

NO-LINEAR RESPIRATION DYNAMICS IN A DEGRADED ALFISOL AMENDED WITH DIFFERENT DOSE OF SALMON SLUDGES

J.E. Celis^{1*}, M.A. Sandoval², and N. Bello²

¹Departamento de Ciencias Pecuarias, Facultad de Ciencias Veterinarias, Universidad de Concepción, Av. Vicente Méndez 595, Chillán, Chile.

²Departamento de Suelos y Recursos Naturales, Facultad de Agronomía, Universidad de Concepción, Av. Vicente Méndez 595, Chillán, Chile.

*Corresponding author: jcelis@udec.cl

ABSTRACT

No research has been conducted regarding respiration model parameters as a consequence of the application of salmon sludge on degraded Alfisols. In order to investigate kinetic parameters representing the decomposition of salmon sludge by means of respiration rates, sediment samples from a land-farmed pisciculture and a lake-salmon facility were applied to a degraded Alfisol at the Biobío region (Chile). Soil microbiological activity was evaluated on a 60-days laboratory incubation experiment under different salmon sludge addition dose: T₁: 25 t ha⁻¹; T₂: 50 t ha⁻¹; T₃: 75 t ha⁻¹; T₄: 100 t ha⁻¹; T₅: 150 t ha⁻¹. A non-amended soil served as control. CO₂-C production was determined in samples under closed incubation. A two-component first-order regression model was applied to CO₂-C curves to obtain kinetic parameter values of C mineralization. The analysis confirmed that microorganisms increased their respiratory activity with time and increased sludge concentration ($p \leq 0.05$). A higher microbial activity was noted when soil was amended with land-farmed sludge at 100 and 150 t ha⁻¹, particularly after 30 days of incubation. The model showed that microorganisms needed more time to decompose labile C compounds in the salmon sludge when dose increased above 100 t ha⁻¹. After 60 days of incubation, land-farmed sludge amended at 25 t ha⁻¹ accumulated 44% of CO₂-C from a labile C source and 56% from a mineralized C pool, whereas at 150 t ha⁻¹ showed a 76% of a labile C and 24% mineralized C.

Keywords: Aquaculture, decomposition of organic matter, k-value, biosolids, soil remediation, biological activity.

INTRODUCTION

The explosive demand for fish in the world has implied Chile increased salmon production, rising from 50,000 annual tons in 1990 to near 400,000 tons in 2007, placing it among the main producer of salmon-farmed around the world (Salazar *et al.*, 2005).

In spite of the temporal brake that affected Chilean's salmon exports in last months, this remarkable development also has generated important volumes of organic residues from intensive salmon-farming facilities, representing a problem of contamination for aquatic ecosystems

(Teuber *et al.*, 2007). Solid wastes correspond mainly of no consumed food and fecal material that accumulates on pool's bottom (in the case of a land-farming salmon pisciculture) or on lake's bottom (case of a lake-salmon farming facility).

Organic materials addition to soil stimulates microbial population so improving the fertility and properties of some Chilean volcanic soils (Millaleo *et al.*, 2006). Also, it has been found that the application of salmon wastes increased microbiological activity in degraded Patagonian soils (Celis *et al.*, 2009). On the other hand, there is an enormous potential for recycling organic residues in Alfisols of the Coastal Mountains at the Biobío region (Chile), even though there is a lack of appropriate studies on the subject. These soils are located in the Chilean Sub-Humid Interior Dryland of the Coastal Mountains, which constitutes one of the regions more affected by erosion, amounting around 2 million hectares between the latitudes 32° 00'S and 37° 00'S (Pérez and González, 2001). These soils were highly impacted in the past 100 years to satisfy cereal demand production, actually having very low OM content in the profile, and usually no A horizon. Chilean Sub-Humid Interior Dryland of the Coastal Mountains is an enormous area considered marginal and poor, which has been severely affected by anthropogenic activities that have practically exhausted almost all its natural resources (Muñoz *et al.*, 2004). In this area, a rural population of approximately 350,000 people lives in very hard living conditions (Santibáñez and García, 2000). A study performed on these soils concluded that degradation due to erosion decreased microbial biomass as directly vegetal cover decreased (Muñoz *et al.*, 2004). This implies a great potential for recovering these degraded soils as being used for recycling salmon sludges.

