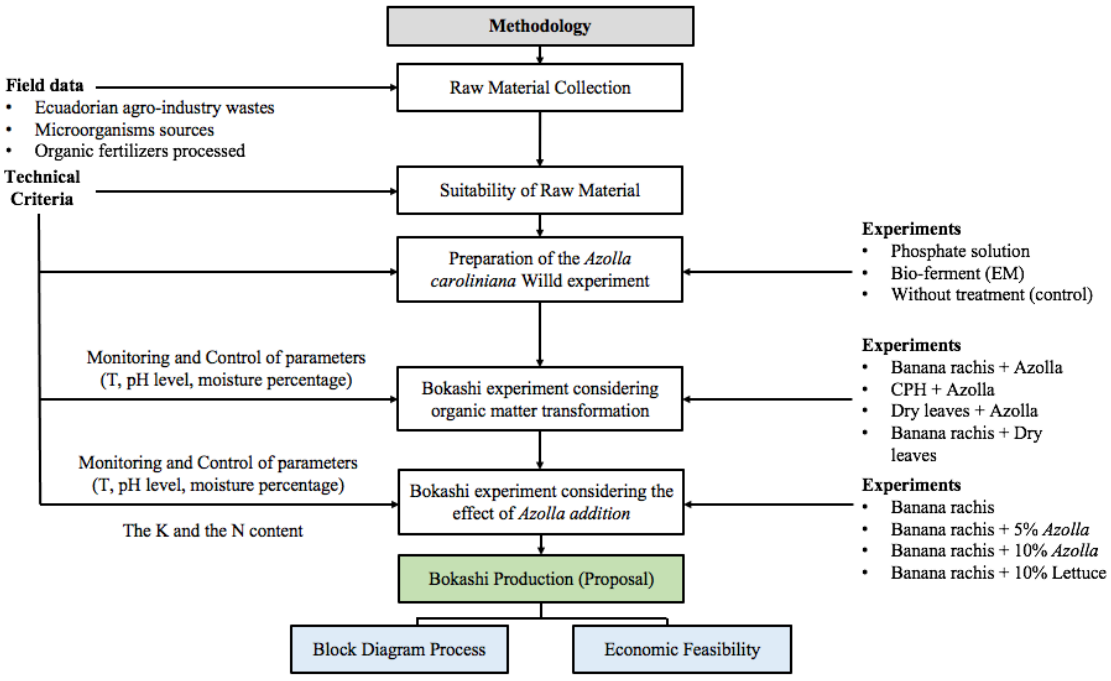

Figure 9. *Project methodology diagram*

3.1 Raw Material Collection

- Several portions of *Azolla caroliniana* were purchased in Aqua Quito. The aquatic fern portions were sent in bags filled with some water.
- Banana empty fruit bunches (banana rachis) were obtained in a dessert banana farm of Giant Cavendish Bananas (*Musa acuminata*) [92, 93], triploid AAA in Machala. Banana rachis was obtained the same day it was discarded after a banana production, guaranteeing freshness of raw material. Cocoa pod shells were obtained in a cocoa farm of Ecuadorian National Cocoa *(Theobroma cacao L.)* [94] in Machala. They were on the soil and looked like a dark cookie. Dry leaves were obtained in Machala from the Fincas de El Oro banana association, located in Machala.
- Goat manure and cattle manure was obtained from a rancher of El Oro province.
- Some rice husk ash was obtained in Machala from the Fincas de El Oro banana association and stored in a sack.
- Biochar, sugarcane molasses, and rice bran were bought in commercial establishments.
- Efficient microorganisms deactivated, or a M-M matrix, were obtained from a bio factory from Fincas de El Oro bananas association.
- Bio-ferment (EM), or M-M activated, was obtained from a bio-factory from the Fincas de El Oro banana association.
- Virgin forest mulch and soil was proportioned from Fincas de El Oro banana association.

3.2 Suitability of raw material

- *Azolla caroliniana* Willd was collocated on settled water in a small tank. After a week, the aquatic plant was fertilized with a soluble solution of chemical phosphate fertilizer (composed by 45% P_2O_5, 12.6% K_2O, 9.1% N), methodology adapted from the pre-grade thesis "*Relación simbiótica de Azolla (Azolla caroliniana, A. filiculoides, A. mexicana) – Anabaena (Anabaena azollae) para la producción de nitrógeno en ecosistemas acuáticos de la zona de Cayambe, 2010*" [95] and the paper "*Blue-Green Algae and Algal Associations*" [44].
- Banana rachises were chopped up using a machete, having cubes with a dimension of one square centimetre. Banana's rachis cubes were stored in a bucket. Cocoa

pod husks and dry leaves were manually shredded into short pieces with a maximum of 2 cm in length. Cocoa pod shells and dry leaves pieces were stored in a sack respectively.

- Biochar was pulverized using a hammer, with this was obtained a mix of biochar fragments of approximately 0.5 cm with biochar dust.
- Goat manure was stored in a sack.
- It is prepared a mix of virgin forest soil and mulch, molasses, and rice bran, then they are stored in an anaerobic tank.

3.3 Preparation of the *Azolla caroliniana* Willd experiment

The *Azolla* experimental design was made to understand what nutritive solution would be better for the aquatic fern reproduction. Also, this experiment was performed focusing on a substrate source to produce *Azolla* bokashi. Thus, three experiments and three randomly repetitions were considered in the design. The following table shows nomenclature and treatments considered.

***Table 3**. Legend of Azolla experiments based on nutritive solution*

AP	*Azolla* treated with phosphate fertilizer solution.
AB	*Azolla* treated with bio-ferment (EM).
A	*Azolla* without treatment (control).

In each *Azolla* treatment was considered to have the same operational conditions. The proportions of substrates in percentages are represented in the following table.

***Table 4.** Model of Azolla experiments according to nutritive solution.*

Solutions	AP (%)	AB (%)	A (%)
Soluble solution of P_2O_5.	7	0	0
Bio-ferment (EM).	0	7	0
Settled water	93	93	100

To increase the *Azolla* production for being used in the bokashi experiment, a used plastic tank (1 x 1 x 0.25 meters) was adapted to reproduce the aquatic fern. The

bioreactor was filled with tap water. The water was settled for a few days waiting it would not have chloride in its content. Then the bioreactor was filled with water and an *Azolla* portion was sown carefully. In the AP treatment, the 7% nutritive solution of P_2O_5 was prepared considering dilution of 3 grams of phosphate in 350 mL of settled water, then it was added in the *Azolla* tank. For the AB treatment 7% of liquid bio-ferment (EM) was added in the *Azolla* tank. The A treatment serves as parameter control, so this sample only required settled water.

3.4 Preparation of the bokashi experiment considering organic matter transformation.

There are many ways to prepare bokashi, it can be prepared by changing percentages of raw material, as well as the kind of raw material used. The majority of authors recommend using the available feedstock in the region [96].

After choosing raw materials for bokashi treatment. A literature review of different kinds of bokashi and of each raw material selected was done in the background information. It allows to conclude and build the following tables. The designed percentages of each raw material followed a theoretical design of C/N ratio, having as a result around 25-30.

The elaboration of the treatments was based on the following model:

Table 5. *Legend of bokashi experiments*

R	Treatment with bananas rachis + *Azolla caroliniana*.
C	Treatment with cocoa pod shells+ *Azolla carolinaina*.
H	Treatment with dry leaves + *Azolla carolianana*.
RH	Treatment with bananas rachis + dry leaves.

Table 5 shows the different treatments to obtain bokashi. The differences between each treatment are due to the raw materials chosen for its preparation. The R, C, and H treatment allows seeing differences between banana rachis, cocoa pod shells, and dry leaves as a bokashi preparation source. On the other hand, the RH experiment is nearly similar to the R treatment, but the first contains dry leaves instead of *Azolla caroliniana*. It is to see what the impact of the aquatic fern in the mixtures to dry leaves is. Dry leaves are in the bokashi experiment to realize the banana rachis and cocoa pod husks as good

nutrient sources. It allows having the design of a constant matrix of bokashi that is comparable between experiments.

Table 6. *Model of bokashi experiments according to vegetal material source*

Raw Materials	R (%)	C (%)	H (%)	RH (%)
Biochar	5	5	5	5
Goat manure	30	30	30	30
Bio ferment (EM)	10	10	10	10
M-M Matrix	17	17	17	17
Rice husk ash	3	3	3	3
Banana rachis	30	0	0	30
Cocoa pod shells	0	30	0	0
Azolla caroliniana	5	5	5	0
Dry leaves	0	0	30	5

Table 6 shows the model of different treatments to obtain bokashi in percentage composition. Raw materials are the ingredients to produce bokashi. The importance of each one in the mixture is because they are good nutrient sources. It improves bokashi nutritional quality. Also, some of them are residues from anthropogenic activities that are being transformed into valuable products. For M-M Matrix and Bio ferment (EM), they are products with a high content of beneficial microorganisms, so they were used in the mixture. The percentage of each raw material was designed according to a comparison of the data of analogies of some researches [14, 79, 97–101].

