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Microcalorimetric qualitative analysis of biofilm development in porous media used in wastewater treatment by constructed wetland

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Abstract In wastewater treatment by constructed wetland, the biodegradation capability of the biomass developed in the soil is one of the most important factors. For this kind of treatment unit, soil properties are studied to improve its filtration capacity and hydraulic residence time of the wastewater. The impact of soil properties like porosity and soil components on biomass development and biodegradation capacity seem to be less studied certainly due to the complexity of microbial identification techniques currently used. The study presented here is a preliminary work to validate that calorimetric technique could be a tool in the understanding of biodegradation capacity of wastewater treatment processes. Biofilm is preliminary developed in columns filled with different porous materials of well known porosity and constitutive components. These columns are fed with the same continuous flow of synthetic solution (C, N, and P) as a substrate amending during 3 weeks. Then each week, 2 mL samples of porous media from these columns are analyzed in isothermal calorimeter for 48 h. Net heat flow is recorded before and after substrate injection. This work results in the definition of the procedure for batch experiments in calorimeter for wastewater process efficiency. The results of these experiments show that the microbial reaction due to substrate amendment is highly depending on the porous material used for biofilm growth. Indeed calorimetric signals recorded lead to conclude that biofilm grown on plastic beads has a faster and more intensive reaction to glucose amendment than biofilm grown on glass beads. At least, two glass beads samples analyzed in the calorimeter after the same duration of feeding with synthetic solution have very different response to glucose or synthetic solution.

Keywords Calorimetric · Biofilm · Wastewater treatment · Porous media

Abbreviations

TOC	Total organic carbon
CW	Constructed wetland
T	Temperature
Q_T	Total heat dissipated by the microbial growth reaction stimulated with glucose in J mL^{-1} of porous media
PT	Peak time corresponding to time before the maximum of the peak of heat flow recorded

Introduction

Constructed wetlands (CWs) are engineered units designed to involve wetland vegetation, soil, and microorganisms to treat wastewater. This system conjugates physicochemical separation of pollutants with the filter composed of the soil and the biodegradation by microorganisms developed in this soil and aerated by macrophytes roots. Macrophytes assimilate compounds for their growth such as nitrogen and phosphorus. Low energy requirement almost restricted to the pumping of the influent and easy maintenance make CWs an alternative to standard wastewater treatment processes especially for small communities. The classification of CW is based on the type of macrophyte grown up and on

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the water flow regime: horizontal flow, vertical flow CW, and hybrid systems (series of horizontal and vertical flow CWs). In these units, organic matter and nitrogen are removed from the sewage by many physical or chemical ways like filtration, adsorption but essentially through aerobic, anaerobic, and anoxic pathways from microorganisms of the soil [1]. Even if performances of CW are affected by seasons [2], Molle et al. have shown that these systems can support hydraulic overload [3]. The good results obtained on removal rate of organic matter, phosphorus and nitrogen make these systems used for more than 30 years for treatment of municipal sewage [4–7]. More recently, they have been used for treatment of industrial, urban, and agricultural sewages even [7–9].

However, clogging of the filter is the biggest operational problem of these systems. Even if several studies have shown that the clogging effect can be accelerated by high organic matter concentration of the influent or hydraulic overload [10, 11], clogging effect is still a complex phenomenon and the mechanisms of clogging effect are not yet understood precisely. Accumulation of organic matter in the filter contributes to the clogging of pore space by filling the porosity of the bed. This phenomenon potentially leads to the decrease of CW removal performance [3, 11, 12]. This organic matter can be constituted by organic materials from the influent but also by biofilm grown on filter porosity. However, Zhao et al. [11] have shown that the accumulation of organic matter particles in the pore space leads to faster clogging than biofilm growth. Moreover, biofilm can help delaying the clogging effect by the decrease of organic matter accumulation due to biodegradation process. In this case, the increase of biodegradation capacity leads at the same time to an enhancement of CW performance on organic matter removal. In the light of these recent studies, qualification of soil properties and the understanding of the associated microbial activity and type of metabolism, in these systems, becomes a fundamental issue to design and improve CW performance and stability. Few studies have been published on the effect of porous media on CW removal efficiency [13]. Akratos et al. have shown that size and chemical components of the gravel used as porous media in pilot scale CWs, had an effect on nitrogen and phosphorus removal but none on organic matter removal. Accordingly, the association of the porous media of the filter and the biofilm grown on it has to be explored together and not separately.