For biosolids can be successful applied in soils as organic amendments, a high degree of OM decomposition is needed to reach their biological stabilization, task that is carried out by a variety of microorganisms, as microfauna such as protozoa, bacteria, fungi, actinomycetes and yeasts (Pinochet *et al.*, 2001). Biological stabilization implies an inhibition of the nitrification process. Nitrate, resultants of nitrification, is a very important polluting agent for underground and superficial water (Sawyer *et al.*, 2000). For this reason, its reduction by microbial activity is an indicator of sludge stabilization. Sludge addition to soil increases microbial biomass because it incorporates a new microbial load to soil and also stimulates growth of the native microbiota due to a fresh C source (Ros *et al.*, 2006). On the other hand, other studies reveal a decrease on microbial population for disturbing natural soil communities as a result of sludge addition (Jonsen *et al.*, 2001, Crecchio *et al.*, 2004).

The most important biological activities are those related to the OM and nutrient cycling (Alvear *et al.*, 2007). Respiration is one of more frequently parameters used to quantify microbial activity in soil (Zagal *et al.*, 2002). It is directly related to decomposition of organic residues of different types and sources (Sylvia *et al.*, 1998). Metabolic activity performed by microorganisms in the decomposition process, can be determined by means of CO₂ production or by the O₂ consumed by microorganisms (Alef, 1995).

Chilean environmental regulations are recently being focused on protecting soils, where one of the parameters that need to be very well controlled is quantity of the sludge applied to soils. For that reason, it is extremely important to know the OM decomposition kinetic, thus adding information for designing better

management practices mentioned by these environmental regulations. Unfortunately, there is a lack of research on the application of models in order to determine the values of the kinetic constants participating in C mineralization, a useful tool for estimating OM decomposition. In studying decomposition of organic materials, regression models have been used by several authors (Jenkinson, 1977; Janssen, 1984; Cheshire *et al.*, 1988; Saviozzi *et al.*, 1993). The two-component first-order regression model proposed by Jenkinson (1977) has been used successfully to describe the decomposition of plant residues (Marstorp and Kirchmann, 1991), and it is also expected to be useful to describe decomposition of salmon sludge. Consequently, the aims of this study were: 1) to determine microbial CO₂-C evolution in a degraded Alfisol of the Biobío region amended with different amounts of salmon sludges; 2) to obtain no-linear coefficients from respiration curves to be correlated with OM decomposition.

MATERIALS AND METHODS

***In situ* activities**

Sludge samples were obtained from two production systems of a salmon-farming industry located at southern Chile: (i) sediments extracted from pools of a land-farmed salmon pisciculture (land-farmed salmon sludge) and, (ii) sediments taken at a depth of 20 m beneath lake-salmon cages by using dredge (lake-farmed salmon sludge). The samples of the Alfisol were obtained at a depth of 20 cm, coming from a sector of the Coastal Mountains of the Biobío region (Chile), near to the city of Quillón (36°40'S and

72°27'O). Taxonomic classification of the soil studied corresponded to an Ultic Palexeralfs, a granitic soil highly physical-chemical and biological degraded, clayed texture, Cauquenes series, with slopes higher than 15% (Stolpe *et al.*, 2008). Sampling area belonged to Sub-Humid Interior Dryland of the Coastal Mountains, located in the eastern slope of the mountains, having a typically arborescent coastal scrub vegetation. Climate is Mediterranean with a prolonged dry station (six months), followed by a humid period with annual precipitation averaging 1,100 mm (Celis *et al.*, 2007).

***In vitro* activities**

Physical and chemical characterization of samples (soil and sludge) was determined at laboratories of the Department of Soil and Natural Resources of the University of Concepcion (certified by the Chilean Soil Science Society, since 2000). Table 1 shows some physical and chemical properties of soil and salmon sludge.

