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EFFECT OF DIFERENTLY POST-TREATED DEWATERED SEWAGE SLUDGE ON β-GLUCOSIDASE ACTIVITY, MICROBIAL BIOMASS CARBON, BASAL RESPIRATION AND CARBOHYDRATES CONTENTS OF SOILS FROM LIMESTONE QUARRIES

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Abstract

This work has evaluated the effects of a thermally dried or composted dewatered sewage sludge on β-glucosidase activity, total (TCH) and extractable (ECH) carbohydrate content, microbial biomass carbon (MBC) and basal respiration (BR) of soils from limestone quarries under laboratory conditions. Two doses (low, L and high, H) of the dewatered sludge (DS), or of the respective thermally dried (TDS) or composted (CDS), were applied to a clayey (CL) and a sandy (SA) soil, both coming from working quarries. The soil mixtures and the controls (soils with no added sludge) were incubated for nine months at 25ºC and 30% of field capacity. The addition of sludge increased all the studied soil parameters, and the increase depended on the amount of sludge. Except in the case of TCH and ECH, the enhancing effect decreased with time but, at the end of incubation, parameters of the treated soils were higher than those of the control. The rank order of the initial stimulating effect was soil-TDS ≥

soil-DS ≥ soil-CDS and probably this order depended on the proportion of stable organic matter which was the lowest in the TDS.

Values of metabolic quotient (qCO_2) were higher at the lower dose and they did not change during incubation in the CDS treated soils. Both TCH and ECH were the parameters with the greatest significant sludge and dose effects. Basal respiration, microbial biomass carbon and β-glucosidase activity were the best measured parameters in distinguishing the long term effects of the three sludge types over the soils.

Key Words Soil reclamation. Sewage sludge. Soil metabolic quotient. Soil carbohydrates. Soil enzymes

Introduction

In Catalonia, the reclamation of quarries has started recently, is becoming more and more frequent and generaly it involves the use of sewage sludge (Bonmatí et al. 2000).

The application of dewatered sewage sludge to soils improves soil fertility because it increases the nutrient content (Bonmatí et al. 2000; Gigliotti et al. 2001; Smith and Tibbett 2004), structure and aggregate stability (Sort and Alcañiz 1999; Gigliotti et al. 2001), biological activity (Albiach et al. 2000; Bonmatí et al. 2000; Gigliotti et al. 2001; Saviozzi et al. 2002) and crop yields (Navas et al. 1999; Düring and Gäth 2002; Smith and Tibbet 2004) of soil.

The post-treatment of dewatered sludge by composting or thermal drying is required to avoid sanitary problems and operational drawbacks, and to remove unpleasant odours and the possible presence of organic pollutants (Burés and Soliva 1984; Gross 1993; Villesot and Fery 1994). Co-composting of sewage sludge with pinewood ensures a more stabilized product (Burés and Soliva 1984; Villesot and Fery 1994). The stimulating effect of composted sludges on the biological activity of degraded soils has been shown to be lower but more

persistent than that of some dewatered sludges (Bernal et al. 1998; Moreno et al. 1999; Emmerling et al. 2000; Pascual et al. 2002). Effects of thermally dried sludge on physical and chemical properties of soil have been studied by Ojeda et al. (2006a, b). However, as far as we know, it is not known the effect of thermal drying or composting of a dewatered sludge on microbial and biochemical properties of soil.

β-glucosidase is the rate-limiting enzyme in the microbial degradation of cellulose to glucose. It plays a crucial role in the C cycle of soils (Pérez de Mora et al. 2005), is sensitive to soil management effects (Bandick and Dick 1999) and has been suggested to be an integrative measure of physico-chemical and biological soil properties (Turner at al. 2002).

Soil microbial biomass and soil respiration have been used to determine the effect of sludge amendment on soil microbiological properties (Alvarez et al. 1999; Debosz et al. 2002; Saviozzi et al. 2002; Barajas-Aceves 2005). The metabolic quotient qCO_2 (Pirt 1975), which is the C_{CO2} –to-C_{mic} ratio, has been measured to assess the effect of environmental factors on microbial activity (Anderson and Domsch 1993; Wardle and Ghani 1995; Sparling 1997; Moreno et al. 1999; Saviozzi et al. 2002; Barajas-Aceves 2005).

