



## Change of potential microbial activity and CO<sub>2</sub>-production in acidic sandy soil amended by sewage sludge application in the rhizosphere of field growing common bean (*Phaseolus vulgaris* L.)

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### Abstract

Field growing common bean were cultivated for 50 days in pots containing acidic sandy brown forest soil treated with different sludge rates 0/100, 30/100, 50/100, 70/100 and 100/0 % sludge-soil (w/w). Results showed that microbial populations at all concentrations increased significantly with increasing the ratio of sludge. These increases were significantly positive in case of the treatment with 30% sludge application rate in the total aerobic heterotrophic bacteria, aerobic spore-forming, *Actinomycetes* and microscopic fungi. Most frequent isolates were belonging to *Acinetobacter*, *Azotobacter*, *Rhizobium*, *Brevundimonas*, *Cellulomonas*, *Chromobacterium*, *Corynebacterium*, *Enterobacter*, *Escherichia*, *Flavobacterium*, *Klebsiella*, *Micrococcus*, *Proteus*, *Streptococcus*, *Serratia* and *Zooglea*. *Streptomyces* was the genus of most dominant isolates of actinomycetes. The most fungal isolates were belonging to the genera of *Alternaria*, *Aspergillus*, *Cephalosporium*, *Cladosporium*, *Fusarium*, *Geotricum*, *Mucor*, *Penicillium*, *Rhizopus* and *Trichoderma*, the ratios between the counted Gram negative to Gram positive were 7/3 and 8/5, respectively. While the ratios of rod shape bacteria to cocci forms were 4/1 and 5/2. The maximum root-nodule number found at 30% sewage sludge, the highest number of isolated *Bacillus* species was found in a medium of 50% sewage sludge, and the aerobic spore-forming bacteria determined at 100% of sludge. The results demonstrate that the amount of CO<sub>2</sub>-production from the soil bulk was more than ½ of the amount released from the rhizosphere. These increases were laid between 30% and 50% more than produced at control.

### Introduction

Recycling of organic wastes within agriculture may help maintain soil fertility via positive effects on physical, chemical and biological properties. Efficient use, however, requires an individual assessment of waste products, and effects should be compared with natural variations due to climate and soil type. Application of different types of organic wastes may have a marked effect on soil microbial biomass and its activity. Biosolid, however, are rich in organic matter and nutrients, and the recycling through application of biosolid to agricultural land has been promoted as a means of avoiding the environmental and economic costs of disposal (Bright & Healy 2003). The organic matter in biosolid improves the soils structure, increases its water holding capacity, and feeds essential soil microorganisms. The most prominent concern is the presence of heavy metals and therapeutic agents in the biosolid and the affect they will have on the environment surrounding the application site and how they will be incorporated into the



food chain (Jjemba 2002). Excessive biosolid application may lead to a reduction in crop yields due to plant nutrient deficiency and/or imbalances. Also excessive application contributes to a range of environmental problems including the nitrification of ground water systems (Cooke et al. 2001).

## Materials and Methods

### Materials

Soil sample of acidic sandy brown forest soil of low humus content was taken from non-cultivated area of Gödöllő town, which is situated 30 km north-east of the capital Budapest, in Hungary.

Soil sample was collected from the upper 250 mm layer after removing the top 20 mm from a sample site. Soil sample was ground and sieved (2 mm) and stored in plastic bags at cold room temperature ( $10 \pm 2^\circ\text{C}$ ) for one week. The physical and chemical characteristics of the above-mentioned soil are mentioned in Table 1.

Table (1).

