BIOFERTILIZERS AND COMPOSTING ACCELERATORS OF POLLUTING MACROPHYTES OF A COLOMBIAN LAKE

P. Martínez-Nieto[1]* J. Bernal-Castillo[2], M. Calixto-Díaz[2], M.A. Del Basto-Riaño[2], and B. Chaparro-Rico[1]

[1]Corporación Autónoma Regional de Cundinamarca. Bogotá, Colombia, (Cundinamarca - Colombia). [2]Facultad de Ciencias, Pontificia Universidad Javeriana
Carrera 7 No. 43-82, Bogotá, Colombia.
*Corresponding autor: patingli@yahoo.com

ABSTRACT

Composting of the macrophytes *Eichhornia crassipes* (water hyacinth) and *Egeria densa* (Brazilian elodea) has been proposed as a final disposal, following their mechanical removal in the lake Fúquene (Ubaté, Cundinamarca). Microorganisms, isolated and selected by antagonism, were evaluated as inoculants to accelerate the composting and to stimulate plant growth. In microbial biopreparations of bacteria, actinomycetes and fungi, maximum amylolytic activities were found of 2,422 U L^{-1}, 1,744 U L^{-1} and 1,426 U L^{-1}, respectively; cellulolytic activities of 233.2 U L^{-1}, 668 U L^{-1} and 701.4 U L^{-1} and proteolytic activities of 660 U mg^{-1}, 520 U mg^{-1} and 400 U mg^{-1}. In test of these biopreparations in windrow composting of aquatic macrophytes waste, the best result was obtained with a concentration of 2%. The assay conducted on seedlings of radish (*Raphanus sativus* L) under greenhouse conditions (P <0.05), using the following treatments: microbial inoculants, compost inoculated to 2%, pure or mixed with soil (1:1), peat 50% and chicken manure at 33% mixed with soil, showed that actinomycetes stimulated plant growth significantly, as did chicken manure and bacteria. Microbial inoculants role for growth, development and nutrients assimilation in radish, was evident, and also for accelerating polluting macrophytes composting process.

Keywords: Composting, microbial inoculants, biomanure, biofertilizers, submerged fermentation.

INTRODUCTION

Lake Fúquene (Cundinamarca, Colombia), has massive proliferation of aquatic plants especially water hyacinth (*Eichhornia crassipes*) and Brazilian Elodea (*Egeria densa*). This, coupled with other factors, has contributed to eutrophication, desiccation and progressive deterioration of the environmental conditions of the lake ecosystem (Andrade and Franco, 2007). As a control and useful technique, the macrophytes is harvested by mechanical means and subjected to composting (Quintero and Otero, 2006; Consejo Nacional de Política Económica y Social, República de Colombia, 2006). In 1999, the Japan International Cooperation Agency (JICA) and the Corporación

Autónoma Regional de Cundinamarca (CAR), obtained compost from Brazilian elodea, water hyacinth and rushes, at temperature not exceeding 37 °C; in 2004, elodea and water hyacinth wastes, were degraded more effectively by inoculating mixtures of microorganisms (Martínez-Nieto, 2004).

The use of bacteria, actinomycetes and fungi in the decomposition of organic matter produces good results by taking advantage of their enzymatic activities, favoring the elimination of organic waste and providing beneficial metabolic products to the soil (Tiquia *et al*., 2002; Singh and Sharma, 2003). The enzymatic activities of these microorganisms play an important role in the degradation of complex substrates such as lignin and cellulose (Nakamura *et al*., 2001; Tiquia *et al*., 2002), which facilitates degradation of aquatic macrophytes in the composting process.

In the research reported here, microbial inoculants were prepared from microorganisms isolated from composting processes of macrophytes, water hyacinth and Brazilian elodea, in order to evaluate their enzymatic activity, the role in accelerating the degradation process and action as a plant growth promoting.

MATERIALS AND METHODS

Microorganisms

The used bacterial strains belong to the species *Comamonas acidovorans*, *Sphingomonas paucimobilis*, *Stenotrophomonas maltophilia*, *Bacillus licheniformis*, *Pseudomonas* sp, *B. mycoides and Providencia* sp. The Fungal were *Syncephalastrum* sp., *Aspergillus fumigatus*, *A. terreus*, *A. flavus*, *A. clavatus*, *Aspergillus* sp., *Rhizopus* sp., y *Absidia corymbifera*. The Actinomycetes fit in *Actinomadura* sp., *Streptomyces avermistitis, Streptomyces* sp., *Nocardiopsis* sp., *Nocardiodes* sp., *Kineosporia* sp., *Pseudonocardia thermophila*, *Actinobispora* sp., and *Dactylosporangium vinareum* (Figures 1a, b y c). These organisms were isolated in a process of composting mixture of water hyacinth, Brazilian elodea, bean pods and manure at rates that depended on each treatment (Martínez-Nieto, 2004) and chosen for the production of inoculants by antagonism techniques and qualitative enzymatic assays in starch agar, cellulose agar and skim-milk agar (Eslava and García, 2001; Rodríguez *et al*., 2002; Martínez-Nieto, 2004; Chen-Chin *et al*., 2009; Orji *et al*., 2009).

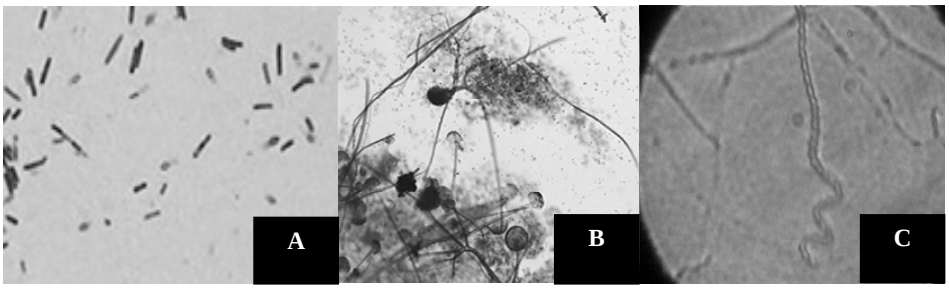

Figure 1. Microscopic features of *Bacillus licheniformis* (A), *Absidia corymbifera* (B) and *Streptomyces* sp. (C).