Carbohydrates of sewage sludge, which have a microbial origin (Urbain et al. 1993), constitute a major factor in stabilizing aggregate of soil (Tisdall 1991; Robert and Chenu 1992; Safarik and Sanctrucková 1992). Extractable (K_2SO_4 soluble) carbohydrates are related to available C in soil (DeLuca and Keeny 1993) and can be related to microbial carbohydrates after cell lysis by $CHCl_3$ fumigation (Badalucco et al. 1990, 1992).

The aim of this study was to compare the effect of thermically dried, composted or no post-treated dehydrated sewage sludge on β-glucosidase activity, total and extractable carbohydrates, basal respiration and microbial biomass of two soils from working quarries, under laboratory conditions.

Material and methods

Soils and sludges

The clayey soil (CL) was a mixture of A and Bw horizons of a soil developed over limestone and dolomite from a quarry located in Alcover (South Catalonia) and had the following characteristics: pH 8.7; lime 25.4%; organic C 0.5%; total N 0.038%; sand 31%; silt 30%; clay 39%.The sandy soil (SA) was the B horizon of a soil developed over limestone and sandstone. It was located in San Fost de Campcentelles (Central Catalonia) and had the following characteristics: pH 8.8, lime 0.6%; organic C 0.25%; total N 0.006%; sand 77%; silt 8%; clay 16%.

The dewatered sludge (DS) was from the waste water treatment plant in Blanes (North Catalonia), a medium-sized coastal town with a low contribution of industrial residues. The sludge was subjected to anaerobic digestion and partially dewatered by centrifugation. It had the following characteristics: moisture 79.7%; total organic matter 669 g kg^{-1} (d.m.); stable organic matter 39.5%; total N 46.6 g kg^{-1}(d.m); total P 18.9 g kg^{-1}(d.m.); pH 8.3; electrical conductivity 1.80 dS m^{-1}. The composted sludge (CDS) was obtained by composting the dewatered sludge with pine wood splinters as a bulking agent, in a tunnel for 15 days; in the tunnel the temperature reached 65ºC; the splinters were then removed by sieving and the compost was left to mature for two months. It had the following characteristics: moisture 33.5%; total organic matter 643 g kg^{-1} (d.m.); stable organic matter 46.2%; total N 33.6 g kg^{-1}(d.m); total P 15.5 g kg^{-1}(d.m.); pH 7.4; electrical conductivity 5.84 dS m^{-1}. The thermally dried sludge (TDS) was obtained by drying the dewatered sludge in a heated cylinder with a rotary system for 15 minutes. It had the following characteristics: moisture 15.3%; total

organic matter 675 g kg^{-1} (d.m.); stable organic matter 37.0%; total N 44.5 g kg^{-1}(d.m); total P 17.8 g kg^{-1} (d.m.); pH 7.0; electrical conductivity 5.28 dS m^{-1}.

Total organic matter was determined by calcination at 560º C. Organic matter stability (that is, the percentage of total organic matter resisting acid hydrolysis) was determined according to the standard method of the Ministere de l'Agriculture de Belgique (1971); briefly 1.5 g of oven dried (110ºC) sludge were hydrolysed with 10 ml of 72% H_2SO_4 at room temperature for 3 h (Stevenson 1982); then the mixture was diluted to 400 ml with distilled water, gently boiled under reflux for 5 h, cooled and filtered; the acid insoluble residue was washed, dried (105º C) and its organic matter content determined by calcination at 560º C.

Incubation experiment

Each sludge was mixed with each soil in a cement mixer at two different C rates: 2% (low dose, L) and 4% (high dose, H) on a dry weight basis in the final mixture. Applied amendments were equivalent to 100 (L) and 200 (H) Mg ha^{-1}(dry weight basis). Each mixture, and the control soils with no sludge added (50 kg each), were then placed in polyethylene containers (9 l) and incubated for nine months at 25º C; soil moisture was kept constant at 30% of the field capacity. Three containers (replications) were prepared for each treatment. Sampling was performed after 7, 67 and 267 days of incubation by taking four soil cores (0-25 cm) from each container; then soil cores from the same container were mixed in a composite sample and sieved (< 2 mm). The long lasting sludge effects over the soils were assumed to be measurable after 267 days.

Analytical measurements

Microbial biomass and soil respiration were immediately determined in fresh samples. Samples were air dried for two days and stored at room temperature for one year before the measurement of total and extractable carbohydrates and β-glucosidase activity

Content of total carbohydrates (TCH) was determined as reported by Cheshire and Mundie (1966) and extractable (soluble in 0.5 M K_2SO_4) carbohydrates (ECH) by Badalucco et al. (1992). Microbial biomass carbon (MBC) was determined by the fumigation-extraction method (Vance et al. 1987) and basal respiration (BR) as the CO_2 produced after 20 days of incubation at 25º C, as reported by Anderson (1982).