Sludge and soil samples were maintained to temperatures below 10°C before their processing in laboratory. After that, samples were dried to room temperature (15% humidity, dry weight basis) and sieved (2 mm mesh). Immediately, different mixtures of sludge/soil based on 1 kg of soil each were prepared. Treatments consisted of different doses of sludge added to soil: T₁ = 25 t ha⁻¹; T₂ = 50 t ha⁻¹; T₃ = 75 t ha⁻¹; T₄ = 100 t ha⁻¹; T₅ = 150 t ha⁻¹. A control (no sludge added to soil) was considered. The amended soil samples were homogenized, packaged in plastic bags, and then placed in a chamber (with temperature and humidity automatically controlled at 25°C and 60-70%, respectively) for a 10-days pre-incubation period.

Table 1. Soil and salmon sludge characteristics (n = 3, ± s.e.m).

Parameter	Alfisol	Salmon sludge	
		Land-farmed	Lake-farmed
Da (g cm ⁻³)	1.4 ± 0.2	0.9 ± 0.1	0.8 ± 0.4
pH (al agua)	5.6 ± 0.5	7.0 ± 0.6	6.7 ± 0.3
OM (%)	2.5 ± 0.2	20.7 ± 1.3	18.3 ± 1.5
N (mg kg ⁻¹)	9.8 ± 0.8	1,695.3 ± 10.4	746.0 ± 11.3
P (mg kg ⁻¹)	5.4 ± 0.9	480.0 ± 9.7	19.9 ± 3.2
K (mg kg ⁻¹)	129.8 ± 2.7	30.0 ± 8.9	120.8 ± 9.5
CIC (cmol (+) kg ⁻¹)	5.5 ± 1.1	1.7 ± 0.7	19.3 ± 1.3
C/N	9.3 ± 0.6	10.5 ± 1.3	7.5 ± 1.1

All the prepared mixtures were used for determining soil respiration, following the procedure described in literature (Zagal *et al.*, 2002; Celis *et al.*; 2009). For the incubation process, samples consisting on 25 g of sludge/soil mixture were placed in glass bottles of 1 L capacity. Then, a test tube with 15 mL of solution 0.1 M NaOH (to absorb CO₂ produced by microorganisms), and a tube with deionized water (to keep humidity constant) were placed in each jar. Following, each bottle was sealed hermetically with parafilm. A total of 44 bottles were set up for the experiment, considering the treatments (5), the control, the type of sludge (2) and replicates (4). Following, bottles were placed in an incubation chamber at 22°C by 60 days. Measurements of no neutralized NaOH along time were accomplished by titration (0.1 M HCl). Then, 1 mL NaOH was extracted (in duplicate), and 2 mL BaCl₂ was added to precipitate the inorganic C as insoluble BaCO₃. Drops of phenolphthalein were added as an acid base indicator, so no neutralized NaOH was titrated directly with HCl (Ianotti *et al.*, 1994). The amount of CO₂-C generated from the

incubated mixtures was calculated by means of the formula proposed by Anderson (1982).

To obtain no-linear kinetic coefficients, the accumulated amounts of CO₂-C measured during the incubation were fitted to a two-component first-order model, following the mathematical equation suggested by Jenkinson (1977):

$$(1) \quad Y = C_1 \cdot (1 - e^{-k_1 \cdot t}) + C_2 \cdot (1 - e^{-k_2 \cdot t})$$

where Y is the microbial respiration (µg CO₂-C g⁻¹ soil), C₁ and C₂ are different pools of carbon subject to mineralization (µg CO₂-C g⁻¹ soil), k₁ and k₂ are rate constants for the mineralization process (d⁻¹), and t is the time involved in the process (d).

Statistical techniques

Results obtained from *in vitro* respirometry trials were expressed as µg CO₂ - C g⁻¹ soil dry matter basis, oven at 105°C (24 h). Cumulative curves on CO₂-C evolution were subjected to non-linear regression analyses using the NLIN procedure of SAS (SAS Institute Inc, Cary, NC, USA). Mean values for each

sampling time were used. Data were processed by means of variance analysis ANOVA, and comparison among means was performed according to Turkey's test. In all cases the level of significance used was 95% ($p \leq 0.05$). The values were processed by means of statistical software SAS version 8.1 for Windows (SAS Institute Inc, Cary, NC, USA).