Physical and chemical analysis of used soils

Different determination	Values of soil parameters
$\text{pH}_{\text{KCl}}$	5.31
$\text{pH}_{\text{H}_2\text{O}}$	5.82
$\text{K}_\text{A}^*$	25
Total salt (%)	0.02
Humus (%)	1.29
$\text{NH}_4^+\text{-N}$ (mg/kg)	1.80
$\text{NO}_3^-\text{-N}$ (mg/kg)	3.50
AL- $\text{K}_2\text{O}$ (mg/kg)	194
AL- (mg/kg)	63.5
Oxalate Fe (mg/kg)	1820
Dithionite-citrate Fe (mg/kg)	8178

\* cohesion determined by the "Number of Arany" (usual method in Hungary)

### Biosolid material

The biosolid material was received from Nyíregyháza and Regional Domestic Wastewater Treatment Plant Ltd., Nyíregyháza. The sample (code 1/2/2003/2) was aerobically digested and sand bed-dried. The chemical characteristic analysis of sewage sludge is shown in Table 2.

Table (2). Chemical analysis of used sewage sludge

Different determination	Sewage sludge
$\text{PH}_{(\text{KCl})}$	6.28
Content of solid matter (%)	75.4
Total nitrogen (mg/kg)	3200
Total phosphorus (mg/kg)	3202
Total iron (mg/kg)	7792
Cadmium (mg/kg)	0.7
Calcium (mg/kg)	9500
Total chromium (mg/kg)	18.5
Copper (mg/kg)	78.1



Lead (mg/kg)	22
Manganese (mg/kg)	161
Magnesium (mg/kg)	1810
Nickel (mg/kg)	13.5
Potassium (mg/kg)	1630
Zinc (mg/kg)	455

#### *Preparation of experimental soil-biosolid mixture*

In two-kg pot capacity, the agro-ecosystem for plant growth was formed according to the following mixture, shown in Table 3.

Table (3). Different ratios of soil and biosolid mixtures

Ratio of soil added to the mixture	Ratio of biosolid added to the mixture
100%	0%
70%	30%
50%	50%
30%	70%
0%	100%

The moisture content of the agro-ecosystem was kept constantly (approximately  $60 \pm 2\%$ ) throughout the 50 days as the time of cultivation.

#### *Greenhouse conditions*

The plant-soil-biosolid agro-ecosystem was conducted in greenhouse under automatically setup conditions to be consisted of a 12 h day maintained with two paired day-light and warm white fluorescent tubes at 10000 lux, day-time temperatures of 22°C, and night temperatures of 16°C. The experimental pots were watered as necessary to maintain the soil moisture content at approximately  $60 \pm 2\%$  field capacity.

#### *Test plants*

Field growing common bean (*Phaseolus vulgaris* L. cv.) which was grown in greenhouse in pots containing various rates of the soil-biosolid mixture.

#### *Cultural media*

For microbiological investigations, the following cultural media were used for the isolation of the more abundant of different rhizo-microorganisms and some of functional rhizomicrobial groups in the soil bulk and the rhizospheres of the growth plant. The media were Nutrient agar and Minimal medium. The following media were used for isolating fluorescent *Pseudomonas* species: King-B agar and Pseudosel agar.

The following media were used for the isolation of common rhizospheric fungi: Tryptone-glucose-yeast extract agar, Malt agar and Selective agar medium for *Trichoderma* species. The medium was used for the isolation of symbiotic nitrogen fixing bacteria is Congo red yeast extract mannitol agar. The following media were used for isolating *Actinomycetes*: Jensen agar and Küsten Williams agar.

Following media were used for counting the cellulose decomposition and phosphate solubilization: For counting the cellulose decomposers, the medium of Hendricks et al. (1995) was used. The cellulose-Congo red agar medium (used to provide essential

trace elements for bacteria). For counting the phosphate solubilizers, medium of Goldstein (1986) was used.