Microbial inoculants production by Submerged Fermentation

The bacterial fermentation process was carried out in a 14 L bioreactor (designed by a Colombian company, Procesos Biotecnológicos) to produce 5000 mL of inoculant in liquid phase at 32 °C, 1 vvm of airflow rate and 120 rpm. Fermentation of fungi and actinomycetes was performed by duplicate, in bioreactors for 5 days at 28 °C and 150 rpm, with air supplied by an aquarium air pump. In each process, substrate consumption was determined by 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959), biomass formation by plate count and measurement of pH values.

Quantitative Enzymatic Activity

Batch fermentation were carried in 500 mL Erlenmeyer flasks, containing 360 mL of soluble starch (1% w/v) milk (1% v/v) or cellulose (1% w/v), with 40 mL of inoculum and 150 rpm agitation during 52 h for bacteria, and 96 h for fungi and actinomycetes. Samples were taken during fermentation and inoculated on skim-milk agar, starch agar or cellulose agar for dilution plate counts; concentrations of reducing sugars were determined using the DNS method and protein content by Bradford assay (Bradford, 1976). One unit (U) of α-amylase activity was defined as the amount of enzyme required to release one micromole of reducing sugar, expressed as glucose per minute under the assay conditions (Orji *et al*., 2009).

The protease activity was calculated on the proteolysis unit, equivalent to a halo of hydrolysis equal to 5 mm in diameter, and relating this value to the extracellular protein content, reporting to proteolysis specific activity as units per milligram of protein (Bradford, 1976). One unit of Carboxymethyl cellulase activity was defined as the amount of enzyme releasing one micromole of reducing sugar, as glucose, from the substrate (carboxymethyl cellulose) per minute under standard assay conditions (Chen-Chin *et al*., 2009).

Water hyacinth and Brazilian elodea Composting

The composting process was carried out in a farm located at the town of Nemocón (Cundinamarca, Colombia). Ten piles were mounted (5 treatments and their repetitions) of 82 kg each, made up of a mixing of elodea (21 kg), water hyacinth (42 kg), husk of rice (2 kg), pea and bean pods (9 kg), molasses (500 g), lime (500 g) and soil (7 kg). Previously, the Brazilian elodea and water hyacinth were cut to obtain a particle size close to 3 cm. Microbial inoculants were added to the first three treatments, T1, T2 and T3, and their repetition, until a final concentration of 1%, 2% and 0.5% respectively. In T4 treatments additions were of 750 mL of sterile liquid culture medium used in the preparation of the inoculants; and in the T5 controls no inoculation was made. During the composting the temperature and humidity were controlled every third day, while pH, bacteria, actinomycetes and fungi counts were weekly. At the end of the process, an evaluation was made of the Carbon/Nitrogen ratio (C/N), dry matter percentage, moisture content, reduction of organic matter, foreign matter, germination of radish seeds (Phytotoxicity test) and human pathogenic organisms content (*Escherichia coli* and *Salmonella* sp.) (Ge *et al*., 2006).

***In vivo* assay to determine the plant growth-stimulating ability**

The microbial inoculants and compost that showed the best result were tested as a vegetable growth-stimulant on radish

plant, using a completely random design, with seven treatments, two replicates and four experimental units per replicate, for a total of 56 experimental units (Table 1).

Table 1. Treatments used in the completely random design.

Treatment	Description
T1	T2 Compost
T2	T2 Compost + Soil (1:1)
T3	Soil + 5 mL bacterial inoculant
T4	Suelo + 5 mL fungi inoculant
T5	Suelo + 5 mL de actinomycetes inoculant
T6	Soil + Canadian peat (1:1)
T7	Soil + chicken manure at 33%

The vegetable parameters evaluated were aerial dry weight, foliar area, bulb weight, bulb formation percentage and total nitrogen content in the substrates and plants. Radish seedlings were obtained from seeds and transplanted, when they reached an average height of 5 cm, in plastic bags with 300 g of the substrates according to the different treatments. Five ml of microbial inoculants were added for each experimental unit, with the highest concentration obtained for each functional group in the fermentation process, according to treatment (Table 1). For controls, Canadian peat was used (supplier: Naturpaipa LTDA) mixed with soil in 1:1 ratio and soil with chicken manure 33% mixture (supplier: Homecenter Sodimac, Bogotá). The data were treated statistically by one-way analysis of variance (ANOVA) ($p < 0.05$) and Duncan's multiple-range test, to determine significant differences between the means. For the interpretation of the results, the percentages of bulb formation and nitrogen assimilation were transformed using the formula $Y=\sqrt[2]{x+0.5}$, where, Y are the transformed values, and x are the percentages data obtained in the experiment, to reduce the coefficient of variation and detect significant differences (Orozco and Thienhaus, 1997).