RESULTS AND DISCUSSION

Figure 1 shows the C-CO₂ evolution in the Alfisol amended with different doses of land-farmed sludge. In Figure 2 is shown microbial respiration in soil amended with lake-farmed salmon sludge.

When comparing both figures, it was noted amendments at 100 and 150 t ha⁻¹ (T₄ and T₅, respectively) notoriously produced more CO₂-C than other treatments ($p \leq 0.05$). It was particularly certain for land-farmed wastes after 30 days incubation. Similarly, lake-farmed sludge accumulated a significantly higher CO₂-C, but only 15 days incubation. In both cases a non-linear increasing trend on microbial activity along time was observed, similar to other studies carried out in amended Andisols using other organic residues (Zagal *et al.*, 2002; Guerrero *et al.*, 2007). It stated that amendments with salmon sludge clearly stimulated microorganism population when adding a new source of C to soil, in agreement with Ros *et al.* (2006).

In general, there was a higher microbial activity as the dose of sludge amended to the soil also increased, all the treatments being higher than the control. In the case of amendments with land-farmed sludge after 20 days incubation, amended treatments accumulated between 2 to 30 more CO₂-C than control. It seems that higher CO₂-C values had a greater C and energy availability for soil

heterotrophic microorganisms, as a consequence of an abundant provision of OM and N, so stimulating microbial activity (Zagal *et al.*, 2002). For that reason and following the same pattern, treatments with lake-farmed sludge showed lower CO₂-C values than land-farmed sludge amendments (sludge with higher N and P content, as shown in Table 1).

Evolution of CO₂-C due to sludge addition to the Alfisol suggest a positive effect on microbial activity, as similarly has been reported by some studies in other soils (Zagal *et al.*, 2002; Ros *et al.*, 2003; Varnero *et al.*, 2004; Teuber *et al.*, 2007). According to Esparza *et al.* (2004) soils with low OM content, as the Alfisol here investigated (2.5% OM), respond well to sludge addition. Similar results have been found in degraded Patagonian soils amended with salmon sludge (Celis *et al.*, 2009). It can be explained because the addition of organic wastes stimulates microbial growth so increasing OM decomposition (Barrena *et al.*, 2006).

Applying the two-component first-order model to the result of the respirometry experiment clearly fitted the non-linear form as also found by Marstorp and Kirchmann (1991). The two-component first-order model represents the data in Figures 1 and 2. These expressions lines are used in the discussion to define two sludge decomposition rates: a pool of labile C, and a pool of mineralized C. In Table 2 are shown estimated kinetic parameters, i.e. the rate constants and pool sizes as a result of the non-linear regression fitted to cumulative curves on CO₂-C evolution. Model seemed to be an acceptable two-component of C mineralization from sludge amendments in the Alfisol, based on high adjusted r² values. Carbon mineralization dynamics showed a decomposability of labile C compounds in the sludge material and a stabilized C not

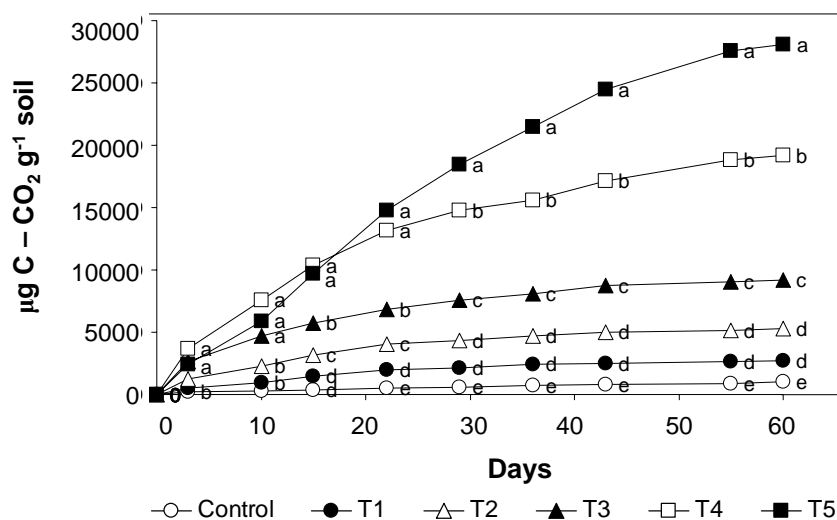


Figure 1. Accumulated C-CO₂ evolution during laboratory incubation of an Alfisol under different amendments with land-farmed salmon sludge (T₁: soil amended at 25 t ha⁻¹; T₂: soil amended at 50 t ha⁻¹; T₃: soil amended at 75 t ha⁻¹; T₄: soil amended at 100 t ha⁻¹; T₅: soil amended at 150 t ha⁻¹). Different letters at the same time are significantly different ($p \leq 0.05$).