### **Methods**

A known weight of air dried of soil sample was mixed well with the desired amount of biosolid and placed in plastic pots of two-kg capacity. The final mixing rates of biosolid with the soil samples were 0 (control: soil without addition of biosolid) 30, 50, 70, and 100% (biosolid without soil) per pot. Seeds of field growing common bean plant were selected for uniformity without any injury, and surface sterilized with 0.2% of acidified mercuric chloride followed by 75% ethanol for three minutes and thoroughly washed several times by changing of the sterile distilled water. Seeds were then soaked for a few hours at room (22°C) in a sterile distilled water (soaking water was changed every 3 h). The five seeds of field growing common bean were planted per pot and covered with layer of approximately 20 mm of soil-biosolid agro-ecosystem. Between one and two week of emergence, the seedlings were thinned to three seedlings per. Seedlings were watered with sterile distilled water when required, and the plants grown under natural illumination (12 h photoperiod) at around 22°C. The experiments were carried out in three replicates.

Plant cultivation was carried out for 50 days, than the plants were carefully uprooted. Roots were thoroughly washed with light tap water for 2-5 minute to remove all loosely adhering soil particles followed by washing with sterile 0.85% saline Milli Q water. The roots were cut sharply. Plants were carefully uprooted, after root length measurements, the root macerated in sterile 0.85% saline Milli Q water with a sterile mortar and paste. The samples were shaken horizontally with 150 rpm for ½ h in shaker machine. Serial dilution technique was carried out for all samples of soil-biosolid cultivation agro-ecosystem in sterile distilled water. The  $10^4$ ,  $10^5$ ,  $10^6$  and  $10^7$  dilutions were used for further investigations.

Plate count technique was done to estimate the total number of soil and rhizospheric aerobic bacteria and aerobic spore-forming bacteria, *Actinomycetes* and fungi as well. Rhizospheric bacterial, actinomycetal and fungal representatives of different morphological characters present on the cultural plates were selected and purified by streaking on minimal medium, Küsten Williams agar and Malt agar respectively to obtain pure colonies. After 2 and 5 days of incubation at 30°C, individual colonies from each isolate were again streaked, and the process was repeated until a plate with pure microorganisms was obtained. The pure colonies were stored in respective medium and maintained at 4°C for further studies.

The selected bacterial colonies were re-isolated and purified using dilution technique and streak plate method. Purified bacterial colonies were used for further bacteriological identification.

The following microbiological tests were carried out to help in the processes of identification of isolated microbes: Gram strain, cell morphology, oxidase test, catalase test, indole test and aerobic spore forming test.

The common microflora associated with the rhizosphere of the plants roots was identified according to Bergey's Manual of Systematic Bacteriology (1984), BBL Crystal Programme for identification systems (Publ. Decton and Dickinson Co. Ltd.). Microflora associated with the rhizospheres of tested plant was identified as described earlier (Nautiyal & Dion 1990).

### **Assay for detection of phosphorus solubilizing microorganisms**



One ml of homogenous soil-sludge agro-ecosystem extraction of low dilution ( $10^5$ ,  $10^6$  and  $10^7$ ) was placed on the surface of the agar plate containing a medium described by Pikovskaya (1948) and according to the procedure of Goldstein (1986). After incubation for 5 days at 28°C, colonies surrounding with clear zones were counted. The technique was repeated to make sure that the selected colonies were phosphate solubilizers, each of the selected colonies was seeded on a straight line, in a medium described by Pikovskaya (1948), and all plates were incubated at 26°C for 5 days. Colonies showing solubilization halos over 0.5 mm in diameter were selected.

#### *Assay for detection of cellulose-decomposing microorganisms*

The detection of cellulose-decomposers in the rhizospheres was carried out according to Hendricks et al. (1995). One ml of the extraction of soil-biosolid agro-ecosystem was placed on the surface of the agar plate containing cellulose Congo red medium. Following the incubation, the colonies exhibiting zones of clearing were counted. Replicate plates of bacterial cultures were flooded with a 1% solution of hexadecyltrimethylammonium bromide (HAB) to examine the ability to enhance visualisation of the zones of clearing.