Biochar, goat manure, Bio ferment (EM), M-M Matrix and rice husk ash were constants for every treatment. It is because the model was designed to analyse what effect causes the residues from agricultural activities. For that reason, in the R experiment it has 30% of banana rachis in its formula, and 0% of cocoa pod shells and dry leaves. This percentage selection is similar for the C experiment, but it has 30% of cocoa pod husks in its formula without the addition of banana rachis and dry leaves.

Raw materials such as banana rachis, cocoa pod shell, *Azolla caroliniana*, and dry leaves were variables for the four experiments. It is because they are ingredients which are changing for each sample.

To prepare 500 grams of bokashi for each sample, three repetitions were made for R, C, H, RH experiments based on a completely randomized design. To differentiate samples, 12 vessels were tagged according to the repetition and material used. The following procedure was applied to obtain each sample:

The bokashi feedstock are pile up starting in the bottom with the vegetable material source, then the goat manure, rice husk ash, biochar, M-M Matrix, one above the other, respectively. In the end, bio-ferment (EM) is sprayed. Then of having every ingredient in the vessel, these are mixed, ensuring correct homogenization. Finally, the vessels are stored in a dry and airy place.

Figure 10. *Bokashi stored.*

3.4.1 Monitoring and Control of parameters of bokashi evolution(development).

Daily measurements of temperature, pH, and moisture were made for 14 days. Data obtained was recorded in a table. To obtain data of temperature, a laboratory thermometer was used, it was inserted in the middle of the samples and then for 5 minutes, temperature data was recorded. On the other hand, to get data of pH and moisture percentage, a soil pH and moisture meter was required; this instrument was inserted in the middle of the samples, and data is recorded then of 5 minutes.

The mixture was homogenized to have a more accurate pH and humidity value.

After recording the parameters of temperature, pH and moisture percentage for 14 days, a comparison of organic matter transformation was made for R, C, H, RH samples. This was done to know which raw material had a better physical decomposition.

3.5 Preparation of the bokashi experiment considering the effect of *Azolla* addition

The elaboration of the treatments was based on the following model:

Table 7. *Legend of the bokashi experiments considering the impact of Azolla addition*

RR	Treatment with bananas rachis.
RA	Treatment with bananas rachis + 5% *Azolla caroliniana*.
RAA	Treatment with bananas rachis + 10% *Azolla caroliniana*.
RL	Treatment with bananas rachis + 10% Lettuce.

Table 7 shows the different treatments to obtain bokashi with banana rachis. This experiment is to see the effect of nutritional quality of *Azolla caroliniana* like feedstock in bokashi samples. The RR treatment represents a bokashi made with banana rachis with more manure substituting the aquatic fern as raw material. The RA sample reduces 5% of manure adding 5% of *Azolla caroliniana* into the bokashi formula. The RAA experiment doubles the addition of *Azolla* by 10%. Finally, RL treatment contains 10% lettuce instead of the aquatic fern; it allows seeing differences between *Azolla caroliniana* and lettuce as nitrogen substrates.

Table 8. *Bokashi experiments model according to vegetable material source.*

Raw Materials	RR (%)	RA (%)	RAA (%)	RL (%)
Biochar	1	1	1	1
Goat manure	35	30	25	25
Bio-ferment (EM)	10	10	10	10
Virgin forest mulch	7	7	7	7
Rice bran	7	7	7	7
Molasses	7	7	7	7
Rice husk ash	3	3	3	3
Banana rachis	30	30	30	30
Azolla caroliniana	0	5	10	0
Lettuce	0	0	0	10

Table 8 shows a new model of different treatments to obtain bokashi in percentage composition. The new model changed biochar percentages from 5% to 1% to increase sources of C of easy assimilation such as molasses. Goat manure is a variable that serves to add or reduce *Azolla caroliniana* in the mixture. It allows to understand how the aquatic fern influences the product. Virgin forest mulch, rice bran, and molasses were added instead of M-M matrix for their nutritional qualities. It is because virgin forest mulch is a rich source of microorganisms and rice bran a source of microelements and cellulose [102]. Rice husk ash, banana rachis, Bio-ferment (EM), were constants for every treatment. In this case, the bokashi model was designed to analyse the effect in nitrogen content of *Azolla caroliniana*, goat manure, and lettuce in the samples. The percent of each raw material was considered according to the last bokashi design.

To prepare 3000 grams of bokashi for each sample, three repetitions were made for RR, RA, RAA, and RL experiments and were made by a randomly design. To differentiate samples, 12 bags were tagged according to the repetition and material used. The following procedure was applied to obtain each sample:

To obtain a bokashi pyramidal pile, banana rachis is added in the bottom, then the variable feedstock (iceberg lettuce, *Azolla caroliniana* Willd, or goat manure), goat manure, rice husk ash, biochar, the mix of virgin forest mulch, rice bran and molasses. Bio-ferment was sprayed on the top of the pyramidal pile and while were mixed the materials. To achieve a right moisture level the “fist test” [97] was applied during the

process. Then of having every ingredient in the bag, they are mixed, ensuring correct homogenization. Finally, the bags are stored in a dry and airy place.

Figure 11. *Bokashi samples stored*

3.5.1 Monitoring and Control of parameters.

The same procedure was followed as in section 3.7.1.

Finally, it is necessary to do a Nitrogen and Potassium characterization to the samples that meet the established thesis objectives.

3.5.2 Nitrogen and Potassium content.

From each bokashi repetition was selected a sample formed by aliquots of the repetitions. Thus, four bokashi sample generalized were characterized in the chemical laboratory of NEMALAB. Then of 14 days, nitrogen content was determined using Kjeldahl method, and potassium content by flame atomic absorption spectrophotometry.

3.6 Bokashi Production

3.6.1 Bokashi process description.

The diagram (figure 12) represents the transformation processes of natural resource into the bokashi cake. It shows four essential steps. They are the storage of feedstocks, pre-treatment, aerobic treatment, and the product diversification.

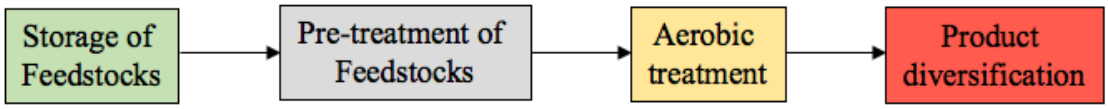

***Figure 12**. Transformation processes agro-industry wastes into the bokashi cake*

The storage of feedstocks means the reception of every raw material. In the pre-treatment of feedstocks, the raw materials are prepared to be transformed, here it is a physical transformation of the feedstocks. In the aerobic treatment, there is a biochemical transformation of the biomass. The product diversification brings two organic fertilizers, bokashi cake and leachates. The comprehension of inputs and outputs in figure 12, allow to continue to a feasibility study.

3.6.2 Economic Feasibility.

The economic feasibility is an analysis applied to laboratory projects to see if the project is viable for its escalation. The main economic parameters are direct and indirect costs and the selling price of the product. This section is for making decisions. The project profits are the new data for discussing the project sustainability.

CHAPTER 4. RESULT AND DISCUSSION

4.1 *Azolla caroliniana* Willd experiment considering nutritive solution

Azolla is a plant that needs nutrients for its growth and development as a crop. The results of these experiment showed AP was the better treatment for *Azolla caroliniana* Willd reproduction, growing and weight (figure 13). It was surely because phosphorus promotes the aquatic fern growth [44]. After 10 days, the aquatic fern of AP treatment had already reproduced exponentially. On the contrary, AB and A treatment did not show changes in growth in the small-free floating fern. Also, for AB and A treatments after two months the aquatic ferns died. Thus, the results show nutrients are needed but not a bio-ferment solution during the free-floating fern evolution. The temperature parameter of optimal temperature range of 18-28ºC was not considered because this condition is common in tropical areas. Although it was not evaluated parameters of photoperiod, salinity, and water pH, the methodology applied in this experiment allowed to meet the proposed objectives of *Azolla* reproduction for its application as substrate in the bokashi design.

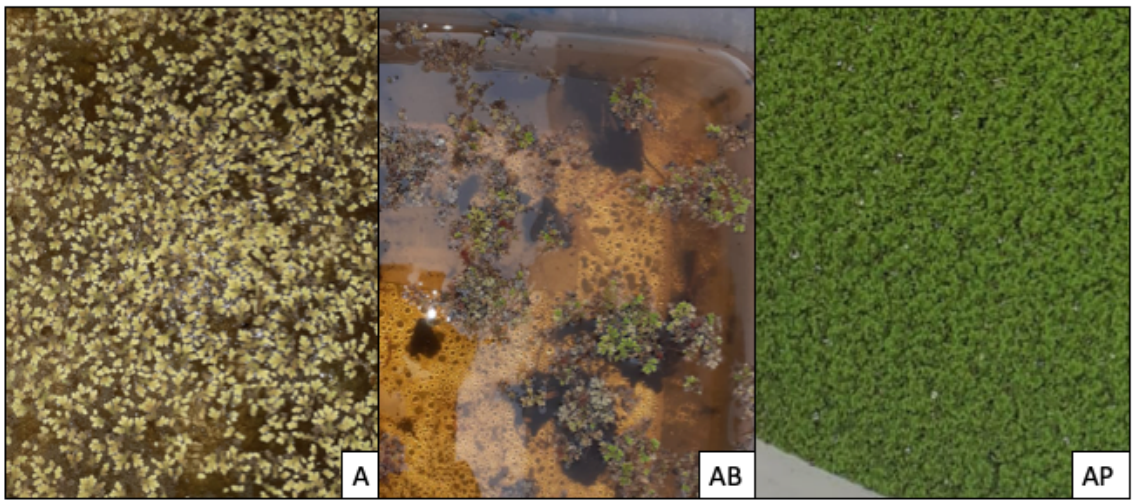

Figure 13. *Azolla experiments according to nutritive solution after 10 days*

4.2 Bokashi experiment considering organic matter transformation

Inside bokashi process there is an oxidative fermentation of biomass. The biomass ("CH_2O") is composed by cellulose, lignin and phenolic polymers. Uncharacterized organic matter (UM-OM) which is constituted by complex compounds resistant to