RESULTS AND DISCUSSION

Microbial inoculants production by Submerged Fermentation

The highest bacterial growth rate was obtained at 24 h with 2 x 10^{12} CFU mL^{-1}. Best biomass formation occurred at 80 h for actinomycetes with 3 x 10^{16} CFU mL^{-1}, and for fungi with 8 x 10^{8} CFU mL^{-1}. Obtained concentrations for bacteria and fungi were higher compared with those claimed in other studies (Eslava and García, 2001; Martínez-Nieto, 2004; Cariello *et al*., 2007) which implied bacterial counts to 10^{9} CFU mL^{-1} and fungal counts of 10^{5} CFU mL^{-1}. Actinomycetes biomass found was among the reported intervals (Eslava and García, 2001; Martínez-Nieto, 2004). Appropriate populations of bacteria, fungi and actinomycetes are important in the composting process, since a higher microbial activity favors organic matter degradation and allows decomposition of complex substrates like lignin and cellulose (Singh and Sharma, 2003).

Quantitative Enzymatic Activity

Bacteria showed maximum activity of amylase production of 2,422 U L^{-1}, a 12 h, with 4 x 10^{12} UFC mL^{-1}; actinomycetes of 1,744 U L^{-1} at 72 h with 1 x 10^{7} UFC mL^{-1} and fungi of 1,426 U L^{-1} at 80 h with

a count of 2 x 10^7 CFU mL^{-1}; these values were high compared to reported values which are between 47 U L^{-1} and 1,038 U L^{-1} (Arnedo and Parrado, 2002; Sarmiento *et al*., 2003). Higher protease production in the bacterial inoculants (660 UL^{-1}), followed by actinomycetes production (520 U L^{-1}) and fungi production (400 U L^{-1}) was observed. This activity took place at 48 h, for the first; at 104 h for second and third, with 2 x 10^{13} CFU mL^{-1}, 4x10^6 CFU mL^{-1} and 2 x 10^5 CFU mL^{-1}, respectively. In a study with thermophilic proteolytic bacteria, isolated in a composting process of flower residues, a proteolytic activity of 1,906 U mL^{-1} at 10 h of discontinuous fermentation a 70ºC was found (Rodríguez *et al*., 2002). Inoculants that showed highest cellulolytic activity were 701.4 U L^{-1} with fungi, at 104 h (4 x 10^6 CFU mL^{-1}), followed by actinomycetes with 668,1 U L^{-1} at 82 h (3 x 10^6 CFU mL^{-1}) and bacteria with 233.2 U L^{-1} at 32 h (2 x 10^{12} UFC mL^{-1}). These results demonstrate cellulolytic capacity of fungi and actinomycetes, which are renowned as good decaying agents particularly of complex substrates (Khalid *et al*., 2006; Chen-Chin *et al*., 2009). Cellulolytic activity obtained with bacterial inocula was lower, compared with amylolytic activity. However the presence of the two enzymatic systems is a competitive advantage for residue degradation; it has been found also that the bacterial cellulolytic enzymes promote amylase activity due to starch-to-glucose hydrolysis increase (Apun *et al*., 2000). Maximum value obtained with actinomycetes in this study is higher compared with Ramírez and Coha study (2003) of 330 UI L^{-1} in exoglucanases. All microbial enzymatic test counts demonstrate that the activity is associated with biomass formation, since its higher concentration occurred with maximum enzymatic activity (Rua *et al*., 1997).

Water hyacinth and Brazilian elodea composting

Treatment T2 was the one with less duration, followed by T3, T4, T5 and T1. In T2, the process was reduced 10 days compared with control T5 (Table 2). Several studies have found reduction up to half time in the composting process of municipality residues using microbial inoculants (Ichida *et al.*, 2001; Cariello *et al*., 2007). During decomposition process, T1 showed rainy water infiltration through the plastic container 15 days after experiment setting, which increased humidity to 85%. Even though turnover was done twice a week to lower water content, this event possibly affected composting duration. High humidity percentage produces low efficacy in degradation process due to the absence of a true thermophilic phase in the upper and medium layers of the pile (Luo *et al*., 2008) and by limiting oxygen content, degradation takes longer (Kroner *et al*., 2002). At the beginning of composting humidity averaged 75%, with no bad odor. At the end of the process, high humidity levels persisted (> 65%, see Table 2), which made necessary to spread all the piles and turn them over every three days, to obtain values less than 35%, according to the guideline for organic fertilizers (NTC 5167, *Instituto Colombiano de Normas Técnicas y Certificación*, ICONTEC, 2004). For composting with fiber materials, like water hyacinth, humidity ranks between 75% and 85% beginning the process are allowed (Hargreaves, 2003). The high humidity percentage and the lower-than - 1 m^3 pile size, could cause low ability to reach temperatures of 55ºC or higher during more than two days. This could be due to the incapacity at those systems to reach an adequate balance between the heat generated by microorganisms and that loss by

conduction, convection and radiation (Cornell Waste Management Institute, 2000). The pile size used in this research is intended for demonstrative or exploratory studies so it is not recommended for industrial purposes, because bulk with lower-than-2 m^2 bases exhibit sharp fluctuations. The highest temperature obtained during composting was observed in T2 with a 56°C average and the lowest value with a less than 50°C en T5, (Figure 2). T2 maintained temperatures equal or higher than 55°C during two days (Figure 2), complying with the Canadian standards for the type B compost; which requires 40°C during 5 days with an increase up to 55°C for at least 4 h (Ge *et al*., 2006). However; compared with standards of Chile, Australia and Canada for compost type AA and A, it did not comply with the required time, since these countries recommend that if the piling-with-manual turn-over systems is used, temperature must be higher or equal to 55ºC during at least 72 h, to obtain reduction of pathogens (*Comisión Nacional del Medio Ambiente*, Chile, 2000; Hogg *et al*., 2002; Ge *et al*., 2006). Despite of temperatures over 55ºC were kept only for 48 h in T2, *E. coli* was within the required ranks, and there was absence of *Salmonella* sp. (Table 2). Temperature and inoculated microorganisms, favored elimination of human pathogens, in contrast with the piles with sterile natural substrate (T4) and those non-inoculated (T5), in which *E. coli* populations were higher; besides in T5 suspicious of *Salmonella* sp., colonies occurred (Table 2). This results are in accordance with what was found in a composting process with cow manure, where *Salmonella enteritidis* an *E. coli* did not survived with increasing temperature higher than 45°C after 48 h and 72 h respectively (Lung *et al*., 2001). Ichida *et al*. (2001) observed that *E. coli* and *Salmonella* sp., found initially in poultry residues, were not detected after 4 d composting inoculated with a mixture of *B. licheniformis* and *Streptomyces* sp. Several studies indicate that pathogenic control is complex and not just the result of a thermal treatment, because in pathogen destruction from microbial