Since the beginning of the 20th century, thermodynamics of microorganism's metabolism are studied by microcalorimetric technique [14–17]. Recently, this technique has been demonstrated to be suitable to qualify microbial growth and activity in soils [18–22]. In these studies, the calorimetric signal recorded corresponds to the metabolic response of microorganisms of the analyzed soil following a glucose feeding. Very few studies are published about the response of soil microorganisms to another substrate than glucose closer than one of substrates naturally found in soil [23].

The aim of this study is to validate isothermal microcalorimetry as a suitable technique to qualify microbial activity in porous media of CWs. Porous media of a CW have been simulated in laboratory by columns fed with recycled synthetic wastewater for 3 weeks. A dedicated calorimetric analysis procedure has been defined to record calorimetric signal of different porous media. Two substrates have been tested to induce a metabolic response of microorganisms, glucose—as the reference in studies of microbial activity in soils by calorimetric measurements—and a synthetic solution which composition is closed to sewage concerning carbon and nitrogen concentrations.

Materials and methods

Biofilm growth

Biofilm is formed in PVC columns on aerobic conditions at ambient temperature. The columns are filled with 200 mL of beads as biofilm growth support. Then they are fed with a synthetic solution as substrate for biofilm growth and biodegradation (Fig. 1). Synthetic solution (Table 1) is used for inoculation and biofilm growth. Feeding substrate is continuously circulated by the means of a peristaltic pump with a flow rate of 1 L h^{-1}.

Experiments are followed on a 3-week period. Two materials have been tested (glass and plastic beads) to study the influence of the material on microbial development and/or activity. Specifications of the experimental setup are presented in Table 1.

Once a week a sample of the effluent has been collected at the bottom of the column and analyzed to determine its

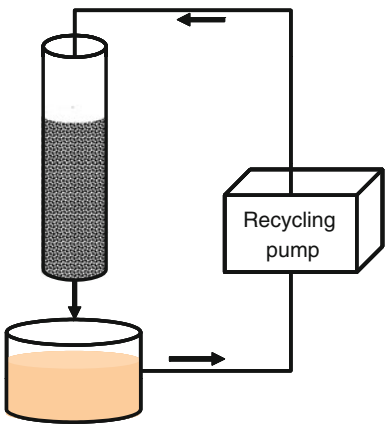

Fig. 1 Experimental setup for biofilm growth

Table 1 Specification of the experimental setup

Columns	
Diameter	30 mm
Volume	200 mL
Porous media	
Glass beads	
Porosity	0.4
Density	2300 kg m^{-3}
Diameter	1.6 mm
Surface-area-to-volume-ratio (calculated)	3750 m^{-1}
Plastic beads	
Porosity	0.4
Density	1000 kg m^{-3}
Diameter	1 ± 0.2 mm
Surface-area-to-volume-ratio (calculated)	6000 m^{-1}
Synthetic solution	
Components	
Organic carbon (from meat extract and sucrose)	380 ± 40 mg L^{-1}
Nitrogen (from ammonium chloride)	12 mg L^{-1}
Phosphorus (from phosphoric acid)	2 mg L^{-1}
Volumetric flow rate	1 L h^{-1}

total organic carbon (TOC) contains. At the same time a sample of beads with the associated biofilm has been analyzed by calorimetric measurements.

Calorimetric measurements

Calorimetric experiments are carried out into an isothermal 3D calvet calorimeter (SETARAM C80) equipped with a sample and a reference chambers. The reference chamber is empty and closed tight after have been dried. A blank run is regularly done to check base line deviation with both empty and dried cells.