biological degradation is known has humic substances. The humic substances are humin, humic acid and fluvic acid [103]. Microorganisms produces enzymes that allows organic matter transformation [97]. The organic matter transformation parameter serves to understand the substrate variability. The substrate nature, the lignocellulose content, and the different cell wall structures and components [104] affect in the conversion of the biomass selected. In this case, the banana rachis (R) has simpler degradation in comparison with the cocoa pod shell husks (C) and dry leaves (H). Banana rachis is constituted by uniformity in its structure. The CPH has three layers, this characteristic means highly diversity in the lignocellulose content, so it generates degradation resistance [105]. Dry leaves are a substrate with zero humidity, this characteristic affects in the bokashi process making it easily losses moisture level (figure 14). Water is essential in the biomass hydrolysis because it supplies a diffusion medium for enzymes, and it is part in the hydrolysis reaction [106]. Particle size, distance between particles also affect the solid-state fermentation, because changes porosity creating big spaces between particles generating an aerobic slower degradation [97]. The management of dry leaves and CPH needs the addition of new strategies to exploit their biomass. For example, for the cocoa pod husk (CPH) it is necessary the addition of specific cellulase producing microorganisms and a milling process to achieve the required particle size in the bokashi mixture. Also, the water retention capacity of raw materials affects in the organic matter degradation. For the H bokashi sample were no significant changes between the initial and final biomass. In the case of the RH sample the addition of dry leaves instead of *Azolla caroliniana* Willd showed density increases in comparison to R sample. Dry leaves similarly to CPH need pre-treatments to enhance its degradation.

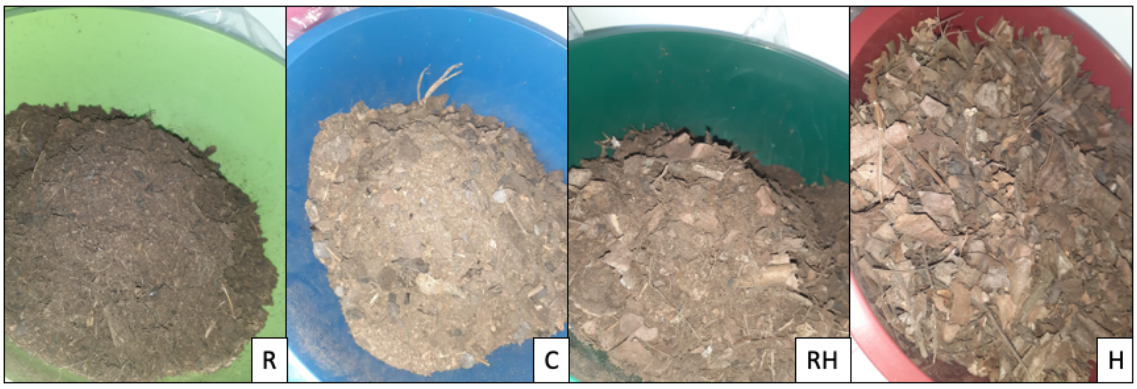

Figure 14. *R, C, H and RH samples after 14 days.*

4.3 Bokashi experiment considering the effect of *Azolla* addition

4.3.1 Temperature

Figure 15 is the graphical representation of the daily temperature record (ºC). It is possible to notice two stages for every bokashi sample, the first is an increasing temperature stage, the second is a decreasing temperature stage. The bokashi samples followed the same behaviour of temperature increases at the first day having temperatures closer to 35ºC. The second day, the RR, RA and RAA samples had temperature increases around 40ºC. Then, they decrease temperature until stabilized in 26ºC to the sixth day. In contrast, on the second day RL sample decreases temperature to 32ºC and it continued decreasing temperature until stabilized in the sixth day. The notable difference between the samples was the addition of extra manure (RR) *Azolla* (RA and RAA) and iceberg lettuce (RL). It could be a correlation between temperature increases by the mesophilic bacteria between the addition of iceberg lettuce, because the same methodology was applied for every sample.

According to Darwel, et al composting time decreases while increases the addition of microorganism cocktails [107]. Maturation time finished in six days, and it shows it is possible to obtain the bokashi cakes at very short time (figure 15). Similar to Darwel results, the organoleptic conditions were not unpleasant, especially to the smell. Further, every bokashi sample had a blackish brown colour, and a smooth but still a little rough texture. Microorganisms' cocktails such as bio-ferment (EM) could be an alternative to the biomass degradation. The addition of virgin rainforest mulch and soil and bio-ferment (EM) in the bokashi formula could create an environment of containing high amounts and diversity of cellulase enzymes could optimize the organic matter transformation process. It is because could be the presence of potential cellulose degraders such as Proteobacteria, Firmicutes, Actinobacteria [108]. For example, Garvey, et. al claim *Bacillus subtilis* has small amounts of native cellulolytic activity by its surface and secretion display properties [104], so a cocktail of microbial consortium could increase the cellulases amounts.

There were temperature increases during the solid-state fermentation. Temperature was less to 40 ºC, so there was mesophilic organisms influence [107]. Further, Wiegel, Ljundahl & Demain claim mesophiles are used as biological catalysts, and the Gram-positive mesophilic bacteria generate amino acids in the fermentation process [109]. Data presented in figure 15 and figure 16, promotes that could be a correlation between mesophiles and acid tolerant, due to none bokashi samples showed a

thermophilic fermentation. It is advantageous because avoid unit operations of turning in a large-scale process. Also, maturation time is related with temperature decrease, due to mesophilic fermentation less time needed to reach maturation (figure 15). However, Wu et al, suggests that lignocellulosic decomposition is made in the thermophilic phase [110].

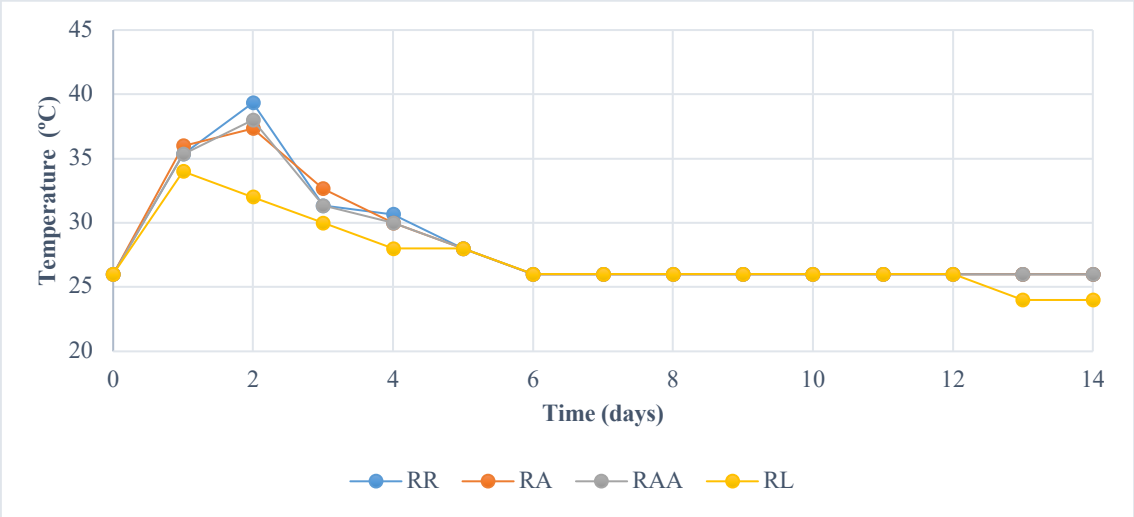

Figure 15. *Daily temperature record (ºC) in the bokashi samples*

4.3.2 PH level

Figure 16 is the graphical representation of the daily pH level record (ºC). The pH level for every treatment had the same behaviour and started in a very acidic environment that was changing trough time by microbial activity. According to Restrepo and Hensel the pH self-correcting during fermentation evolution (the bokashi maturation phase) [97]. In the second day, pH level increases for every bokashi sample, and it was modifying until to reach stability. The pH decreasing in the sixth day was due to the methodology applied; every bokashi sample was homogenized because there were many variations at the time of recording the data.