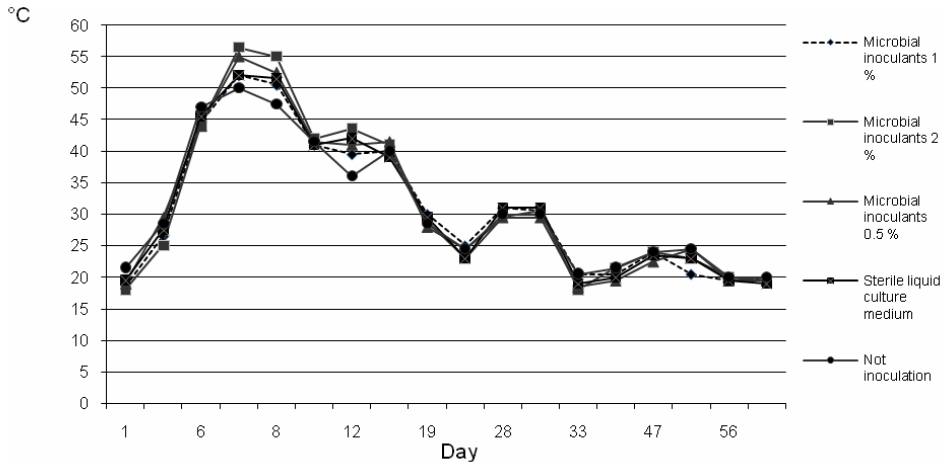

Figure 2. Temperature changes in the composting Treatments.

competence is important and avoids recolonization when temperatures fall, either by antibiotics production, antagonism or other factors (Cornell Waste Management Institute, 2004).

The final pH interval in all treatments was between 7.8 and 8.4**,** and complying with Colombian Standard NTC 5167 (ICONTEC, 2004). However, international standards have dictated the maximum pH between 7.5 and 8.0 (Hogg *et al*., 2002; Jackson and Campbell**,** 2003). Microbial biomass formation was favored by addition of microbial inoculants, as the higher final concentrations indicated in T2 and T3 in comparison with the sterile substrate and non-inoculated checks, related with fungi and actinomycetes populations (Table 2). The bacterial populations found in all the treatments were high compared with studies carried out by Nakamura et al. (2001) and Moreno and Oñate (2001), in which those authors obtained maximum count between 10^5 and 10^9 CFU g^{-1}; which demonstrates that nutritional conditions used in the process were appropriate. These microbial counts at the end of composting process were also high related to those established by BBC laboratories (2005) between 10^8 and 10^{10} UFC g^{-1} for bacteria, 10^3 and 10^5 UFC g^{-1} for fungi and between 10^5 and 10^6 CFU g^{-1} de actinomycetes. As indicated by Czaczyk *et al*. (2001), inoculation effect was observed in mesophilic bacteria count, where there was a rise in microorganism number related to the non-inoculated control.

Temperature rise beyond 50ºC in most treatment, high production of microbial biomass and lack of bad odors in this study, indicate that the prepared compound mixtures had appropriate carbon and nitrogen content, which allowed a fast use of these nutrients by the microorganisms in protein synthesis and energy obtaining for cellular division (Martínez-Nieto, 2006; Cariello *et al*., 2007; Abaunza *et al*., 2008). At the end of the process, C:N ration fell to 12:1 approximately for all treatments, but for control T5 that was 9:1; this trend was according Colombian and international quality standards, that have established ratios lower than 25:1 (Hogg *et al*., 2002; ICONTEC, 2004; Ge *et al*., 2006). For some authors, a compost to be incorporated to the soil must not have a C:N ratio below 10:1; to avoid high ammonia concentrations, which are generally phytotoxic (Bio-Logic Environmental System, 2001; Samudro and Hermana, 2007).

Parameters such as organic material reduction percentage, foreign matter, total coliformes, *Salmonella* sp. And phytotoxicity in radish, are found in the interval required in quality standard; except treatment T5, which showed two *Salmonella* sp. suspicious colonies and treatment T1, with a radish germination percentage below 90% (Table 2). Heavy metal content was not determined in this study. Previous research analyzed these elements concentrations in Water hyacinth, Brazilian elodea, bean pods and the compost from these compounds, obtaining in all cases concentrations below the allowed limit (Martínez-Nieto, 2004).

Considering all evaluated parameters and based on Colombian, Chilean, Canadian and Australian Standards (*Comisión Nacional del Medio Ambiente del Gobierno* from Chile, 2000; Hogg *et al*., 2002; ICONTEC, 2004; Ge *et al*., 2006) the treatment with best results was T2; thus, T2 was chosen for the *in vivo* assay.

Table 2. Evaluation of physicochemical and microbiological quality compost obtained in the different composting treatments.