#### *CO<sub>2</sub>-release from soil-plant ecosystem*

Basal respiration (CO<sub>2</sub>-production) of the biosolid treated soil at different mixing rates was determined by incubating the soil samples for 9 days. The measurement of CO<sub>2</sub>-production was carried out in 0.5 kg of the treated soil sample which was filled in 1.5 l gas-tight glass vessel, and in the middle of the soil a plastic tube of 4 cm in diameter containing 50 ml of 10 M NaOH solution was fixed for trapping the evolution of CO<sub>2</sub> and glass vessel was closed tightly.

The NaOH was titrated with HCl (1 M) to calculate the volume of CO<sub>2</sub> of soil respiration, which represented the respiration due to litter decomposition, root respiration, rhizomicrobial respiration (i.e. microbial respiration utilising C directly derived from living roots), and microbial respiration utilising native soil organic matter.

Applied method of Wardle & Parkinson (1991) was used for simultaneous determination of NaOH and Na<sub>2</sub>CO<sub>3</sub> content in our experimental soil samples.

#### *Statistical analysis*

All tests were carried out at least in triplicates, group differences across metric dependent variables based on set of categorical (non-metric) variables were assessed by multiple analyses of variance (MANOVA), and differences in means were evaluated by F-probe according to Sváb (1981). Excel 5.0 statistical functions were used for calculations and graphic presentation of data. Standard deviation (SD) and Least Significant Difference at 5% level (LSD<sub>0.05</sub>) were calculated as well, statistical interpretation was made by reference to Sváb.

## **Results**

The results demonstrated that the addition of biosolid to soil increased the pH to be about favourable for plant growth (the results indicated that when the soil with pH 5.31 was treated with different concentrations, the pH of the soil ecosystem was increased to be near to pH 6). The soil treated with biosolid kept the moisture for more time than the control soil. The plant growth showed healthier and grew faster than the control.

The rhizomicrobial population size of the rhizosphere of the field growing common bean (Table 4) was increased with the increases of the biosolid concentration. This



increase was significantly positive when the soil treated by 30% applied dose of biosolid in the following cases: total aerobic heterotrophic bacteria, aerobic spore-forming, *Actinomycetes* and microscopic fungi, but, in the rhizosphere the plant at the concentration, also, it was found that population size of fungi was significantly different with control. At 50% biosolid treated soil all counted rhizomicrobial groups were significant with control.

Table (4) Effect of different applicable doses of biosolid on the different microbial groups in the common bean rhizosphere

Sewage sludge doses	Mean of three replicates of different microbial counts / g soil					
	Aerobic bacteria (x10 <sup>6</sup> )	Aerobic spore-forming (x10 <sup>3</sup> )	Actinomycetes (x10 <sup>3</sup> )	Fungi (x10 <sup>4</sup> )	Aerobic phosphate solubilizers (x10 <sup>2</sup> )	Aerobic cellulose decomposers (x10 <sup>3</sup> )
0%	74	5,1	1,3	5,6	4,5	4,9
30%	136	11,1	3,5*	8,9*	7,3	9,1
50%	193*	18,4*	4,3*	10,7*	9,6*	12,2*
70%	253*	24,1*	5,2*	12,7*	13,2*	15,1*
100%	381*	29,3*	6,3*	14,7*	19,7*	21,4*
SD (P < 0,05)	114,37	9,03	1,22	2,14	5,16	5,67

The numbers labelled with (\*) within the column is significantly different from the control at  $p \leq 0.05$