All calorimetric measurements are performed in hermetic 12.5-mL stainless steel cylinder cells. Batch experiments are carried out for 48 h at 30 °C. 2 mL bead samples are introduced in the calorimeter sample cell. After 12 h under endogenous respiration, 1 mL of glucose solution (1 g L^{-1}) or a synthetic nutrient solution is introduced in the sample cell to stimulate microbial activity. The nutrient solution is made from meat extract and sucrose, as well as synthetic solution, to contain the same carbon concentration as the glucose solution i.e., 0.4 g C L^{-1}. Because of current configuration of the calorimeter, the cell has been taken out of the calorimeter for substrate introduction. Before closing the cell again, ambient air is renewed by the means of insufflated air.

Oxygen demand for biodegradation of 0.4 mg of organic carbon (contained in 1 mL of solution) is calculated in order that aerobic metabolism during calorimetric experiments could not be limited by chemistry. Oxygen diffusion in substrate solution is not considered as a limiting transfer due to the long period of measurement. Global reaction of glucose oxidation due to aerobic metabolism is assumed in its simplest way [15]:

$$C_6H_{12}O_6(aq) + 6O_2(aq) \rightarrow 6CO_2(aq) + 6H_2O(l). \quad (1)$$

It shows that a volume of 4 mL of air is necessary to oxidize all the glucose contained in the 2 mL volume of substrate. So, available air volume in the selected cells is sufficient to carry out these batch experiments.

After 48 h of experiments, analytical cell is weighted to be sure that no mass has been lost by vaporization.

Results and discussion

Influence of the porous media

Preliminary experiments

Before recording the behavior of biofilm samples, the different materials and substrates have been tested one by one in the calorimeter at 30 °C for 48 h.

Recorded signals for sterile porous media show very low or no heat exchange as well as the two substrates used to amend samples: glucose and synthetic nutrient (Fig. 2). These signals confirm that there is at least very weak or no biological activity in the different samples at initial time.

TOC analyses

The TOC analyzer (TOC-V Shimadzu) uses the combustion by catalytic oxidation. TOC is measured by injecting few milliliters of the sample into a heated combustion tube ($T = 680$ °C) packed with an oxidation catalyst. The water

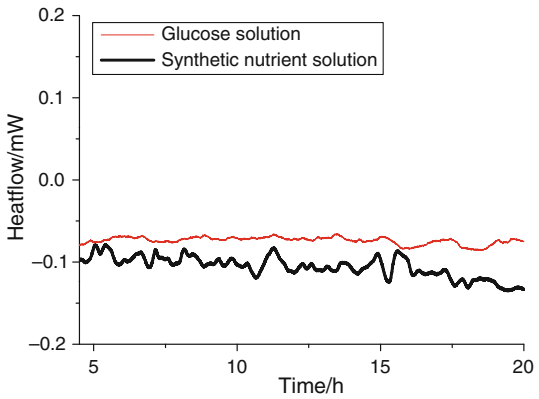

Fig. 2 Power-time curves of isothermal recordings of the two substrates ($T = 30$ °C)

is vaporized and TOC, the organic and inorganic carbon, is converted to carbon dioxide (CO_2). The CO_2 is carried with the carrier gas stream from the combustion tube to a non-dispersive infrared gas analyzer (NDIR) and concentration of CO_2 is measured. The TOC concentration of the sample is obtained by using the calibration curve prepared with standard solutions. The result is the mean of five analyses of the sample. Results of TOC analyses of the collected samples are presented in Table 2.

These results show that biofilm developed on the porous media leads to TOC removal. Three quarters of organic matter have been removed in 1 week for both samples. After 1 week, the kinetic of organic matter removal decreases but a removal rate of 90% can be observed after 3 weeks of experiments.