Bio-ferment (EM) as product of anaerobic digestion process could have lower pH condition by the accumulation of volatile fatty acids (VFA) [111]. Bacteria manifest high stability and tolerance under harsh conditions of lignocellulose conversion [105]. Fungi and yeast are acid-tolerant microorganisms which have pH optima nearer to neutrality [112]. This could be a reason why 3 pH was changing though time until to reach 6 pH level. Also, the addition of bio-ferment is reflected in the lower pH at the beginning of the bokashi samples (figure 16), this methodology was made to reduce pathogens contents

for the consideration of goat manure in the bokashi formulas. Although the presence of indicators of faecal matter microorganisms was not evaluated, the methodology could have inhibited the growth of these pathogens. This thinking was supported by the research of Akkermans, et. al, where they show *Escherichia coli* is tolerant to mesophilic conditions but no in very acidic conditions of 3 pH [113].

Although the fermentation time is reduced, Shu-guang, et al claim the lower pH condition is correlated with mesophilic digestion, and it would inhibit protein degradation in anaerobic environments [111]. This could have occurred at the beginning of the solid-fermentation of the bokashi samples, but the change in pH on the second day by microbial activity could promotes the protein degradation, which is optimal in 4-6 pH range (figure 16). That is to say, while protein is being hydrolysed producing amino acids, there could be an ammonia releasing [107, 113].

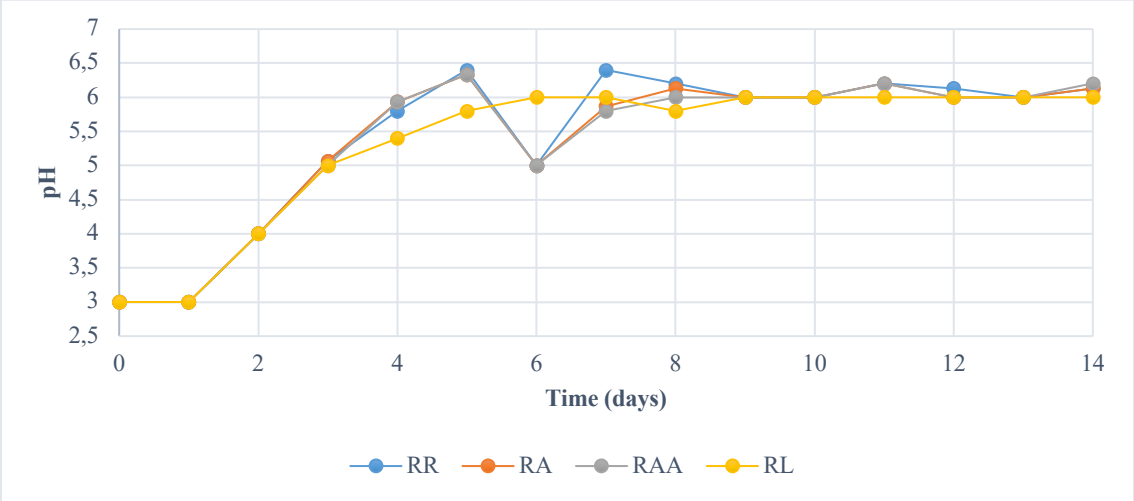

Figure 16. *Daily pH level record in the bokashi samples*

4.3.3 Moisture (%)

Figure 17 is the graphical representation of the daily moisture level record (ºC). The first days the moisture level for every bokashi treatment was high around 80%. This could be the result of the bio-ferment (EM) addition. There were no significant differences the first five days for the RR, RA, and RAA bokashi treatments. The RR, RA, RAA samples reach 40% moisture content in five days. In the sixth day, moisture level increases for the RA and RAA samples due to the methodology applied, it also occurred for the RR sample but in smaller magnitude. This behaviour could be by the water retention capacity of the feedstock. Then the moisture level decreases and stabilize in 50%

in eight days for the treatments. On the other hand, the RL treatment had a constant moisture level decrease thought time. After ten days the RL sample reaches a moisture content of 50%.

Moisture level of the bokashi samples was decreasing during the fermentation process. It could be by water and volatile gases evaporation, or the consumption of the organic liquids by microbiota activity, generating bokashi leaches on the bottom of the bokashi samples. Formation or accumulation of organic acids during fermentations generates acid environments [112]. In the bokashi design the production of bokashi leaches was not considered, so when the methodology was applied, surely the bokashi cakes were mixed with the bokashi leaches. It causes a pH decrease and moisture level increase in the bokashi samples (figure 16 and figure 17).

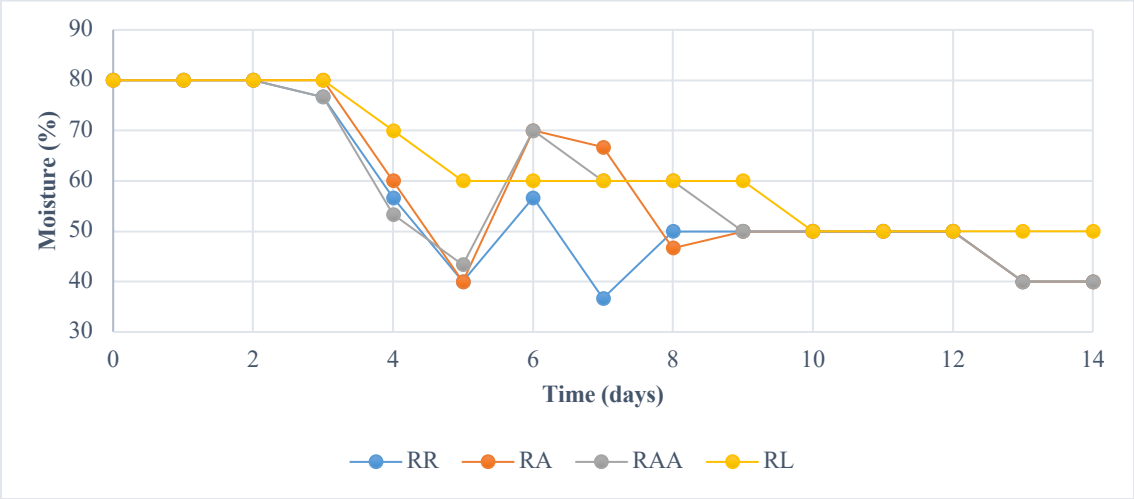

Figure 17. *Daily moisture record in the bokashi samples*

4.3.4 Nitrogen and Potassium content

Table 13 shows nitrogen and potassium content of the bokashi samples. N and K were considered as quality parameter because the bokashi cake is focused on banana nutrition. Every bokashi sample had relatively higher potassium content, it could be due to the addition of banana rachis as substrate in the bokashi formula [20, 88]. Comparing the bokashi samples, the RR bokashi sample had the highest potassium content, and it is followed by the RAA, RA, and RL, respectively. The results of potassium content show how could vary the potassium content of the substrates. For example, considering the addition iceberg lettuce (RL) instead small amounts of goat manure in the sample, reduces potassium content because this does not provide enough nutrients (K, and N) [114]. On

the other hand, considering the *Azolla* (RA and RAA) addition instead of small amounts goat manure, has no significative differences. Further, the RA, RAA, and RR comparison show the variability of potassium content could have the goat manure.

In the case of nitrogen content, comparing the bokashi samples, the RAA bokashi sample had the highest nitrogen content, and it is followed by the RA, RR, and RL, respectively. To achieve higher nitrogen contents in the bokashi samples the novel addition of *Azolla caroliniana* Willd was considered into the bokashi formula. The results of the table 13 show there is a possible correlation between nitrogen content and the amounts of Azolla substrate. This is the reason why the RAA sample has the highest nitrogen content. The cyanobiont which is living inside Azolla [44] enriched the aquatic fern with highest nitrogen contents. On the other hand, the RL bokashi sample showed the less N content because this sample was made considering iceberg lettuce, which has lower nitrogen content ranged from (4 – 5) % [114].

Table 9. *Nitrogen and potassium content of the bokashi samples*

Treatment	K (%)	N (%)
RR	4,15	1,09
RA	3,75	1,23
RAA	4,10	1,29
RL	3,40	0,53

A histogram allows a broader view of nitrogen and potassium content of the bokashi samples. Thus, figure 18 and, figure 19 were made using data from a review of different bokashi made with similar substrates [42], focusing on their potassium and nitrogen content. These histograms allow us to compare K and N contents from RR, RA, RAA and RL bokashi with the contents obtained by other authors.