The most frequent isolates were belonging to, *Acinetobacter*, *Azotobacter*, *Rhizobium*, *Brevundimonas*, *Cellulomonas*, *Chromobacterium*, *Corynebacterium*, *Enterobacter*, *Escherichia*, *Flavobacterium*, *Klebsiella*, *Micrococcus*, *Proteus*, *Streptococcus*, *Serratia* and *Zooglea*. *Streptomyces* was the genus of most dominant isolates of actinomycetes. The most fungal isolates were belonging to the genera of *Alternaria*, *Aspergillus*, *Cephalosporium*, *Cladosporium*, *Fusarium*, *Geotricum*, *Mucor*, *Penicillium*, *Rhizopus* and *Trichoderma*. Also, many isolates of the genus *Saccharomyces* were isolated from soil samples of the rhizospheres of the tested plants amended with communal biosolid comparing with the control soil. Percentage of Gram negative and Gram positive rhizobacteria was determined and it was found that the ratios between the counted Gram negative to Gram positive rhizobacteria were 7/3 and 8/5, respectively. While the ratios of rod shape bacteria to cocci forms were 5/2. The symbiotic nitrogen fixing bacteria was determined by counting the root-nodule existing on the roots. Also, the results showed that the maximum root-nodule number was found on the roots grown in the agroecosystem of 30% biosolid. The reason is that because of high nitrogen applied to the soil, the root-nodules were inhibited, the highest number of isolated *Bacillus* species was found in a medium of 50% biosolid, and the aerobic spore-forming bacteria were determined at maximum at 100% of biosolid.

The results of this study demonstrate that the amount of CO<sub>2</sub>-production from the soil bulk was more than 1/2 of the amount released from the rhizospheres of the plant; the CO<sub>2</sub>-production was higher than control. These increases were laid between 30% and 50% more than produced at control. The results (Table 5) indicated that by increasing the applied doses of the biosolid to the soil, the amount of CO<sub>2</sub>-released was increased.



Table (5) Effect of different applicable doses of biosolid on the soil respiration (CO<sub>2</sub>-production, WHC 60%, 25 °C) in the rhizosphere of common bean in brown forest soil (Gödöllő)

Applicable doses of sewage sludge (%)	Amounts of CO <sub>2</sub> -production (mg C/100 g soil) in the rhizosphere	
	Soil bulk	Common bean
0	104,1	157,6
30	127,3	287,4
50	194,8*	473,3*
70	242,1*	683,3*
100	297,5*	761,6*
SD (P ≤ 0,05)	77,61	202,29

The numbers labelled with (\*) within the column is significantly different from the control at P ≤ 0.05

These results indicated that the application of biosolid activated the soil respiration due to the availability of the nutrients to the living forms in the soil.

Also, there was a significant difference between the results obtained at the control and the results at 50% or over 50%, but no significantly different between control and the result obtained at 30% of biosolid in soil.

## Discussion

Katai (1999) summarised the results of changes in the microbiological population size in long-term fertilisation experiment with mono-, and triculture of maize. It was found that the total bacterial and cellulose decomposing bacteria in Hajdúböszörmény soil was increased by increasing the fertilisation doses in mono-, and triculture, while maximum microscopic fungi was obtained at medium dose applied to the monoculture. But in Látókép, the total bacterial count was increased by increases the fertilisation dose in triculture, and it was maximal at low to medium rate of fertiliser. The maximum population of microscopic fungi was at medium to high rate of applied fertilisation both mono-, and triculture. Also, it was found that the population size of cellulose decomposing bacteria was increased by increases the dose in monoculture and it was at maximum when the triculture treated with medium to high dose rate. Our results showed that in the rhizospheres of field growing common bean, the population sizes of the total aerobic bacteria, aerobic spore-forming, Actinomycetes, fungi, aerobic phosphate solubilisers and aerobic cellulose decomposers were increased by increasing the applied dose of biosolid

Most of the recent literature containing microbial solubilization of minerals in soil and their potential use for enhancement of soil fertility deals with phosphate solubilizing bacteria and vesicular arbuscular mycorrhizal fungi. However, the ability of a few filamentous nonmycorrhizal fungi, especially *Aspergillus* spp. and *Penicillium* spp., to solubilize phosphate has also been shown (Illmer & Schinner 1992, Molla et al. 1984). A commercial formulation of *Penicillium bilaii* Chalabuda has been registered in Canada as a biological enhancer of plant nutrition (Cunningham & Kuiak 1992). It was mentioned that using agar media with calcium phosphate and the pH indicator alizarin red S, the influence of the medium composition on phosphate solubility and medium acidification was recorded. The major acidic metabolites produced by the fungus in a sucrose nitrate liquid medium were found to be oxalic and citric acids. The release of



organic acids that both sequester cations and acidify the microenvironment near roots is thought to be a major mechanism of solubilization of phosphorus by plants and non-vesicular arbuscular mycorrhizal fungi.