The β-glucosidase (E.C 3.2.1.21) activity was determined as reported by Tabatabai (1982) without using toluene because of the short incubation time (1 hour); calibration plots of p-nitrophenol were prepared by using each sludge treated soil, so as to keep into consideration the relative adsorption of p-nitrophenol by each soil (Vuorinen 1993).

The qCO_2 and the ECH/TCH ratio of each treated soil were also calculated.

Depending on the measured parameter, measurements in each container were not replicated or replicated three times.

Statistical analysis

For each soil, the influence of three fixed main factors (sludge type, sludge dose and incubation days) and one random factor (container) on the studied parameters were considered. General Linear Models (GLM) were used to evaluate the influence of the different factors on the measured variables. Data were analyzed using the Statistics Analysis System software, and the GLM procedure was performed using variance tests (SAS 1990). Separation of means was made according to the Tukey-Kramer procedure (at the level of α=0.05).

Results and Discussion

Global effects

ANOVA analysis shows that sludge type and dose had (with a few exceptions) significant effects on the measured parameters of the two soils at three incubation times (Table 1). The

ECH and TCH contents were the most affected by the type and dose of sludge. Both BR and MBC were more affected by dose than by sludge type and the highest effects on these parameters were observed at the longest incubation time. Since values of the measured parameters were always higher at the higher dose (data not shown) we decided to present the mean values of the two doses. Generally, mixtures containing TDS had the highest values and those containing CDS the lowest values (Tables 2 and 3).

Several authors (Giusquiani et al. 1989, Moreno et al. 1999, Pascual et al. 1999, García et al. 1993; Emmerling et al. 2000; Saviozzi et al. 2002, Usman et al. 2004, García-Orenes et al, 2005) have found that the increase in soil microbial biomass, carbohydrates content and biological activity of soil depend on the application waste rate and on the proportion of decomposable organic matter of the waste. Our results confirm these findings, since TDS had the lowest and CDS the highest proportion of stable organic matter. Moreover, Mejía (2005, unpublished Ph.D. thesis) found that thermal drying of our dehydrated sewage sludge caused a depolymerazition of the protein N of DS by using SDS-PAGE and HPLC separation techniques. It may be hypothesized that thermal decomposition (Irwin 1982; Blazsó and Jakab 1985) occurred in DS organic matter during thermal drying and that this decomposition made the organic matter more easily decomposable. In the case of basal respiration and β-glucosidase activity (Tables 2 and 3), the enhancement effect of the added sludge decreased with time, probably because microbial activity was affected by the amount of available organic matter which decreased with time.

Changes in the parameters over the incubation time

Values of microbial biomass carbon, basal respiration and the metabolic quotient are shown in Table 2, whereas β-glucosidase activity and contents of extractable and total carbohydrates and their quotient are shown in Table 3.

Soil microbial biomass carbon (MBC) was initially 1.5 times higher, by considering the average value of CL and SA soils, when TDS treatment was compared with the other two treatments; this was probably due to the higher content of more easily decomposable organic compounds in the former than in the latter sewage sludges. By considering the average values of both CL and SA treated soils, basal respiration (BR) was initially 20, 20 and 10 times higher when TDS, DS and CDS were added to soil, respectively. The qCO_2 values were higher in soils treated with the lower sludge dose throughout the incubation period (data not shown), probably due to the presence of substances inhibiting microbial activity in the sludge. There was a decrease in MBC and BR in all mixtures by prolonging the incubation. The decrease was greater in TDS treated soils especially between 7 and 67 days, when the intense mineralization probably occurred. A decrease was also observed in the qCO_2 of the TDS treated soils because the degradable organic C was probably decreasing. In CDS treated soils the decrease in BR was small and the metabolic quotient, which was initially lower than in the other treated soils, showed no significant change. The results of the CDS treated soils may be explained by the higher organic matter stability of CDS than the other used sludges.

Suprimit:

Soil β-glucosidase activity and total and extractable carbohydrates were initially enhanced (although less than soil respiration) by sludge addition. The effect was higher in sandy than in clayey soil probably due to the initial lower values of the former than the latter soil. The enhancement was similar for the three mentioned parameters (about 12, 9 and 6 times) by considering the average values of both soils treated with TDS, DS and CDS respectively.

