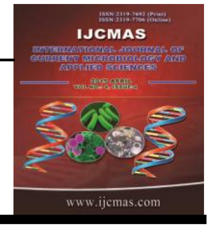

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Original Research Article

Enhanced BM Inoculant Using Bio carrier for Bioremediation

Gloria Y. Ponciano*

Tarlac College of Agriculture Institute of Agriculture and Forestry Malacampa, Camiling, Tarlac Philippines
**Corresponding author*

A B S T R A C T

Keywords

Bacteria-matrix
BM Inoculant,
Bio carrier,
Bioremediation

The study aimed to enhance the BM Inoculant through the use of a natural carrier which can act synergistically together to produce useful compounds for agricultural and bioremediation applications over extended period of time. Results showed that immobilized BM inoculants coated with zeolite and grown anaerobically for 2 weeks period had higher viable microbial cells. The immobilized BM inoculants grown aerobically showed a greater than 1 log loss in population. The anerobically processed Immobilized BM inoculant recorded a very high Ammonia-N degradation from 50.0 mg/kg to 6.0 mg/kg during the 1 to 2 weeks treatment period. The aerobic type Immobilized BM inoculant also exhibited Ammonia- N reduction of 66.33mg/kg during the 1 week period of treatment. The untreated pig manure showed a very high Ammonia-N concentration of 482.50 mg/kg as compared to the other treatments Results showed that immobilized BM inoculant with natural zeolite as biological carriers are very effective in reducing the ammonium- N concentration when applied in pig manure.The composted pig manure was found to be highly enriched with nitrogen, phosphorus and potassium based from the result of the analysis on the NPK components of the pig manure treated anerobically with immobilized BM inoculants.

Introduction

Bioremediation is the use of microorganisms to remove contaminants from soil, water and wastes. Indigenous microbes commonly degrade pollutants but also produce persistent degradation products in the environment through incomplete metabolism or transformation (Stelting,S ,et al, 2009). Hence, storage and delivery of these bio products are important factors in determining their effectiveness.

The encapsulation of microorganisms into a polymer matrix is still experimental in the field of bacterial-inoculation technology. At present there is no commercial bacterial product using this technology. The concept underlying immobilized microbial cells, is to entrap beneficial microorganisms into a matrix.

The formulation (bacteria-matrix) is then fermented in a bacterial growth medium. These formulations can produce many useful compounds for industrial and environmental applications (such as organic acids, amino acids, enzymes) and biodegrade toxic materials (bioremedation) over extended periods of time.

Immobilized microbial cells are easy to produce, store, and handle during industrial operations. The main goal of these formulations is to maintain the cells entrapped in an active form for as long as possible. Any premature release of the microorganisms from these encapsulated forms is undesirable. Encapsulated bacterial formulations in agriculture have at least two distinctly different goals from those of the fermentation industry: (i) to temporarily protect the encapsulated microorganisms from the soil environment and microbial competition, and (ii) to release them gradually for the colonization of plant roots (Bashan, 1986a; Bashan and Carrillo, 1996; Digat, 1991

The control of animal manure odours is one of the major headaches in raising farm animals. The main culprit for the bad smell is the ammonia released by the manure. Zeolite has been used in the Far East most notably in China for thousands of years for medicinal purposes. Over the years researchers have found out that Zeolites absorb ammonia and other toxins which can be applied in animal husbandry.

Immobilization of BM Inoculant has been formulated using zeolite as biological carriers. Natural zeolite has been shown as a promising material for the immobilization due to its high porosity and large surface area (Shindo et al, 201). The extent of bacterial colonization depends on the chemical properties and particle size of zeolite.

The Tarlac College of Agriculture in full support to Organic Agriculture Act 2010 is committed in promoting organic agriculture in Tarlac through its research, extension and training programs. The College has pioneered organic technologies which have been disseminated to farmers and other clientelles which contributed to farm productivity and livelihood generation.

Nowadays, organic- based agricultural production is a rapidly emerging technology in the Philippines which partly solves waste disposal problem. Driven by the desire to preserve the living soil and the environment as a whole, TCA has developed organic zeolite-based technologies for agricultural, and environmental applications.