The efficacy of various *Pseudomonas* spp. in dissolving rock phosphate from suspension, agar, and soil has received considerable attention during the last two decades (Azcon et al. 1976, Ralston & McBride 1976, Gaur et al. 1980, Illmer & Schinner 1992). The dissolution of rock phosphate involves two steps: first production of the monocarboxylic acids (gluconic and 2-ketogluconic acids) by the bacteria and second dissociation of these acids (Moghimani & Tate 1978) and subsequent dissolution of the rock phosphate by the resulting protons.

Maximum crop yields require sufficient P fertilisation. Current fertiliser technology supplies the soil solution with soluble P via the application of large amounts of phosphate salts. Problems with this technology include energy-intensive production processes with associated environmental consequences (Goldstein 2000). The most promising approach is the phosphate solubilization by heterotrophic microorganisms producing organic acids. Microbial mediated solubilization of rock phosphate can be performed in condition of solid-state fermentations utilizing solid-industrial wastes (Vassilev & Vassileva 2003). The resulting fermented materials, containing mineralised organic matter, soluble (plant-available) P, and microbial biomass, were further introduced into typical soil-plant systems. Analyzing the plant responses, plant growth increases of at least 300% were registered as compared to non-amended controls (Vassilev et al 1996,).

The large surface area increases the secretion of P solubilizing compounds into localised regions of the rhizosphere (Gardner et al. 1982). This is important in the natural environment where phosphorus acquisition is most likely to be limited by the low solubility and diffusion of phosphorus across the soil to the root absorbing surfaces. Phosphorus is the least available element to sugar cane and to plants in general. This is due to two phenomena occurring when contacting the soil: the first phenomenon is called immobilisation, and is carried out by those microorganisms that populate the mineral's deficient regions and which the nutrients amounts needed to perform their vital processes (Jungk et al. 1993). The second phenomenon is called precipitation or fixation to insoluble complex minerals, and is due to the union of phosphorus with elements such as iron and aluminium in acid soils, and calcium in alkaline soils, denying the plant up to 75% of all soluble phosphorus (Goldstein 1986, Kucey et al. 1989), and thus, generating a 0,002-0,5% concentration of mineral in the soil (Chabot et al. 1993). This has forced many crop raisers to apply up to four times the required amount of phosphorus to plants. Higher plants have access to phosphate only a few millimetres around the root. There are several potential mechanisms for phosphate solubilization. These include the modification of pH by the secretion of organic acids or protons. Secretion of  $H^+$  from roots into the medium in response to uptake of  $NH_4^+$  and of  $OH^-$  for  $NO_3^-$  uptake are well documented, and the decrease in pH of the medium in the presence of  $NH_4^+$  has been correlated with solubilization of inorganic phosphates (Marschner 1991). Kwabiah et al. (2003) established that soil microbial biomass is a major sink and sources of plant available phosphorus and transformer of soil organic phosphorus.

Datta et al. (1982) extracted an auxin named acid 3-indol acetic from a phosphate-solubilizing genus of *Bacillus firmus*. It is possible that microorganisms may have the ability to produce certain substances that act in a similar way; in fact, *Enterobacter* is used in soil recovery processes, due to its ability to transform nutrients and to produce vegetable growth stimulating substances. While using *Enterobacter* genus in corn





seeds inoculation, Chabot (1993) observed root elongation due to production of auxins. Wenzel et al. (1994) isolated bacteria from cluster and non-cluster roots of waratah (*Telopea speciosissima*) seedlings are able to acidify the medium and solubilize calcium phosphates when grown in culture in the presence of ammonium salts and an appropriate carbon source. It was mentioned that this activity was not detected when nitrate ion was substituted for ammonium ion, and it is proposed that protons were secreted in exchange for ammonium ions. Cation exchange between these protons and calcium in the medium is a possible cause of the calcium phosphate solubilizing activity.