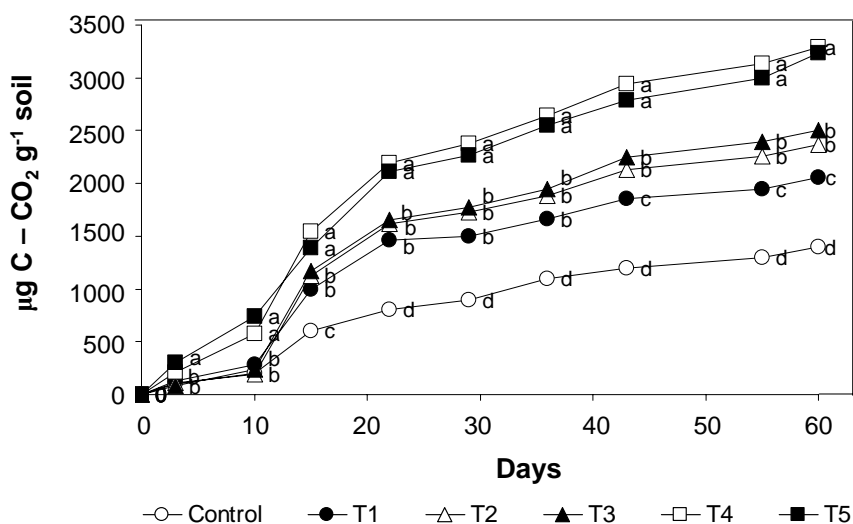


Figure 2. Accumulated C-CO₂ evolution during laboratory incubation of an Alfisol under different amendments with lake-farmed salmon sludge (T₁: soil amended at 25 t ha⁻¹; T₂: soil amended at 50 t ha⁻¹; T₃: soil amended at 75 t ha⁻¹; T₄: soil amended at 100 t ha⁻¹; T₅: soil amended at 150 t ha⁻¹). Different letters at the same time are significantly different ($p \leq 0.05$).

subject to mineralization during the period measured.

In the assessment, OM decomposition is considered to proceed in two steps: (1) decomposition of a labile C; followed by (2) decomposition of a mineralized C. The model used by Jenkinson (1977) assumes that the sludge can be approximated to two different pools of constant but different decomposability. The first term of the equation ($C_1 \cdot (1 - e^{-k_1 \cdot t})$) represents a pool of labile C and the second term ($C_2 \cdot (1 - e^{-k_2 \cdot t})$) a pool of mineralized

C. Thus, at the end of incubation, land-farmed sludge applied to soil at a rate of 25 t ha⁻¹ (T₂), produced an accumulated respiration of 2,947.9 µg CO₂-C g⁻¹ of soil. At this time, the proportion of labile C available for mineralization was about 44%, whereas the proportion of mineralized C was 56%. At the highest dose (T₅), an accumulated respiration of 29,611.1 µg CO₂-C g⁻¹ of soil was produced by microbial respiration, which was 10 times greater than T₂. The labile C was 76% as opposed to a mineralized C of 24%.

Table 2. Values of the constants for the equation ($Y = C_1 \cdot e^{-k_1 \cdot t} + C_2 \cdot e^{-k_2 \cdot t}$) describing C-CO₂ production from microorganisms in an Alfisol amended with different doses of salmon sludges (T₁: soil amended at 25 t ha⁻¹; T₂: soil amended at 50 t ha⁻¹; T₃: soil amended at 75 t ha⁻¹; T₄: soil amended at 100 t ha⁻¹; T₅: soil amended at 150 t ha⁻¹).