The Tarlac College of Agriculture has been promoting the use of microbial inoculants and vermin in the production of organic fertilizers for crop production in the province of Tarlac. It can be said that the College has extended the technology to the different municipalities. This has also contributed to the increased in yield and provided additional income for the farmers. Massive waste management information campaign was launched in every barangays. Waste segregation and composting was done using the technologies.TCA has made contributions in the proliferation of information and products for environment-friendly agriculture while ensuring enhancement of farm income for the farmers

Science branded zeolite as a mineral of the 21st century, it used practically in all spheres of human activity. More than 150 types of zeolite have been synthesized. There are 48 known naturally occurring zeolites. They are basically a hydrated alumino-silicate minerals with an "open" structure which can accommodate a wide variety of positive ions, like Na^+, K^+, Ca^{2+}, Mg^{2+} and others.

The shift from the use of synthetics to organic zeolite-based products is an important step in balanced and selfregulating agricultural system. Organic-based crop and vegetables production, feed additives and bio disinfectants provide opportunities for producing high quality food which support the Natural Tarlac program of the province. Likewise, help farmers and livestock and poultry growers to increase their income and improve farm productivity. Likewise, the technologies can contribute to a clean and safer environment.

This study aims to enhance the BM Inoculant through the use of a natural carrier which can act synergistically together to produce useful compounds for agricultural and bioremediation applications over extended period of time.

To determine the capacity of natural zeolite as a biological carrier impregnated with BM inoculant. To evaluate the viability of the immobilized BM inoculants using zeolite as biological carrier. To determine the NPK composition of the enhanced BM inoculants with zeolite. To provide a metabolically active biological agent for bioremediation.

Materials and Methods

Microbial cultures

Beneficial Microorganism (BM) is a mixed culture of beneficial microorganisms that can be applied as inoculants. This was formulated by the Tarlac College of Agriculture with many applications such as compost, odor eraser and bio pesticide. The inoculants consist of Active ingredients: microorganisms which includes 1 million colony forming units/cc (units/ml)... 1% Lactobacillus Saccharomyces, Actinomycetes, Bacillus subtilis, Acetobacter,Rhodopsuedomonas. Other ingredients are: 96% Water and 3% Molasses. medicinal plants and aromatic herbs.

Pre-cultures were prepared by re suspending a culture stock microcentrifuge tube using a universal culture media and returning the entire contents to the vial. Vials were incubated at 30°C and 200 rpm. Pre-culture vials were harvested after 1h and 500 mL flasks and were inoculated with 1 mL (1% v/v) of pre-culture. Cells from flasks were enumerated after 24 h growth on a shaker (200 rpm) at 30°C.

Immobilization and stability assessment of BM Inoculant

Mixed culture of BM inoculants was coated onto zeolite. Samples of culture were immobilized with and onto zeolite and stored at ordinary room temperature in open and closed containers.

Determination of the number of viable cells in a culture by plating methods

Serial dilution of the broth culture was made. Based on the total count, the number of viable cells were about 1.0 x 10^8cels/ml of each microorganisms. To achieve this concentration, 8 tubes each were set out, which contained 9 ml of sterile diluents (pH 6.8). About 1 ml of broth culture was diluted in steps, tenfold each time (10^1through10^8).

Fresh pipette should be used for each strain and for each dilution in the series. Highest dilution in the series should be the starting point with the aid of the suction bulb, fill and empty the pipette by sucking in and out 5 times with diluted culture and then transfer 1ml aseptically to sterile petri dish of respective medium. The pipette should be opened sufficiently to allow the pipette to enter and deliver the sample. The pipette should be flamed by passing through the Bunsen burner flamed each time prior to successive removal of aliquots for replication (2 per dilution)from the same tube. About 1 ml aliquots should be removed with the same pipette in duplicated from the 10^7 and 10^6 dilutions into more petri dishes(Chandra C., 2009)

Plates were incubated at 30°C for 24 h prior to counting. The microbial cell count was done using a high power objective microscope and a hemocytometer.

Analysis of the Nitrogen-Phosphorus-Potassium (N-P-K) Content

The samples were analyzed for their nitrogen, phosphorus and potassium contents.

Ammonia- N degradation

Fresh manure was treated with powdered immobilized BM inoculants with zeolite. These were allowed to decompose for 1 to two weeks. The compost was analyzed for Ammonia- N.

Analysis of the NPK components of the Treated Manure

The NPK components of the treated manure was also analyzed

Results and Discussion

Immobilization and stability assessment BM inoculants into zeolite

Immobilized bacteria using zeolite as a biological carrier were processed aerobically and anaerobically at 35°C. Bacteria were extracted and enumerated for a 3 weeks period. Beneficial Microorganism adsorbed into zeolite and incubated at 35°C anerobically survived in higher numbers compared to aerobically grown samples. Poor survival of beneficial microorganisms immobilized into zeolite aerobically and is shown by a greater than 1 log loss in cfu/ml after 3 weeks. Results of the experiments conducted by Swaminathan (2008) showed that in a longer-term experiment zeolite was again found to influence the stability of *Pseudomonas* sp.strain ADP in closed containers, with at least 10 weeks survival and less than 1 log loss in population .