Altomare et al. (1999) investigated the capability of the plant growth promoting and biocontrol fungus *Trichoderma harzianum* to solubilize *in vitro* some insoluble or sparingly soluble minerals via three possible mechanisms: acidification of the medium, production of chelating metabolites, and redox activity. The fungus was able to solubilize calcium phosphate in a liquid sucrose-yeast extract medium. Phosphates were also solubilized by cell-free culture filtrates. According to Weaber (1980) cytokinins induce leaf elongation. And, according to a survey by Barea et al. (1976) cytokinins were extracted from the RNA of a phosphate-solubilizing genus of *E. coli*. Solubilization of  $\text{CaHPO}_4$  was positively correlated with bacterial acidification of the medium below about pH 5. There are many reports of secretion of organic acids such as oxalic, malic or citric acids from bacteria or roots that solubilize phosphate (Marschner 1986, Marschner et al. 1987).

From the above mentioned reports, we have found that when the field growing common bean was grown in media containing different mixing rates of biosolid for 50 days, the enumeration of the calcium phosphate solubilising rhizomicrobes were increased by increasing the sludge rate in the models.

Several different approaches for the selective enumeration of cellulose-utilising bacteria in soil have been described (Mahasneb & Stewart 1980, Smith 1977). The common basis of most methods is the hydrolysis of cellulose substrate. (Hankin & Anagnostakis 1977) looked for zones of clearing surrounding colonies growing on agar containing cellulose. Others have flooded agar plates containing carboxymethylcellulose with a solution of Congo red to enhance the detection of bacterial colonies able to use cellulose (Wood et al. 1988). Furthermore, the colonies of cellulose-utilising bacteria are often difficult to differentiate from other organisms on solid media (Stotzky et al. 1993), even with polysaccharide precipitants, e.g., hexadecyltrimethylammonium bromide (Cruden Markovetz 1979).

De Melo et al. (2002) mentioned that cellulase activity increased until 90 days after sewage sludge application and then decreased. Sewage sludge used in the experiment should already contain some amylase activity or a substance that was a soil enzyme activator and also a substance that was an inhibitor of soil cellulase inhibitor. Some of the plant nutrients contained in sewage sludge, mainly P, did not migrate down the soil column, an indication that sewage sludge should be incorporated into the soil to improve nutrient bioavailability.

Our speed and precision in enumerating cellulose-decomposing bacteria in a system were improved with the cellulose Congo red agar. We found the cellulose-utilising bacteria were more distinguishable on the used medium by definitive zones of clearing around the colonies that were generally red and, therefore extremely easy to count. The results were confirmed by the Hendricks et al. (1995).

Quemada & Menacho. (2001) stated that soil respiration was greater in soils that received 80 tons  $\text{ha}^{-1}$  of sewage sludge for 1 year prior to the study than in unamended soils. Soils that received 40 tons  $\text{ha}^{-1}$  of sewage sludge respired more than unamended



soils on only two sampling dates, probably because soil temperature and water content favoured high microbial activity. These results showed that 1 year after sludge amendment, soil respiration still depends on sludge application rate. Our results showed that by increasing sewage sludge in the mode the rate of CO<sub>2</sub>-released was increased (Table 14). Soil microorganisms that catabolize the cellulose material of plants (40 to 60 % of plant residues) influence the flow of energy from plant material to higher trophic levels and ultimately the release of CO<sub>2</sub> to the atmosphere (Paul & Clark 1989).

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