Calorimetric measurements

As it is explained in "Preliminary experiments", calorimetric experiments have been carried out in batch mode for 48 h at a temperature of 30 °C. This period is separated into two phases; the first (named 1 in Fig. 3) corresponds to an endogenous respiration period of 2 mL of beads extracted from the biofilm experimental setup, the second (named 2 in Fig. 3) corresponds to the response of microorganisms after substrate introduction (simple arrow).

For the following graphs, reference time is chosen to be the substrate injection time to compare easily the heat flow recordings. Figures 4 and 5 show the exothermic response to glucose introduction of the microorganisms grown on the porous media compared to the same sterile media.

For the same sample volume, signal recorded in the case of plastic beads is much higher than in the case of glass beads as shown in Fig. 6. However, porosities of these two materials are the same so must be hydraulic pathway and dedicated volume to biofilm development.

These results could be explained by two possibilities. The first hypothesis is that more biofilm has grown on

Table 2 TOC amount in collected samples

Time	TOC/mg L^{-1}	
	Glass beads column	Plastic beads column
0	356 ± 36	356 ± 36
1 week	92 ± 8	98 ± 10
2 weeks	76 ± 8	57 ± 6
2.5 weeks	46 ± 5	–
3 weeks	43 ± 5	–

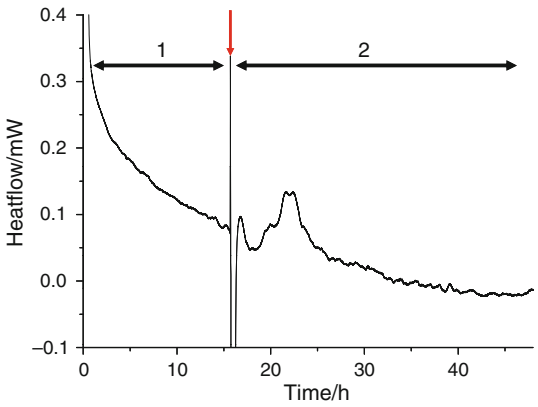

Fig. 3 Power-time curve of isothermal glucose response of 1 week glass beads ($T = 30$ °C) *1* endogenous respiration, *2* glucose response, simple arrow: glucose introduction

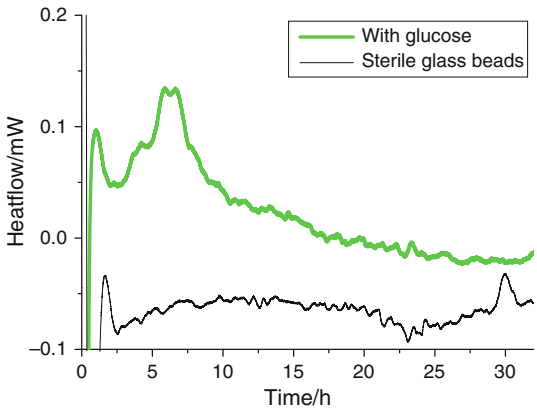

Fig. 4 Power-time curves of isothermal glucose response of sterile and 1 week glass beads ($T = 30$ °C) showing microbial growth reaction recorded from 2 mL of glass beads sample amended with 1 mL of a solution containing 1 mg glucose

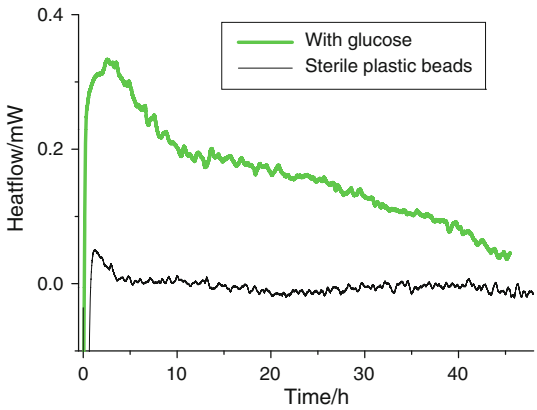

Fig. 5 Power-time curves of isothermal glucose response of sterile and 1 week plastic beads ($T = 30$ °C) showing microbial growth reaction recorded from 2 mL of plastic beads sample amended with 1 mL of a solution containing 1 mg glucose