It is shown that naturally occurring zeolite enhanced absorptive growth in the encapsulated microorganisms and were able to achieve high cell density and longer viability. The extent of bacterial colonization can be attributed to thehigh porosity and large surface area of the zeolite as shown by several conducted studies.

Nitrogen-Phosphorus-Potassium (N-P-K) Contents of immobilized BM

Table 1 shows immobilized BM treated anerobically showed higher nitrogen, phosphorus and potassium contents. Based from the researches conducted, zeolite has been used extensively as soil amendment for high value crops such as vegetables, fruits, potatoes etc. Zeolite can hold fluids and plant nutrients. It can absorb up to 55% of its weight in water or other liquids. Water, ammonium(nitrogen), and other plant nutrients that are held and are water soluble. Zeolite reduces the amount of water needed for irrigation by up to 35%. Zeolite can hold cations such as ammonium (nitrogen), potassium, calcium and other plant nutrients within the mineral lattice, where it is not water- soluble. However, the cations and plant nutrients are plant accessible on a plant demand basis. The zeolite will hold nitrogen fertilizers in the growth zone where they are plant accessible. Typically, 30 to 50% of the conventional nitrogen fertilizers leach directly to the water table where they cause pollution Also, when composted with manure, it becomes a natural fertilizer.

Ammonia- N degradation

Table 1 shows the result of the ammonium-N concentrations on the pig manure treated with immobilized BM inoculant during a 1 to 2 weeks period of treatment. Untreated pig manure exhibited a very high ammonia-N concentration. The anerobically processed Immobilized BM inoculant recorded a very high Ammonia-N degradation from 50.0 mg/kg to 6.0 mg/kg during the 1 to 2 weeks treatment period.

The aerobic type Immobilized BM inoculant also exhibited Ammonia- N reduction of 66.33mg/kg during the 1 week period of treatment. The untreated pig manure showed a very high Ammonia-N concentration of 482.50 mg/kg as compared to the other treatments Results showed that immobilized BM inoculant with natural zeolite as biological carriers are very effective in reducing the ammonium- N concentration when applied in pig manure.

The most important property of zeolites is the removal of ammonia (NH_3) and ammonium (NH_4^+).Zeolites remove ammonium ions by means of ion-exchange and, at higher concentration, adsorption. The ammonium ions present in pig manure are exchanged for sodium ions.

The dynamic capacity of zeolites for ammonium is about 0.9 meq/g. If there are a number of different cations present in the wastewater, the adsorption capacity per ion will be lower as a consequence of competition between the different cations. The adsorption will depend on relative selectivity of zeolites for the different ions, the composition of water and the temperature. The relative selectivity is determined by the hydrated diameter, the charge and the mobility of the ions. (lennetech, 2011).

NPK and Ammonia- N concentrations of Immobilized BM inoculants (anaerobic) 2 weeks treatment

The composted pig manure was found to be highly enriched with nitrogen, phosphorus and potassium based from the result of the analysis on the NPK components of the pig manure treated anerobically with immobilized BM inoculant Also, there was a high ammonium degradation after 2 weeks of treatment The synergistic effects of BM inoculant and Zeolite was responsible for the degradation of ammonia. This result was confirmed based from the findings of Bernal et al(1993) who composted anerobically swine manure using zeolite within 3 days and significantly reduced airborne noxious odor. Also the compost qualified as organic fertilizer for use in crop production.

The immobilization of BM Inoculants have been conducted to provide a dependable source of beneficial microorganisms that can survive inthe soil and become available to the plant and also to provide an active biological agent in bioremediation. Semiarid conditions, make survival difficult for the introduced bacteria due to harsh conditions including frequent droughts, lack of sufficient irrigation, high salinity and soil erosion which may quickly diminish the population of any microorganisms. However, in this type of agriculture, immobilized beneficial microorganisms may make the greatest contribution, being cheap and easy to use with high water-holding capacity, ability to deliver the right number of viable cells and containing effective microbial strain. Based from the result of the study, the cells are still viable within the 2 weeks period. Other works conducted showed that immobilized microorganisms have sufficient shelf life for about one or two years at room temperature.