Treatments	k ₁	k ₂	C ₁	C ₂	r ²
	(d ⁻¹)		(µg C-CO ₂ g ⁻¹ soil)		
i) Land-farmed sludge					
Control	0.030	0.029	240	940	0.90
T ₁	0.037	0.031	1,460	1,950	0.91
T ₂	0.039	0.023	4,750	2,150	0.92
T ₃	0.049	0.033	7,110	4,160	0.90
T ₄	0.037	0.025	17,150	7,130	0.91
T ₅	0.027	0.021	27,870	10,130	0.90
ii) Lake-farmed sludge					
Control	0.031	0.029	1,170	470	0.89
T ₁	0.035	0.026	1,430	970	0.91
T ₂	0.034	0.025	1,640	1,090	0.92
T ₃	0.036	0.027	1,870	980	0.90
T ₄	0.031	0.024	2,870	1,080	0.92
T ₅	0.029	0.025	2,860	1,040	0.93

Comparatively, lake-farmed sludge amended at the lowest dose (T_2) showed an accumulated respiration from a labile C source of $1,426.7 \mu\text{g CO}_2\text{-C g}^{-1}$ of soil (63%), and a respiration from a pool of C mineralized of $846.8 \mu\text{g CO}_2\text{-C g}^{-1}$ of soil (37%), for an accumulated of $2,273.5 \mu\text{g CO}_2\text{-C g}^{-1}$ of soil. The highest dose (T_5) tested showed a 75% from a labile C and 25% from a C mineralized, amounting $3,165.9 \mu\text{g CO}_2\text{-C g}^{-1}$ of soil, which was 39% higher than the accumulated $\text{CO}_2\text{-C}$ of T_2 . A cumulative $\text{CO}_2\text{-C}$ evolution along time clearly showed two stages representing the decomposition of the active and less readily decomposable organic carbon, respectively. This response was also reported by Saviozzi *et al.* (1993) when studying the decomposition of vegetation-water sludge in soil. Also, a similar kinetic trend was found by Marstorp and Kirchmann (1991) on determining $\text{CO}_2\text{-C}$ from decomposing plant material in a pot experiment.

Previous data showed microorganisms needed more time to decompose labile C compounds in the salmon sludge when the dose increased. The model proposed by Jenkinson (1977) described well the decomposition kinetics of salmon sludge. The parameter values calculated can be used for comparisons of different sludges applied to soil and for predictions of the kinetics under changing environmental conditions. The results also contribute to the development of kinetic models to describe decomposition and oxidation of sludges over a wide range of temperatures (Shanableh and Imteaz, 2008). Finally, this information seems to be very useful for making decisions on applying salmon sludge in Alfisols, and therefore is necessary to take more precautions when using salmon sludge as amendments to soil at rate higher than 100 t ha^{-1} .

CONCLUSIONS

Salmon sludge addition stimulated soil microbial population in the degraded Alfisol for an incubation period of 60 days. Higher microbial activity was directly correlated with increased dose of salmon sludge, showing a notorious impact on biological activity when land-farmed sludge was amended to soil at 100 and 150 t ha^{-1} . Amendments represented a new C source to the Alfisol, so stimulating microbial activity.

The two-component first-order model showed a good correlation with data of the $\text{CO}_2\text{-C}$ evolution along time. In this experiment C mineralization dynamics showed the presence of a source of a labile C and a pool of a mineralized C. The first term of the equation ($C_1 \cdot (1 - e^{-k_1 \cdot t})$) represented the pool of a labile C, whereas the second term ($C_2 \cdot (1 - e^{-k_2 \cdot t})$) refers to a pool of a mineralized C. At the end of the incubation period, land-farmed sludge amended to 25 t ha^{-1} accumulated 44% of $\text{CO}_2\text{-C}$ from a labile C source and 56% from a mineralized C pool, whereas at 150 t ha^{-1} showed a 76% of a labile C and 24% mineralized C.

The model showed that microorganisms needed more time to decompose labile C compounds in the salmon sludge when dose increased, so more care is needed when amending at high rates. The resulting kinetic model adequately represented OM decomposition of salmon sludge, in terms of $\text{CO}_2\text{-C}$ produced by soil microorganisms during respiration.

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