Parameter	Unit	MI 1 %	MI 2 %	MI 0.5 %	SLCM	NI	NTC 5167
pH		7.9	7.8	7.9	8	8.4	4.0-9.0
Moisture content	%	67	65	72	72	68	< 35
Self heating	^{o} C	20	20	20	19	20	PNR
Reduction	%	61	67	68	60	69	PNR
Organic matter							
T ^{o} maxime	^{o} C	52	56	55	52	50	PNR
T ^{o} ≥55 ºC	h	0	48	24	0	0	PNR
T ^{o} 45-50 ºC	day	8	8	8	7	7	PNR
Foreign matter	%	0	0	0	0	0	Plastic, metal and rubber: <2 Glass: Absent Stone: <2
Phytotoxicity	%	83	93	97	90	93	PNR
Total Nitrogen	%	1.54	1.71	1.48	1.43	1.59	>1 %
Carbon	%	19	22	17	17	15	≥15
Carbon/nitrogen ratio		12	13	11	12	9	≤25
Organic Matter	%	34	35	27	30	32	PNR
Faecal coliform	CFU g^{-1}	6	3	2	15	156	<1000
Salmonella sp.	CFU g^{-1}	0	0	0	0	2	Undetected
Bacteria	CFU g^{-1}	1 x 10^{16}	7x 10^{15}	7 x 10^{15}	2 x 10^{14}	1 x 10^{14}	Beneficial microorganisms count report
Fungi	CFU g^{-1}	4 x 10^{6}	2 x 10^{7}	8. x 10^{6}	7 x 10^{6}	3 x 10^{6}	
Actinomycetes	CFU g^{-1}	9 x 10^{7}	4. x 10^{7}	3 x 10^{7}	1 x 10^{6}	2 x 10^{7}	
Composting period	day	70	54	62	68	64	PNR

MI: Microbial inoculants; SLCM: Sterile liquid culture médium; NI: Not inoculation Tº: Temperatura; PNR: Parameter is not required.

***In vivo* assay to determine the plant growth-stimulating ability**

Soil inoculated with actinomycetes produced radishes with higher foliar area and weight, bulb formation percentage despues si sigue and foliar nitrogen content (Table 3). On foliar area, actinomycetes and bacteria treatment, did not show significant differences related to treatments added with chicken manure and peat. The growth longitudinal of radish in actinomycetes, fungi (lower foliar area average), peat at 50% and chicken manure at 33% treatments, it is shown in figure 3. Related to foliar biomass, treatments with higher averages and with no significant differences among them were: soil plus actinomycetes, chicken manure at 33%, compost at 50%, bacteria and peat 50% (Table 3). Only in soil with actinomycetes maximum (100%) bulb formation was obtained. In other treatments percentages were lower. Average weight in bulb with chicken manure was of highest value, although with 50% bulb formation. Analysis of variance did not show significant differences among treatments related bulb formation percentage (P = 0.7288), but differences were detected on bulb weight (P = 0.0141) (Table 3). Actinomycetes treatment showed the highest nitrogen assimilation percentage and third percentage of this element loss in soil, while the mixture compost-soil showed the lowest nitrogen loss with 8.5% assimilation. Soil fertilized with chicken manure showed the highest nitrogen loss with second in assimilation (Table 4), followed by treatment with bacteria. These results give an approximation of percentage of mineralized nitrogen assimilated by plants, and are of relevance since a healthier and more fertile soil is that in which the highest percentage of mineralized nitrogen from organic matter is absorbed by plants (Orozco-Jaramillo and Martínez-Nieto, 2009).

Obtained results show that bacteria and actinomycetes used in this research play an important role in nitrogen flux and plant growth promotion. Cummings *et al*. (2006) and Orozco-Jaramillo and Martínez-Nieto (2009) observed that inoculation of nitrogen fixing bacteria, use alone or in mixtures, increased nutrient recovering by the plant, reducing the gas loss of nitrogen fertilizers in the soil. Many microorganisms used in this study are reported as nitrogen fixers, phosphate solubilizing agents and plant growth stimulators due to the production of substances excreted to the media, among which are found phytohormones, vitamins, phospholipids, antibiotics and siderophores, among others (Páez *et al*., 2005; Urrego *et al*., 2006; Orozco-Jaramillo and Martínez-Nieto, 2009; Chen-Chin *et al*., 2009).

In the treatments with compost could be observed the lower nitrogen loss with

A B C D

Figure 3. Radish plants growth in treatments with fungi (A), actinomycetes (B), Canadian peat (C) and chicken manure at 33% (D).

Table 3. Averages of different plant parameters evaluated *in vivo* radish seedlings experimental assay.

Treatment	**Foliar area (cm^2) * *P*=0,002**	**Foliar biomass (g) * *P*=0,015**	**Bulb weight (g) * *P*=0,014**	**Bulb formation (%)* *P*=0,729**	**Foliar nitrogen (g) * *P*= 0,022**	**Total nitrogen substrate (g) * *P*= 0,001**
T2 Compost	33.4 **b, c**	5.14 **b, c**	1.84 **b**	37.5	0.158 **b, c**	2.46 **a**
T2 Compost: Soil (1:1)	43 **b, c**	9.84 **a**	4.81 **b**	62.5	0.345 **a, b**	1.89 **b**
Soil + 5 mL bacterial inoculant	149.8 **a**	9.63 **a**	1.25 **b**	62.5	0.375 **a, b**	1.41 **c**
Suelo + 5 mL fungi inoculant	8.7 **c**	1.15 **c**	0.73 **b**	37.5	0.029 **c**	1.92 **b**
Suelo + 5 mL actinomycetes inoculant	153,5 **a**	14.87 **a**	2.93 **b**	100.0	0.502 **a**	1.35 **c, d**
Soil + Canadian peat (1:1)	90.1 **a, b**	6.61 **a, b, c**	2.95 **b**	62.5	0.275 **a, b**	1.92 **b**
Soil + chicken manure at 33%	150.4 **a**	12.41 **a**	10.47 **a**	50.0	0.445 **a**	1.08 **d**

The amount of substrate was 300 g according to treatment.