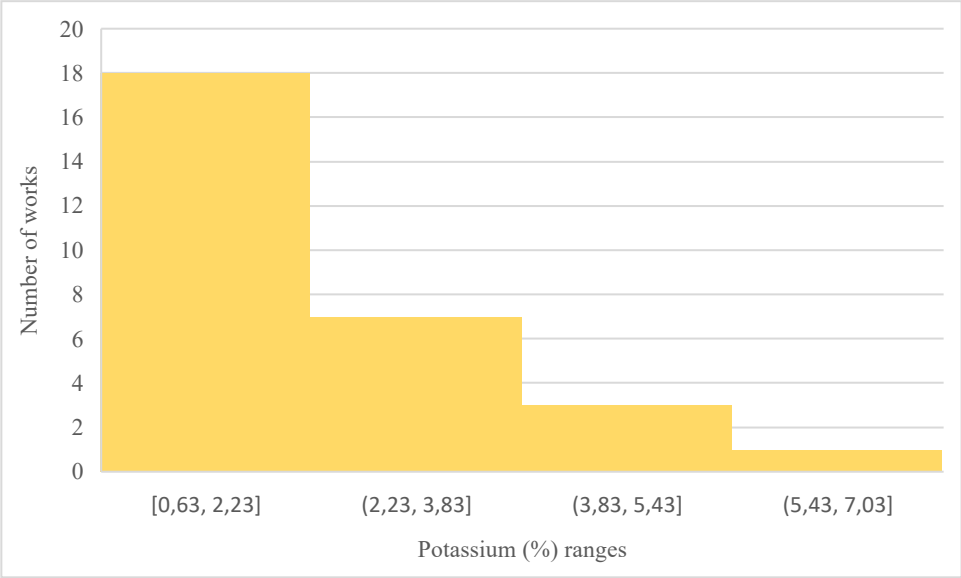

Figure 18. *Potassium content histogram*

The RR, RA and RAA samples are in the 3,83-5,43 range of potassium content. They are the only ones representing the block of this range. The RL sample is in the 2,23-3,83 range (figure 18). Potassium content was relatively higher in the bokashi sample, in comparison with bokashi samples from the literature review [42]. The majority of the samples presented in the figure 18 was made with banana rachis, so this data show that could be a correlation between the potassium fertilization of the banana plant, the banana variety where rachis comes from, and the K content rachis have.

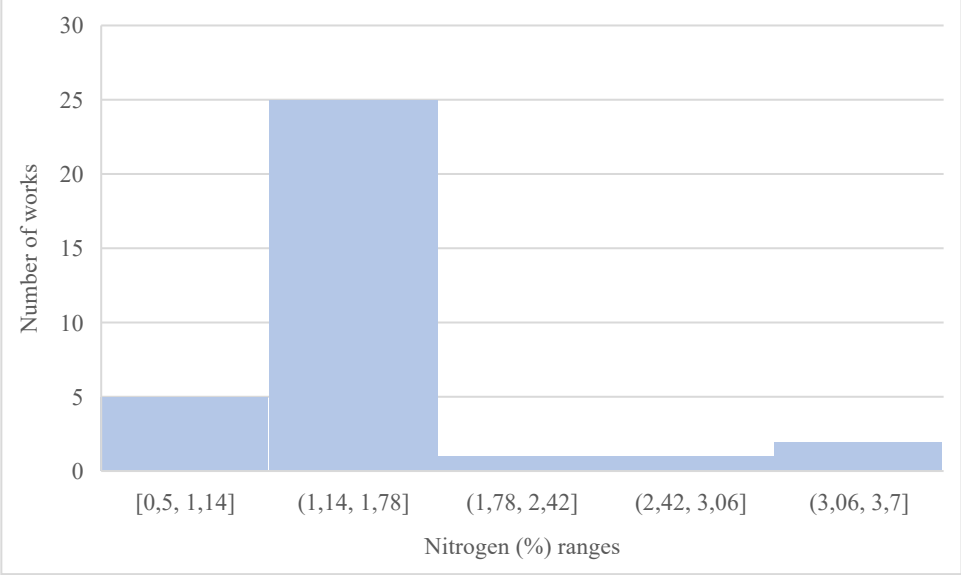

Figure 19. *Nitrogen content Histogram*

The RR, RA and RAA samples are in the 1,14-1,78 range of nitrogen content. They are in the second block, sharing nitrogen content similitudes with 22 bokashi samples. The RL sample is in the first range of 0,5-1,14 (figure 19). According to figure 18, nitrogen content of the bokashi samples made with *Azolla* are in the common range of bokashi samples made without the addition of the aquatic fern. It could be by other factor that could affect the nitrogen losses, which is the manure storage method [28]. The goat manure was not pre-treated, so it is possible to nitrogen loss due to volatilization. It could support the fact the RL bokashi sample had a very low nitrogen content. Despite these possible factors of nitrogen losses, the bokashi obtained has similar nitrogen contents to 23 bokashi samples made with similar substrates by the addition of the aquatic fern [42]. Thus, there is a possibility to increase nitrogen content of the bokashi samples if the goat manure is pre-treated [29].

4.4 Bokashi process

4.4.1 Block diagram (BD)

Data obtained from the RAA bokashi sample allows the building of the block diagram of the bokashi cake production. The graphical representation followed the methodology of chapter 3. The BD is constituted of four coloured blocks. They are storage (green), pre-treatment (grey), aerobic treatment (yellow), and product diversification (red). Storage is the reception of the substrates, then pre-treatment is the crushing of banana rachis and *azolla*. Subsequently, the aerobic treatment includes the bedding of the feedstocks. Then, in the same block, leaching and blending of the biomass accumulated. Finally, there is a product diversification that includes the RAA bokashi and bokashi leachates.

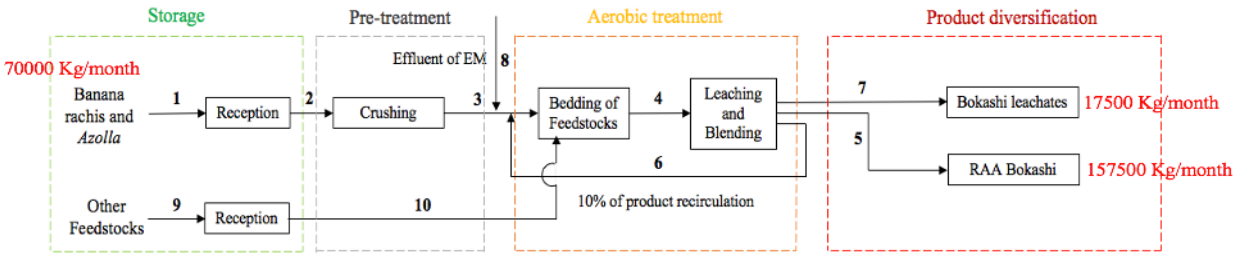

Figure 20. *Block diagram proposed for bokashi production.*

In the complete bokashi process diagram, there are ten flows. The first flowrate (1) is the reception and management of banana rachis and *azolla*. It also happens with other

feedstocks. The second flow rate (2), reduces the particle size of banana rachis and the aquatic fern. In the third flowrate (3), there is bedding of the feedstocks according to the technical criteria. Leaching and blending are the unit operations that separate bokashi leachates (7) from the RAA bokashi cake (flow 5). The sixth flow represents the recirculation of 10% of the bokashi cake produced. It is for the reuse of the microbiota present in the bokashi product. The effluent of EM is the Bio-ferment (EM); the flow (8) is the addition of bio-stimulants to the final product. The ninth flow (9) is very similar to the first flowrate, but it is for the reception of other feedstocks. They go in the last flow to be bedding together with the banana rachis and Azolla.

4.4.2 Economic Feasibility

The economic feasibility study was done by using data from the RAA bokashi experiment. The economic study considered direct and indirect costs. Direct costs were feedstock costs, electrical consumption, workers, and maintenance. The indirect costs consisted of rent and basic services.

***Table 10**. Direct costs*

Passives	Total $(USD)
Feedstock cost	35612.50
Electrical energy consumption	583.78
Workers	3200.00
Maintenance	223.33

Direct costs (table 10) are associated with the costs of feedstock. They are very close to the real market prices of the raw materials and biomass. The electrical energy consumption is an estimate of how much energy will be consumed by industrial activity (machines). It was considered to have four employers, each with an $800 USD salary. Also, it is recognized that the instrument and the place will need maintenance work.

Table 11. *Indirect costs*

Passives	Total $(USD)
Rent	1000
Basic services	300

Indirect costs (table 11) correspond to the rent payment and the basic services such as water, light, phone, and internet services.

An organic fertilizer factory should be able to transform at least 158.1 tonnes of feedstock. Values above 160 tonnes of feedstock management generate profits for the RAA bokashi production. For the economic viability of the agro-industry, a feedstock management of 175 tonnes was chosen.

Table 12. *Feedstocks description for the RAA bokashi production*

Feedstocks	(%)	Amount (Kg)	Price per Kg	Total $(USD)
Banana rachis	30	52500	0.02	1050.00
Goat manure	25	43750	0.20	8750.00
Rice husk ash	3	5250	0.20	1050.00
Sugarcane molasses	7	12250	0.20	2450.00
Rice bran	7	12250	0.25	3062.50
Virgin Forest mulch	7	12250	0.75	9187.50
Biochar	1	1750	1.75	3062.50
Bio-ferment (EM)	10	17500	0.20	3500.00
Azolla	10	17500	0.20	3500.00
Total	100	175000	3.77	35612.50

Although it is surprising to have 52.50 tonnes of banana rachis, it is a quantity that represents 7500 empty fruit bunches. They can easily be obtained after banana harvesting, when it is common to have empty banana bunches every day. Although the majority of the feedstocks are easy to obtain, *Azolla caroliniana* Willd is the exception. To have 17500 Kg of the aquatic fern, it is necessary to build a giant tank filled with a nutritive solution. It is because the free-floating fern is very expensive; a small portion of 5 grams of the plant has a cost of $5.

Table 13. *Profits*

	Monthly $ (USD)	Annual ($)
Annual fixed profit at the second year.	7608.69	91304.34

Considerable profits are estimated for every month and for the second year. It is relevant to have a vision of the bokashi project. Nevertheless, data presented in profits belongs to a competitive standard value for the bokashi. Similar imported products on the market sell for $0.26 USD per kilogram. Thus, there is an opportunity to Bokashi to enter into the fertilizer market with economical price of $0.246 USD per kilogram.