During the incubation period the enzyme activity decreased more in the TDS and DS treated soils than in the CDS treated soils. It may be hypothesized that β-glucosidase activity was partly associated with stabilized organic matter since CDS has the highest proportion of stable organic matter. Miller and Dick (1995) found that β-glucosidase was associated with

humus colloids. It is important to underline that we measured the enzyme activity in air-dried soils which were stored for about one year, and probably β-glucosidase activity of non-proliferating cells and immobilized enzyme were prevailing over β-glucosidase activity associated to proliferating cells (Bonmatí et al. 2003). A durable increase in soil β-glucosidase activity by addition of composted sewage sludge was also observed by Moreno et al. (1999).

Total (TCH) and extractable (ECH) carbohydrates content generally showed small changes during the controlled incubation; only a significant increase of TCH could be detected in clayey soil treated with CDS after 267 days. It may be hypothesized that a minor proportion of the recalcitrant (and hence resistant to the acid hydrolysis) organic matter was decomposed during incubation with release of carbohydrates (Gigliotti et al. 2001). Thus depolimerysation of recalcitrant carbohydrates seems to prevail over mineralization in CDS-clayey soil mixtures, which is coherent with the previously stated smaller decrease in respiration in these mixtures during the controlled period. Pascual et al. (1999) also found few changes in TCH values in their 360-day incubation of dewatered and composted sewage sludge-soil mixtures; the authors hypothesized the microbial origin of TCH to explain the stability of this parameter over time.

The initial value of the ECH-to-TCH quotient was always higher in TDS treated soils, a fact that again confirms the higher content of labile organic matter of this sludge. In all the mixtures the quotient generally reached its maximum value after 67 days, coinciding with the supposed most intense mineralization of organic matter.

BR, MBC and β-glucosidase activity were the properties which best distinguished the long-lasting differences (267 days of incubation) among sludge type effect on the soils (Tables 2 and 3). BR was especially interesting since it reproduced the same trend: soil-DS > soil-TDS ≥ soil-CDS.

Conclusions

It has been demonstrated that thermally drying or composting modifies the stimulating effect of the dehydrated sewage sludge on biochemical and microbiological properties of soils from limestone quarries and that the rank order of this effect was initially TDS ≥ DS ≥ CDS. The increases depended generally on the amount of sludge applied and, except in the case of the carbohydrates contents, the effect decreased with time. The increase was also generally higher in SA than in CL soil, probably depending on the lower initial organic matter content of the former than the latter soil. Basal respiration was the only property reproducing the same trend in long term differences among sludge type effects in any of the two soils.

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Table 1. ANOVA table showing F-value of basal respiration (*BR)*, microbial biomass carbon (*MBC)*, microbial metabolic quocient (*qCO_2)*, β - glucosidase activity, and contents of extractable carbohydrates*(ECH)* and total carbohydrates *(TCH)* in soils at three times.

Soil	Time	Factor	BR	MBC	qCO_2	β - glucosidase activity	ECH	TCH	ECH/TCH
CL	7 days	Sludge	36.1***	6.7*	26.9***	22.2***	45.5***	74.5***	7.6**
		Dose	45.7***	83.3***	12.7**	6.5*	71.0***	144.7***	8.2*
	67 days	Sludge	3.0ns	8.8**	8.3**	20.6***	31.0***	148.6***	1.3ns
		Dose	9.3**	39.9***	2.7ns	16.4**	76.5***	170.4***	3.5ns
	267days	Sludge	27.6***	10.8**	0.80ns	14.3***	34.2***	35.0***	94.4***
		Dose	77.1***	36.3***	0.8ns	6.9*	63.7***	277.2***	19.2***
SA	7 days	Sludge	6.2*	12.6**	3.8ns	23.8***	108.0***	12.6***	15.4***
		Dose	6.0*	38.2***	0.5ns	24.0***	165.2***	110.2***	2.8ns
	67 days	Sludge	47.1***	3.3ns	14.2***	23.3***	88.7***	73.6***	28.5***
		Dose	21.1***	27.3***	6.6*	44.2***	64.4***	161.6***	10.3**
	267days	Sludge	13.1***	30.2***	6.5*	26.4***	169.1***	63.6***	17.8***
		Dose	53.2***	52.5***	5.3*	19.1***	139.2***	213.4***	0.1ns

*, ** and*** indicate significance at $p<0.05$, $p<0.01$ and $p<0.001$, respectively. ns:non significant

.*CL*, clayey soil, *SA* sandy soil.

Table 2. Basal respiration, microbial biomass carbon and microbial metabolic quotient of control and sludge treated soils.