*The data represent averages of three replicates. Values with different letters show significant difference as determined by Duncan's Multiple Range Test ($p < 0.05$).

Table 4. Average percentages of assimilation and loss of nitrogen from *in vivo* assay.

Treatment	Foliar biomass (g)	Nitrogen (g)			Assimilation % * (P=0,009)	Retention in soil %	Loss %
		Substrate		Foliar			
		Initial	End assay				
T2Compost	5.14	5.1	2.46	0.158	3.1 **c, d**	48.1	48.8
T2 Compost: Soil (1:1)	9.84	4.1	1.89	0.345	8.5 **a, b**	46.3	45.2
Soil + 5 mL bacterial inoculant	9.63	4.5	1.41	0.375	8.3 **a, b**	31.3	60.3
Suelo + 5 mL fungi inoculant	1.15	4.5	1.92	0.029	0.6 **d**	42.7	56.7
Suelo + 5 mL actinomycetes inoculant	14.87	4.5	1.35	0.502	11.2 **a**	30.0	58.8
Soil + Canadian peat (1:1)	6.61	4.6	1.92	0.275	6.0 **b, c**	41.8	52.2
Soil + chicken manure at 33%	12.41	4.5	1.08	0.445	9.9 **a**	24.0	66.1

The amount of substrate was 300 g according to treatment

*The data represent averages of three replicates. Values with different letters show significant difference as determined by Duncan's Multiple Range Test ($p < 0.05$).

variable assimilation. In the case of plants that grew with compost at 50%, the assimilation was good and there were not significant differences with treatments: actinomycetes, chicken manure and bacteria; in contrast for 100% compost treatment, plants assimilated a low percentage of present-in-substrate nitrogen (Table 4).

Some researchers when adding compost to the soil, observed initial immobilization of nitrogen followed by strong mineralization of this element. This phenomenon occurred between week 2 and 8 after compost was added, depending on soil type. Nitrate loss due to lixiviation is low compared with the amount of nitrogen added to the soil (Burgos *et al*., 2006; Deenik, 2006). Another factor that could influence nitrogen absorption, when 100% compost was used as substrate, is electric conductivity, that was high according to Martínez-Nieto (2004) (13.6-19.1 dSm^{-1}), not allowing a good water and nutrient absorption, mainly in sensible-to-salinity plants like vegetables (Sullivan and Miller, 2004; De Gracia *et al*., 2006).

Plants grown in the mixture soil-compost (1:1) exhibit best records of all plant parameters compared with those grown in only-compost; this case did not show significant differences with treatments of higher averages in aerial biomass and nitrogen foliar (Table 3), according to Ozores-Haptom and Asmad reports (2010), who found negative results in vegetable growth when substrate was 100% compost; specially if it contained high values of soluble salts or if compost was still unstable. Best results were attained when peat was partially replaced by compost between 18% and 52%.

It is important to compare results in compost with those in peat; the latter substrate is the most used material in plantings for the Horticulture Industry (De Gracia *et al*., 2006). Treatment with 50% compost, did not show significant differences in most plant parameters, with plants grown in 50% peat, with the exception of foliar area (Table 3); this results coincide with those found for different vegetables produced at industrial scale in compost mixed with soil or other substrates, by replacing peat and vermiculite (De Gracia *et al*., 2006; Ozores-Haptom and Asmad, 2010).

CONCLUSIONS

Microbial inoculants role for growth, development and nutrients assimilation in radish, was evident, and also for accelerating polluting macrophytes composting process as for the case of Water hyacinth and Brazilian elodea. This due to a high enzymatic activity and probably nitrogen fixation, phosphorous solubilization or production of plant growth stimulating substances or antibiotics, promoted in the substrates by the introduced microorganisms.

ACKNOWLEDGEMENTS

To CAR (Corporación Autónoma Regional de Cundinamarca) for lending of microbial strains used in this study and to *Facultad de Ciencias* of *Pontificia Universidad Javeriana* for the use of its facilities.

REFERENCES

Abaunza, C.A., García, G., Martínez-Nieto, P., Pinto, C. O. 2008. Incorporación de prácticas agroecológicas en los principales sistemas de producción de la localidad de Santa Fe, Distrito Capital. CORPOICA, Alcaldía Mayor de Bogotá D. C., Secretaria de Gobierno, Alcaldía Local Santa Fe. Produmedios. Bogotá, Colombia, 125 p.

Andrade, G., Franco, L. 2007. El complejo de humedales de Fúquene, Cucunuba y Palacio. Un ecosistema estratégico bajo tensión. In: L. Franco, G. Andrade (eds). Fúquene, Cucunuba y Palacio. Conservación de la biodiversidad y manejo sostenible de un ecosistema lagunar andino. Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Fundación Humedales, Bogotá, Colombia, pp: 43-60.

Apun, K., Chian-Jong, B., Azib-Salled, M. 2000. Screening and Isolation of a cellulolytic and amylolytic *Bacillus* form sago pith wastes. J. Gen. Appl. Microbiol. 46, 263-267.

Arnedo, J., Parrado, G. 2002. Aislamiento y caracterización de bacterias termofílicas aerobias, con actividad amilolítica, a partir de pilas de compost en fase termofílica. Tesis pregrado Microbiología Industrial, Pontificia Universidad Javeriana, Colombia, 101 p.

BBC Laboratories. 2004. A summary guide for the microbiological analysis of soil and compost. http://www.proprecycles.org/Documents/msc2004/MFGcompost.doc. Access on June, 2011.

Bio-Logic Enviromental System. 2001. Report on assessing Compost Maturity. A final Report for the Nova Scotia Department of Environment and Labour. 49 p. Available on: http://www.rrfb.com/pdfs/Appendix%20A.pdf. Access on October, 2009.