CONCLUSIONS

The phosphate nutritive solution was the best treatment for *Azolla caroliniana* Willd exponential growth.

The banana rachis lignocellulosic constitution makes it easier to be degraded by microorganisms than other dry and highly lignocellulosic diversity wastes.

The addition of bio-ferment (EM) could optimize the bokashi production time in a maximum of six days.

Microorganisms from the Ecuadorian virgin forest soil and mulch and bio-ferment (EM) are acid-tolerant; they show mesophilic activity.

Manure pre-treatment is substantial for avoiding nutrient volatilization.

The addition of banana rachis and *Azolla caroliniana* increase the potassium, and the nitrogen content of bokashi, respectively.

The bokashi production could be summarized in four stages. They are storage, pre-treatment, aerobic treatment, and product diversification.

The feedstocks management for bokashi production should be 175 tonnes; for generating a viable and competitive product that can compete in the market with similar organic fertilizers.

FUTURE WORK (RECOMMENDATIONS)

- The *Azolla caroliniana* Willd growth with a rock phosphate treatment would be interesting considering the pH medium, salinity, photoperiod, and moisture parameters.
- The effect of milling biomass pre-treatment in the organic matter transformation. Also, the similitudes and differences between dry and wet biomass degradation.
- It should be studied the correlation between mesophiles and acidic conditions in solid-state bokashi fermentation. Also, a temporal characterization of the microbiota and its products from biomass decomposition by thermophiles and mesophiles who started in acid media.
- The effect of microorganism consortium and isolated microorganism in the solid-state bokashi fermentation.
- Identification and phylogenetic analysis of isolated bacterial strains.
- Temporal categorization of microbiota through time, after 2, 4, and 6 days during the solid-state fermentation.
- Metagenomic analysis of native microorganisms used in the bokashi solid-state fermentation.
- Ammonium and ammonia generation during solid-state fermentation with pH changes from acid to neutral or alkaline medium.
- To improve manure storage management for bokashi composting processes.
- Effect of bokashi fertilization in banana crops.
- Effect of adding bio-ferment (EM) in the solid-state fermentation.
- Effect of Bokashi pelleting, and what could be the new bokashi properties and quality parameters.
- Economic feasibility of bio stimulants for banana farming.

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APPENDICES

APPENDIX SECTION A (SA). Data recorded from the bokashi samples

SA 1. Daily temperature record (ºC) in the bokashi samples

Treatment/day	**Days**														
Treatment	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
RR (ºC)	26	35	39	31	31	28	26	26	26	26	26	26	26	26	26
RA (ºC)	26	36	37	33	30	28	26	26	26	26	26	26	26	26	26
RAA (ºC)	26	35	38	31	30	28	26	26	26	26	26	26	26	26	26
RL (ºC)	26	34	32	30	28	28	26	26	26	26	26	26	26	24	24

SA 2. Daily pH level record in the bokashi samples

Treatment/day	**Days**														
Treatment	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
RR	3	3	4	5	6	6	5	6	6	6	6	6	6	6	6
RA	3	3	4	5	6	6	5	6	6	6	6	6	6	6	6
RAA	3	3	4	5	6	6	5	6	6	6	6	6	6	6	6
RL	3	3	4	5	5	6	6	6	6	6	6	6	6	6	6

SA 3. Daily moisture record in the bokashi samples

Treatment/day	**Days**														
Treatment	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
RR	80	80	80	77	57	40	57	37	50	50	50	50	50	40	40
RA	80	80	80	80	60	40	70	67	47	50	50	50	50	40	40
RAA	80	80	80	77	53	43	70	60	60	50	50	50	50	40	40
RL	80	80	80	80	70	60	60	60	60	60	50	50	50	50	50

APPENDIX SECTION B (SB). Chemical characterization of the bokashi samples.

SB 1. Nitrogen and potassium characterization of the RR, RA, RAA, and RL samples

CLIENTE: GOOTMAN JADAN IVAN
REMITE: SR. IVAN GOOTMAN J.
PROPIEDAD: TESIS DE GRADO
LOCALIZACIÓN: MACHALA - EL ORO

Nº DE DOCUMENTO: 55958
FECHA/MUESTREO:06/08/2021
FECHA/INGRESO :06/08/2021
FECHA/SALIDA :14/08/2021

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Laboratorio de análisis agrícola

RESULTADOS DE ANALISIS QUIMICO DE FERTILIZANTE

			%	
Nº LAB	#MUESTRA	IDENTIFIC.	NITROGENO	POTASIO
4020	1	RA	1.23	3.75
4021	2	RR	1.09	4.15

BIOQ. MARTHA MOREIRA
JEFE DE LABORATORIO

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ING. NARCISA PINTADO
SERV. AL CLIENTE

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NOMBRE DEL CLIENTE : GOOTMAN JADAN IVAN
REMITE . SR. IVAN GOOTMAN J.
PROPIEDAD : TESIS DE GRADO
LOCALIZACIÓN: MACHALA - EL ORO

Nº DE DOCUMENTO: 55958
FECHA DE MUESTREO : 06/08/2.021
FECHA DE INGRESO : 06/08/2.021
FECHA DE SALIDA : 14/08/2.021

RESULTADOS DE ANALISIS QUIMICO DE FERTILIZANTES

		%		
Nº LAB	IDENTIFIC.	NITROGENO	POTASIO	M.ORGANICA
4022	RAA	1.29	4.10	30.55

BIOQ. MARTHA MOREIRA
JEFE DE LABORTORIO

NEMALAB
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NOMBRE DEL CLIENTE :	GOOTMAN JADAN IVAN	Nº DE DOCUMENTO:	4023
REMITE .	SR. IVAN GOOTMAN J.	FECHA DE MUESTREO :	16/08/2.021
PROPIEDAD :	TESIS DE GRADO	FECHA DE INGRESO :	16/08/2.021
LOCALIZACIÓN:	MACHALA - EL ORO	FECHA DE SALIDA :	27/08/2.021

RESULTADOS DE ANALISIS QUIMICO DE FERTILIZANTES

		%		
Nº LAB	IDENTIFIC.	NITROGENO	POTASIO	M.ORGANICA
4023	R 1	0.53	3.40	29.03

BIOQ.MARTHA MOREIRA
JEFE DE LABORTORIO

NEMALAB
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APPENDIX SECTION C (SC). Technical report of microorganisms in Bio-ferment (EM) from "FINCAS DE EL ORO" banana association.

SC 1. Technical report about DNA analysis of Bio-ferment (EM)

Programa de Agricultura Orgánica y Convencional en el cultivo de Banano.

Proyecto PIP/CLAC/FLO.

Informe sobre el análisis de ADN / Biología Molecular. 17 / 05 / 2016.

Asociación Fincas del Oro, Macha, Ecuador.

Por: Ing. W. Herrera B. y MBA Y. Gonzales A.

Los resultados analíticos de las 8 muestras analizadas por el Laboratorio LAMA, de los diferentes Biofermentos de la Biofabrica de la Asociación de las Fincas del Oro de Machala, Ecuador se presentan en el cuadro siguiente:

Identificación de la muestra	1- M - Foliar	2- M - Suelo	3- M - M activ
Código Laboratorio	F758	F759	F760
Microorganismo			
Hongos	209374	110923	387432
Bacterias totales	187322	32432	121322
α-Proteobacteria	72	293	285
β-Proteobacteria	293	285	278
Firmicutes	176	87	534
Actinomicetes	298	534	543
Streptomyces sp.	126	1299	845
Bacillus sp.	23	37	5
Bacterias fototrópicas	231	256	432
Desnitrificantes	645	76	32
Fijadores de nitrógeno	435	254	65
Oxidadores de amonio	523	387	232
Algas verdes y protistas	2	42	5
Azospirillum sp.	32	66	76
Bacillus subtilis	123	231	432
Bacillus thuringiensis	321	123	432
Bacillus pumilus	154	243	554
Burkholderia cepacia	21	54	37
Nitrosomonas multiformis	42	22	33
Pseudomonas fluorescens	23	44	65
Streptomyces antibioticus	33	36	45

Streptomyces aureus	423	655	0
Streptomyces globus	312	64	76
Aspergillus penicilloides	0	0	0
Mucor sp	321	254	654
Paecilomyces lilacinus	325	121	543
Trichoderma spp.	435	523	765
PATOGENOS			
Agrobacterium tumefaciens	0	0	0
Erwinia sp.	0	0	0
Pseudomonas agarici	0	0	0
Pseudomonas apii	0	0	0
Pseudomonas corrugata	0	0	0
Pseudomonas delphinii	0	0	0
Pseudomonas maculicola	0	0	0
Pseudomonas syringae	0	0	0
Pseudomonas tagetis	0	0	0
Xanthomonas sp.	0	0	0
Ralstonia spp.	0	0	0
Armillariella mellea	0	0	0
Ceratocystis sp.	0	0	0
Claviceps sp.	0	0	0
Cylindrocarpon sp.	0	0	0
Dematophora sp.	0	0	0
Fusarium oxysporium sp.	**112**	**350**	**278**
Macrophomina phaseolina	0	0	0
Nectria nauritiicola	0	0	0
Phytophthora sp.	0	0	0
Plasmodiophora sp.	0	0	0
Pythium sp.	0	0	0
Rhizoctonia sp.	0	0	0
Thielaviopsis sp.	0	0	0
All Nematodes	0	0	0
Globodera sp.	0	0	0
Heterodera sp.	0	0	0
Meloidogyne sp.	0	0	0
Meloidogyne exigua	0	0	0
Meloidogyne hapla	0	0	0
Meloidogyne incognita	0	0	0
Meloidogyne javanica	0	0	0
Meloidogyne arenaria	0	0	0
Meloidogyne arabicida	0	0	0
Pratylenchus sp.	0	0	0
Radopholus similis	0	0	0