Type of soil:	Type of sludge added to soil:	Basal respiration μg C-CO_2 g^{-1} h^{-1}			Microbial biomass carbon μg Cg^{-1}			Microbial metabolic quotient μg C-CO_2 mg^{-1} C-MB h^{-1}		
		7 days	67 days	267 days	7 days	67 days	267 days	7 days	67 days	267 days
CL	TDS	7.3Aa	1.7Ab	0.9Bb	1234Aa	481ABb	346Ab	7.6Aa	3.7ABb	2.5 Ab
CL	DS	5.1Ba	1.3Ab	1.1Ab	759 Ba	625 Aa	406Aa	6.7Aa	2.1Bb	3.1 Ab
CL	CDS	2.3Ca	1.7Ab	0.6Cc	997ABa	370 Bb	221Bb	2.7Bb	5.5Aa	3.1Ab
CL	Control	0.4a	0.3a	0.4a	nd	nd	nd	-	-	-
SA	TDS	6.6Aa	1.1Bb	0.7Bb	1080Aa	587Aab	456Ab	6.5Aa	1.9Bb	1.5Bb
SA	DS	7.0Aa	2.1Ab	0.8Ab	671Ba	493Aab	286Bb	10.1Aa	4.5Ab	3.1ABb
SA	CDS	2.8Ba	1.9Aab	0.6Bb	626Ba	424Aab	183Cb	5.3Ba	5.3Aa	3.9Aa
SA	Control	0.4a	0.4a	0.3a	nd	nd	nd	-	-	-

Clayey soil *CL*, Sandy soil *SA*. Dewatered sludge *DS*, Thermally dried sludge *TDS,* Composted sludge *CDS*. Overall mean values (including both doses). For each soil, values not followed by the same capital letter/lower case show significant differences ($p<0.05$) between mixtures/sampling dates. Number of observations=6. nd: not determined.

Table 3. β -glucosidase activity, total and extractable carbohydrates of control and sewage sludge treated soils.

Type of soil	Type of sludge added to soil	β -glucosidase activity μmol PNP[(a)] $h^{-1}g^{-1}$			Extractable carbohydrates mg glucose g^{-1}			Total carbohydrates mg glucose g^{-1}			Extractable carbohydrates/ Total carbohydrates		
		7 days	67 days	267 days	7 days	67 days	267 days	7 days	67 days	267 days	7 days	67 days	267 days
CL	TDS	0.71Aa	0.32Ab	0.21Ac	0.35Aa	0.52Aa	0.32Aa	4.22Aa	4.33Aa	5.10Aa	0.08Ab	0.12Aa	0.06Bc
CL	DS	0.38Ba	0.29Ab	0.19Ac	0.16Bc	0.43Ba	0.35Ab	2.98Ba	3.21Ba	3.81Ba	0.05Bc	0.14Aa	0.10Ab
CL	CDS	0.25Ba	0.21Bb	0.15Bc	0.18Ba	0.29Ca	0.19Ba	2.50Cb	2.24Cb	3.85Ba	0.07ABb	0.13Aa	0.05Cb
CL	Control	0.09a	0.12a	0.11a	0.04b	0.06b	0.09a	0.72b	0.85b	1.27a	0.06a	0.07a	0.08a
SA	TDS	0.34Aa	0.26Ab	0.16Ac	0.37Aa	0.24Ba	0.27Aa	2.65Aa	2.80Aa	2.38Aa	0.15Aa	0.09Cb	0.11Ab
SA	DS	0.23Ba	0.18Bb	0.08Cc	0.18Bc	0.36Aa	0.24Ab	2.29Aa	2.57Aa	2.38Aa	0.08Bb	0.14Aa	0.10Ab
SA	CDS	0.19Ba	0.16Ba	0.12Bb	0.21Ba	0.16Cb	0.08Bc	1.76Ba	1.35Ba	1.62Ba	0.12Aa	0.12Ba	0.07Bb
SA	Control	0.02a	0.01b	0.01b	0.02a	0.03a	0.02a	0.24a	0.22a	0.25a	0.07a	0.13a	0.09a

Clayey soil *CL*, Sandy soil *SA*. Dewatered sludge *DS*, Thermally dried sludge *TDS,* Composted sludge *CDS*. Overall mean values (including both doses). For each soil, values not followed by the same capital letter/lower case show significant differences ($p<0.05$) between mixtures/sampling dates. Number of observations=18 . [(a)] PNP, p-nitrophenol