In immobilized inoculants, bacterial multiplication continues during the storage period, as long as sufficient nutrients, moisture , and the correct temperature were maintained. Formulation is *the* crucial issue for inoculants containing an effective bacterial strain and can determine the success or failure of a biological agent. The encapsulation of microorganisms into a polymer matrix is still experimental in the field of bacterial-inoculation technology. At present there is no commercial bacterial product using this technology. However, *a major constraint* for the inoculation industry is that polymers are expensive while Immobilized microbial cells are easy to produce, store, and handle. To the best of our knowledge, almost none have been tested in soil or in the field.

Zeolite is a good carrier or delivery vehicle of live microorganisms. They have the capacity to deliver the right number of viable cells. Zeolites have high water holding capacity suitable for as many bacterial species and strains as possible. Considering the short time period of application in pig manure, the removal of the ammonia-N was remarkable. However, after 14 days of treatment, the ammonia level was greatly reduced from 50mg/kg to 6.0 mg/kg. Further to this the zeolite acts as a buffer for ammonium ions; in case of a large ammonia production, part of the ammonia is adsorbed by zeolite and if the ammonia concentration is low, the bacteria metabolize part of the adsorbed ammonium. This was indeed a breakthrough in bioremediation.

It was clearly shown that the pig manure applied with Immobilized BM inoculant with zeolite as carrier was found to be highly enriched with nitrogen, potassium and phosphorus. This qualifies the treated manure as a quality organic fertilizer.
Future researches should be conducted to improve the formulation on enhanced BM inoculants using zeolite that will consistently provide higher bacterial numbers under field conditions, extended shelf life, protection against the soil environment, convenience of use and cost effective production.

More studies should be done on the formulation process especiallybacterial survival as affected by several variables: the culture mediumused for bacterial cultivation, the physiological state of the bacteria when harvested from the medium, the process of cell encapsulation, the use of protective materials, the type of drying technology used, and the rate of dehydration.

Conduct on-site techno demo on the effectivity of the products in the biodegradation of Ammonia-N to poultry and piggery. Conduct longer period of application to totally eradicate the ammonium N concentration. Undertake researches on other possible application of the formulated products.

Table.1 Nitrogen-Phosphorus-Potassium (N-P-K) Contents of immobilized BM (in %)

TREATMENTS	Nitrogen	Phosphorus	Potassium
Anerobically processed Immobilized BM	1.51	.84	1.04
Aerobically processed Immobilized BM	1.09	-	0.39

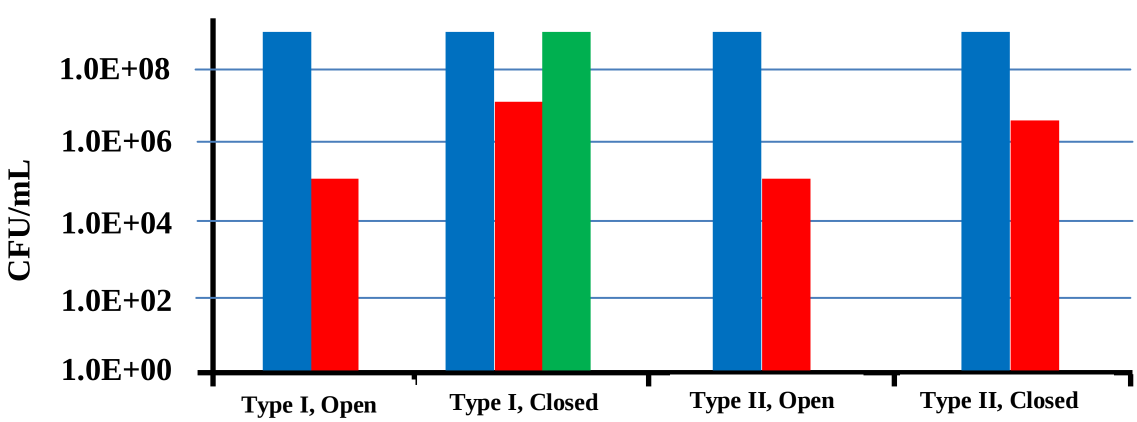
Table.2 Ammonia-N concentrations (mg/kg) of untreated and treated pig manure during a period of 1 week

Treatments	Mean
Control- untreated	482.50
Applied with Immobilized BM (anaerobic)	50.00
Applied with Immobilized BM (aerobic)	66.33

Table.3 Results of Analysis of Immobilized BM inoculants (anaerobic) 2 weeks after treatment

UNITS (mg/kg)	MDLs	
Total Nitrogen	1,440	140
Total Phosphorus	46,900	1250
Total Potassium	4,150	20
Ammonia- N	**247**	**6.0**

Figure.1 Stability and viable cell counts of Immobilized BM inoculants



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