Rotylenchus sp.	0	0	0
Xiphinema sp.	0	0	0

Para interpretación de patógenos

pg ADN/ml	
Bajo	*0-50*
Medio	*50-300*
Alto	*>300*

Resultados de la diversidad y riqueza microbiológica

Identificación de la muestra	Totales		
	Indice de Shannon	No. de especies	Indice de Shannon
1	2,77	16	3,23
2	1,79	6	3,40
3	1,61	5	4,03
4	2,20	9	2,08
5	1,95	7	2,71
6	1,39	4	1,10
7	2,20	9	1,79
8	1,39	4	1,61

Rangos índice de Shannon:

Bajo	0-1,8
Medio	1,8-2,8
Alto	>2,8

Interpretación:

En general en las diferentes muestras analizadas los **Microorganismos Benéficos** se presentan en forma diversa y fluctúan en un rango de **bajo a alto**, y los Patogénicos solo un microorganismos fue detectado el cual fluctúa en un rango bajo a medio, por tanto se deduce que estos resultados son prácticamente inocuos ya que un solo patógeno no puede ejercer daño alguno ante un medio con una gran cantidad de Microorganismos altamente supresores dada su diversidad.

Gráficos sobre la dinámica poblacional:

Con la finalidad de observar en forma más clara las poblaciones de Microorganismos Benéficos vrs los Patogénicos en las diferentes muestras en estudio, se presenta el

siguiente gráfico que permite apreciar la alta carga supresora de los Benéficos contra los Patogénicos.

No obstante se debe trabajar más en la calidad de estos Bioproductos para lograr valores más altos de todos los M. Benéficos en cada Bio producto elaborado.

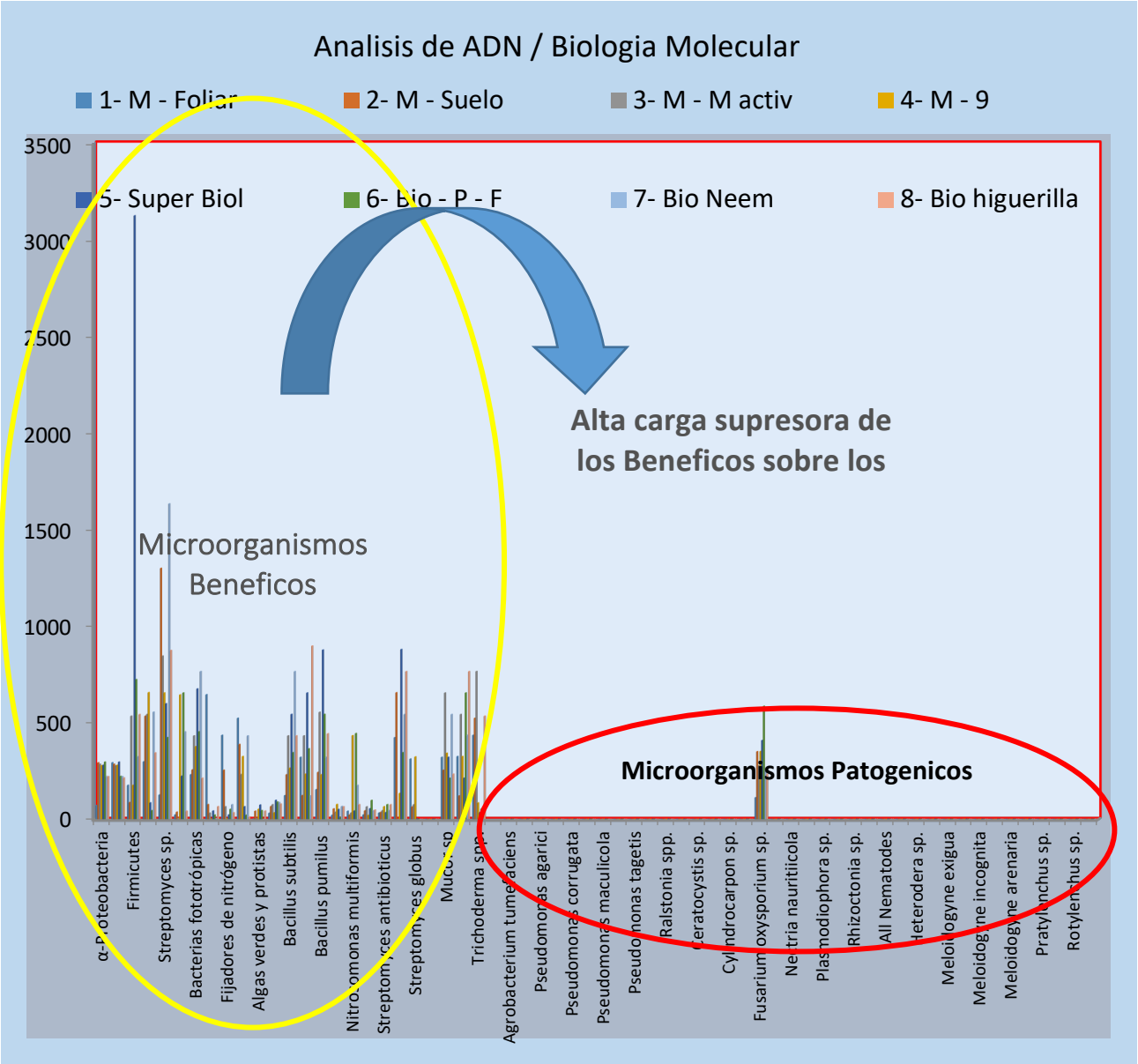


Grafico sobre la caracterización del Filo plano – establecimiento de la línea base.

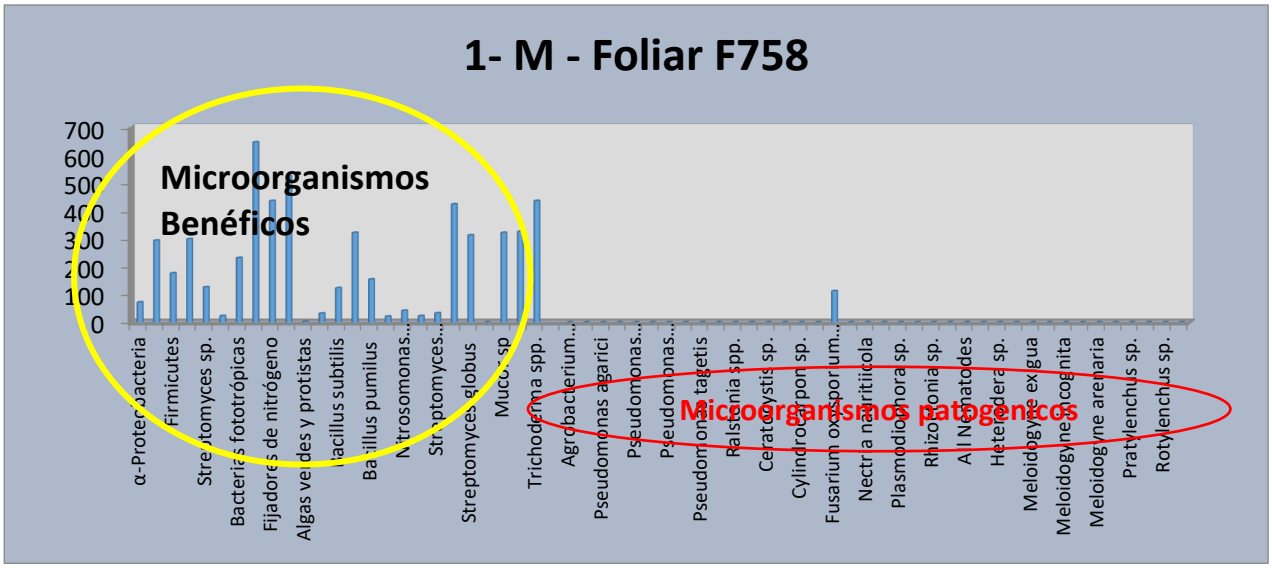
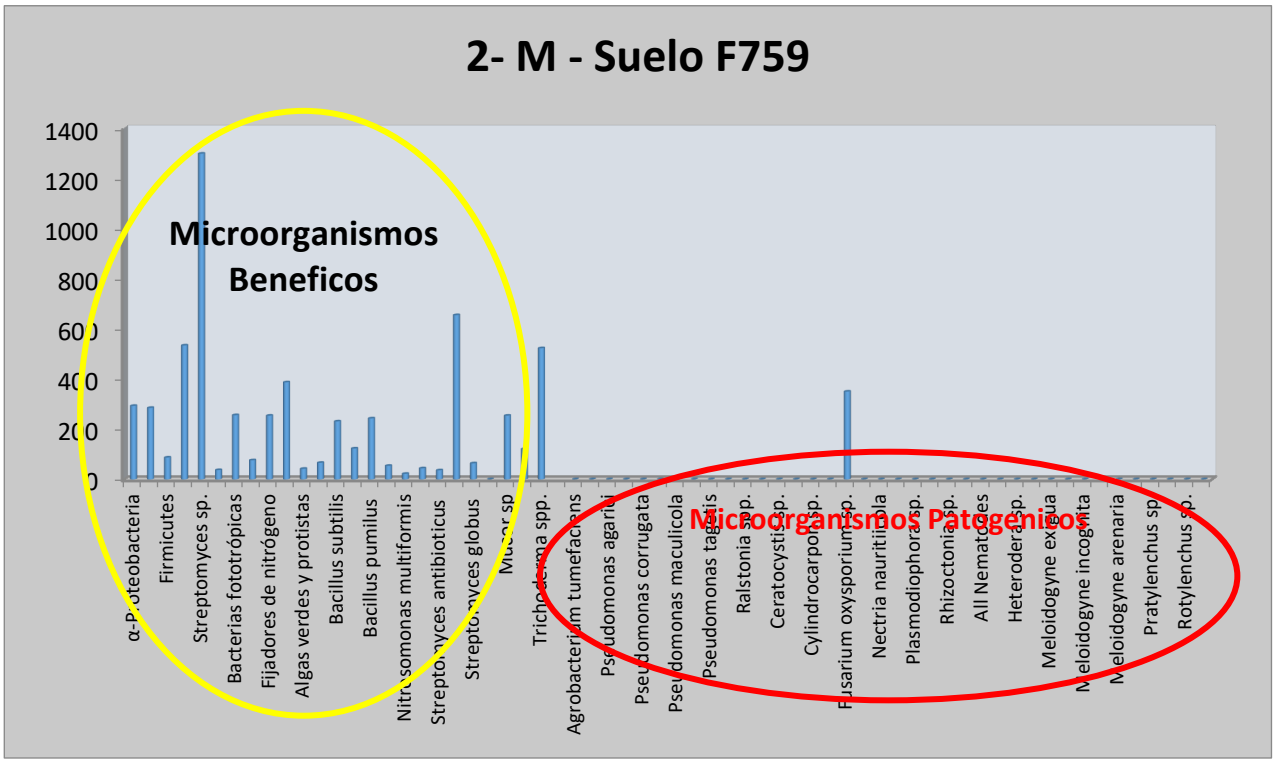