Bradford, M. 1976. A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-DRE binding. Anal. Biochem. 72, 248-254.

Burgos, P., Madejón, E., Cabrera, F. 2006. Nitrogen mineralization and nitrate leaching of a sandy soil amended with different organic wastes. Waste Manage. Res. 24, 175-182.

Cariello, M. E., Castañeda, L., Riobo, I., González, J. 2007. Inoculante de microorganismos endógenos para acelerar el proceso compostaje de residuos sólidos urbanos. J. Soil Sc. Plant Nutr. 7, 26-37.

Chen-Chin, C., Chan-Chai, N., Chung-Yi, W.,Yuan-Tay, S. 2009. Activity of cellulase from *Thermoactinomyces* and *Bacillus* spp. isolated from *Brassica* wates compost. Sci. Agric. 66, 304-308.

Comisión Nacional del Medio Ambiente del Gobierno de Chile. 2000. Norma de calidad de compost. 18 p. Available on: http://www.lombricultura.cl/lombricultura.cl/userfiles/file/biblioteca/normas/Norma%20calidad%20COMPOST.pdf. Access on Mayo, 2006.

Consejo Nacional de Política Económica y Social República de Colombia.2006. Estrategia para el manejo ambiental de la cuenca Ubate-Suarez. Documento CONPES 3451. Bogotá, Colombia, 53 p.

Cornell Waste Management Institute. 2000. The Science and Engineering of composting. Collage of Agriculture and Life Sciences. Ithaca, New York. Available on: http://compost.css.cornell.edu/science.html. Access on May, 2006.

Cornell Waste Management Institute. 2004. Hygienic implications of Small-scale composting in New York State. Cold compost Project. Ithaca, New York, 71 p.

Cummings, S. P., Humphry, D. R., Santos, S. R., Andrews, M., James, E. K. 2006. The potential acompostnd pitfalls of exploiting nitrogen fixing bacteria in agricultural soils as a substitute for inorganic fertilizer. Environ. Biotechnol. 2, 1-10. Available on: http://www.environmentalbiotechnology.pl/eb_dzialy/eb_online/2006/vol2_1/ms011spcummings.pdf. Access on May, 2009.

Czaczyk, K., Trojanowska, K.,Stachowiak, B., Dubisz, H. 2001. Changes in cell number and ATP content during the composting process. Pol. J. Environ. Stud. 10, 149-153

Deenik, J. 2006. Nitrogen mineralization potential in important agricultural soils of Hawaii. Soil and Crop Management. SCM-15. 5 p. Available on http://www.ctahr.hawaii.edu/deenikj/Downloads/SCM-15.pdf.. Access on June, 2010.

De Gracia, J., Tittonel, P.A., Chiesa, A. 2006. Efecto de sustratos con compost y fertilización nitrogenada sobre la fotosíntesis, precocidad y rendimiento de pimiento (*Capsicum annuum*). Cien. Inv. Agr. 34, 195-204.

Eslava, M., García A. M. 2001. Evaluación de un inoculo microbiano en el proceso de degradación de residuos en cultivos de flores. Tesis pregrado Microbiología Industrial, Pontificia Universidad Javeriana, Colombia, 114 p.

Ge, B., Mccartney, D., Zeb J. 2006. Compost environmental protection standards in Canada. J. Environ. Eng. Sci. 5, 221–234

Hargreaves, T. 2003. The water hyacinth. Disponible en: http://ecologist.testing.net-genie.co.uk/investigations /naturalworld /82936 / the_water_hyacinth.html. Access on June, 2010.

Hogg, D., Barth, J., Favoino, E., Centemero, M., Caimi, V., Amlinger. F., Devliegher, W., Brinton, W., Antler, S. 2002. Review of Compost Standards in Australia. Banbury, Reino Unido, 11 p.

Ichida, J.M., Krizova, L., Lefevre, C. A., Keener, H. M., Elwell, D. L., Burtt, E.H. 2001. Bacterial inoculum enhances keratin degradation and biofilm formation in poultry compost. J. Microbiol Meth. 74, 199-208.

Instituto Colombiano De Normas Técnicas Y Certificación (Icontec). 2004. Norma Técnica Colombiana 5167 del 2004. Productos para la industria agrícola. Productos orgánicos usados como abonos o fertilizantes y enmiendas de suelo. Ediciones ICONTEC, Bogotá, Colombia, 40 p.

Jackson, M., Campbell, A. 2003. Producing quality compost. University of New South Wales, Sydney, Australia, 71 p.

Khalid, M., Yang, W., Kishwar, N., Rajput Z., Arijo, A. 2006. Study of cellulolytic soil fungi and two nova species and new medium. J. Zhejiang Univ. Sci. B. 7, 459-466. Available on: Access on: May, 2009. http://www.zju.edu.cn/jzus/2006/B0606/B060607 .pdf Access on May, 2009.

Kroner, I., Braukmeier, J., Herreklage, J., Leikam, K.,Ritzkowski, M., Schlegelmich, M., Stegmann, R. 2003. Investigation an optimization of composting processes-test systems and practical examples. Waste Management. 23,17-26.

Lung, A. J., Lin, C. M., Kim, J. M., Marshall, M. R., Nordstedt, R., Thompson, N. P., Wei, C. I. 2001. Destruction of *Escherichia coli* O157:H7 and *Salmonella enteriditis* in cow manure composting. J. Food Prot. 64, 1309-1314.

Luo, W., Chen, T.B., Zheng, G.D., Gao, D., Zhang, Y.A., Gao, W. 2008. Effect of moisture adjustments on vertical temperature distribution during forced-aeration static-pile composting of sewage sludge. Resour. Conserv. Recycling. 52, 635-642.