Grafico sobre la caracterización de la Salud del suelo – establecimiento de la línea base.



APPENDIX SECTION D (SD). Photographs of the methodology applied.

SD 1. Feedstock collection

SD 2. Azolla reproduction experiment

SD 3. Organic transformation according to Ecuadorian wastes

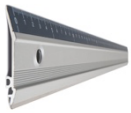
SD 4. Azolla bokashi experiment

SD 5. Safety normative

In the experiments were considered industrial safety in the handling of microorganisms; thus, protective costume, face mask, gloves, were considered during the measurements and even more in the preparation of all bokashi mixtures.

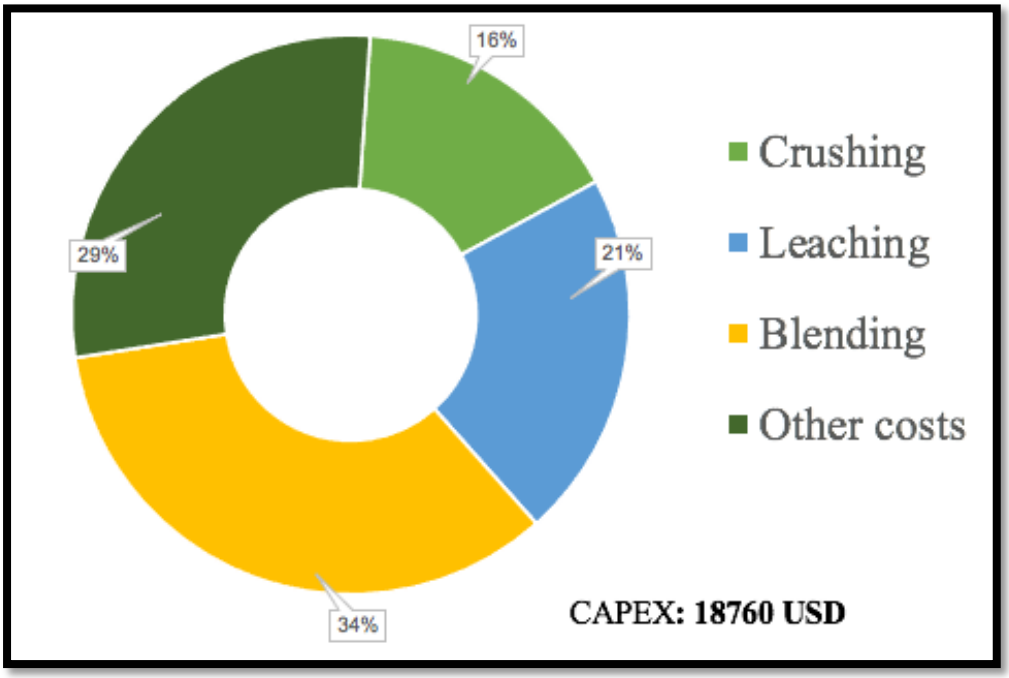
APPENDIX SECTION E (SE). Measurement tools.

SE 1. Feedstock collection.

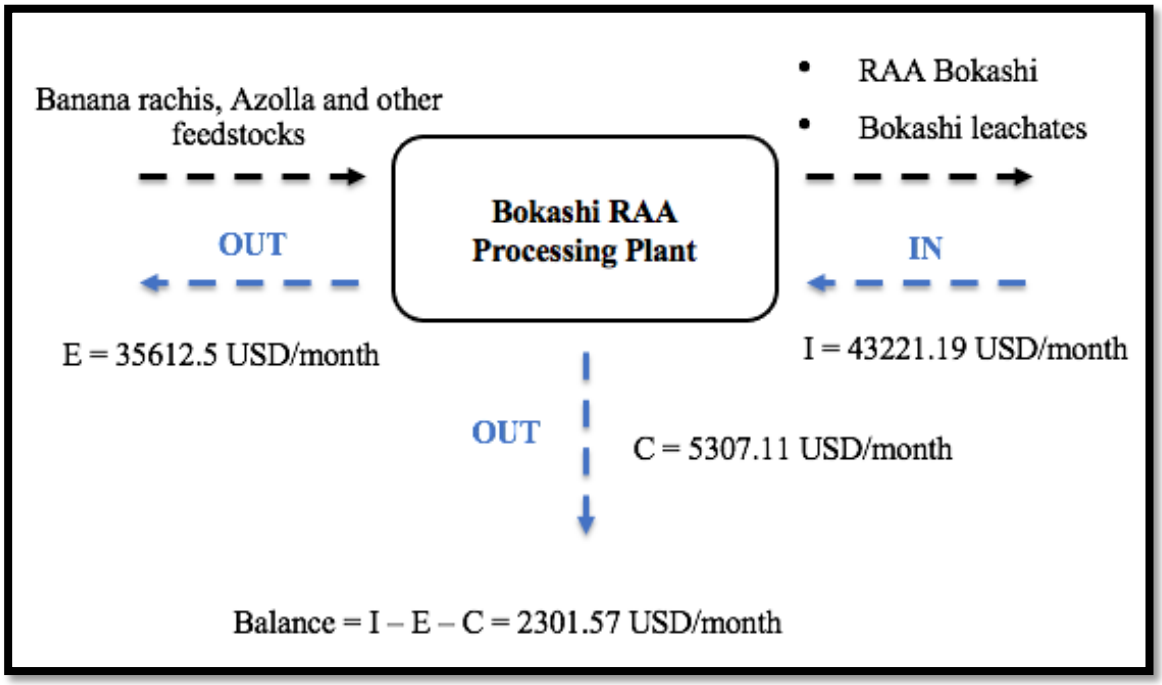
Equipment		Description
Thermometer		A mercury thermometer with a calibration and accuracy of 2 ºC with a scale of -20ºC to 300ºC.
Soil pH and Moisture Meter		Manufactured and distributed by NAVELAGRO ACP. It is a soil pH and moisture meter with a range of pH: 3-8 and Moisture: (10%-80%), in an operation temperature of 5-50ºC
Metal Ruler		Metal ruler with a graduation of 1mm, 1cm.
Electronic Kitchen Scale		CAMRY electronic kitchen scale of high precision Model: EK5055, has a maximum capacity of 5Kg, graduation of 1g.

APPENDIX SECTION F (SF). Economic Feasibility.

SF 1. Capital expenditure for bokashi production



SF 2. Bokashi RAA processing plant.



APPENDIX SECTION G (SG). General block diagram.

SG 1. Block diagram of the organic fertilizers production.