Martínez-Nieto, P. 2004. Evaluación de un inóculo microbiano en un proceso de compostaje con *Eichhornia crassipes* y *Eigeria densa* presentes en la laguna de Fúquene. Informe final contrato No C-0732-03. Corporación Autónoma Regional de Cundinamarca (CAR), Bogotá, Colombia, 86 p.

Martínez-Nieto, P. 2006. Compostaje de elodea, residuos de cebolla y gallinaza. In: C. Herrera, G. Sánchez, V. Peña (eds). Avances de resultados de investigación en cebolla de rama en Aquitania, Boyacá. Corporación Colombiana de Investigación Agropecuaria (CORPOICA), Corporación Autónoma Regional de Boyacá (CORPOBOYACA), Bogotá, Colombia, pp: 27-40.

Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31, 426-428

Moreno, G., Oñate, M. 2001. Producción de un inóculo acelerador de compostaje a partir de bacterias lipolíticas y proteolíticas aisladas del tren de tratamiento de una industria láctea. Tesis pregrado Microbiología Industrial, Pontificia Universidad Javeriana, Colombia, 144 p.

Nakamura, K., Haruta, S., Languyen, H., Ishii, M., Igarashi, Y. 2001. Enzyme production-based approach for determining the function of microorganisms within a community. Appl. Environ. Microbiol.70, 3329-3337.

Orji, J.C., Nweke, C.O., Nwabueze, R.N., Nwanyanwu, C.E., Alisi, C.S., Etin-Osowo, E.N. 2009. Production and properties of a amylase from Citrobacter species. Available on http://www.ambi-agua.net/seer/index.php/ambi-agua/article/view/153/pdf_177. Access on December, 2009.

Orozco-Jaramillo, C., Martínez-Nieto, P. 2009. Evaluación de la inoculación con microorganismos fijadores de nitrógeno asimbióticos aislados de la rizósfera de *Pinus patula* en Colombia. Bosque. 30, 70-77.

Orozco, M., Thienhaus, S. 1997. Efecto de la gallinaza en plantaciones de cacao (*Theobroma cacao* L) en desarrollo. Agronomía Mesoamericana. 8, 81-92.

Ozores-Hampton, M., Asmad, B. 2010. Guía para la Utilización Exitosa del Compost en la Producción de Hortalizas. Available on: http://edis.ifas.ufl.edu/pdffiles/HS/HS40600.pdf. Access on June, 2010.

Páez, M., Martínez-Nieto P., Bernal-Castillo. 2005. Siderophore producing Pseudomonas as pathogenic *Rhizoctonia solani* and *Botrytis*

cinerea antagonists. Universitas Scientiarum. 10, 65-74.

Quintero, M., Otero, W. 2006. Mecanismo de financiación para promover agricultura de conservación con pequeños productores de la cuenca de la Laguna de Fúquene. Su diseño, aplicación y beneficios. Proyecto Regional Cuencas Andinas. Centro Internacional de la papa. Available on: http://www.infoandina.org/node/7616 Access on June, 2011.

Ramírez, P., Coha, J. 2003. Degradación enzimática de celulosa por actinomicetos termófilos: aislamiento, caracterización y determinación de la actividad celulolítica. Rev. Peru. Biol. 10, 37-77.

Rodríguez, E., Rueda, A., Pedroza, A., Poutou, R. 2007. Aislamiento, identificación y caracterización de bacterias termófilas aeróbicas, con actividad proteolítica, a partir de pilas de compost en fase termofílica. Available on: http://redalyc.uaemex.mx/pdf/499/49910969004.pdf. Access on June, 2011.

Rua, L., Wahl, C., Spraver, S., Schimid, R. 1997. Thermoalkalophic lipase of *B. thermocatenulatus* large-scale production and properties aggregation behavior and its effect on activity. J. Biotech. 56, 89-102.

Samudro, G., Hermana, J. 2007. Denitrification efficiency in a compost bed with various carbon and nitrogen content. J. Appl. Sci. Environ. Sanit. 2, 57-62.

Sarmiento, V.C., Vargas, D.H., Pedroza, A.M., Matiz, A., Poutou, R.A. 2003. Producción de α amilasa con células libres e inmovilizadas de *Thermus* sp. MVZ-Córdoba. 8, 310-317.

Singh, A., Sharma, S. 2003. Effect of microbial inocula on mixed solid wasted composting, vermicomposting and plant response. Compost Sci. and Uti.11, 190-199.

Sullivan, D.M., Miller, R. O. 2004. Propiedades cualitativas, medición y variabilidad de los compost. In: P.J. Stofella, B. A. Khan (eds). Utilización de compost en los sistemas de cultivo hortícola. Multiprensa libros S.A., Madrid, España, pp: 95-122.

Tiquia, S., Wan J., Tam, N. 2002. Microbial population dynamics and enzyme activities during composting**.** Compost Sci. Util.10, 150-161.

Urrego-Layton, J., Rodríguez-Aponte, D., Bernal-Castillo, J., Martínez-Nieto, P. 2006. Inmovilización de bacterias diazotróficas y solubilizadoras de fósforo aisladas de un Bosque Alto Andino Cundinamarqués. In: Universidad Pedagógica y Tecnológica de Colombia (ed). Memorias Primer Congreso Nacional en Sistemas de Alta Montaña Tropical. Tunja, Boyacá, pp: 1-11.

Veijalainen, A.M. 2007. Sustainable Organic Waste Management in Tree-Seedling Production. Available on: http://epublications.uef.fi/pub/urn_isbn_978-951-27-0790-4/urn_isbn_978-951-27-0790-4.pdf Access on June, 2011