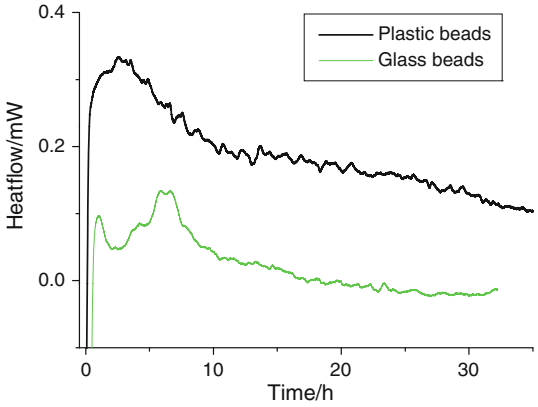

Fig. 6 Power-time curves of isothermal glucose response of 1 week plastic and glass beads ($T = 30$ °C) showing microbial growth reaction recorded from 2 mL of porous media samples amended with 1 mL of a solution containing 1 mg glucose

plastic beads, because of a better affinity and a bigger surface-to-volume-ratio than the glass beads, so higher is the microbial activity recorded. Second, physical properties of the biofilm depends on the media he grew on and in the case of plastic beads, its internal porosity leads to a better diffusion and/or absorption of oxygen and nutritive elements. This last proposal seems to be the more tangible.

Results about total heat flow dissipated and peak time obtained with samples of different porous media, after 1 or 2 weeks of continuous feeding with synthetic solution, and amended with 1 mL of glucose, are shown in Table 3.

First, the calculation of these parameters confirms that microbial activity is higher in the sample of plastic beads than in the sample of glass beads for the same duration of experiments. Second, microbial reaction in the case of plastic beads is faster than in the case of glass beads. These two result show that metabolic pathways are different and influenced by the porous media dedicated to biofilm growth.

Then calorimetric results show that microbial activity decreases between one and 2 weeks under continuous feeding with recirculated synthetic solution. This confirms TOC analyses of the effluent. The decrease of carbon concentration in the recirculated influent leads to a decrease of microbial activity due to substrate inhibition.

Table 3 Values of the total heat flow dissipated, Q_T by the microbial reaction due to glucose addition and peak time, PT which is the time before the maximum of the peak of microbial response to the substrate

	Types of porous media	Q_T/J mL^{-1}	PT/h
1 week	Glass beads	2.18	5.5
	Plastic beads	3.35	3
2 weeks	Plastic beads	1.70	3.25

Table 4 Values of the total heat flow dissipated, Q_T by the microbial reaction due to glucose or synthetic solution addition on a sample of glass beads and peak time, PT which is the time before the maximum of the peak of microbial response to the substrate

Types of substrate	Q_T/J mL^{-1}	PT/h
Glucose substrate	2.18	5.5
Synthetic solution	1.89	4

Influence of the substrate

In order to understand metabolic pathway involved in pollutant biodegradation in wastewater treatment processes, the synthetic solution has been used as nutrient substrate for calorimetric samples. Two samples of glass beads after 1 week of continuous feeding with synthetic solution have been analyzed in the calorimeter. After 12 h under endogenous respiration in the calorimeter, the samples have been amended with 1 mL of glucose for one and 1 mL of synthetic solution for the other one and the heat flow recorded (Table 4).

Figure 7 shows that the signal recorded with synthetic solution amendment is still exothermic like glucose response but very different from the previous one. The biofilm has been grown up with this synthetic solution so it is acclimated to this substrate. It can explain why microbial reaction is faster than in the case of glucose.

Moreover, this synthetic solution is containing more nutritive components (N, P…) than glucose solution that certainly induces different metabolic pathways of substrate degradation [19, 23].

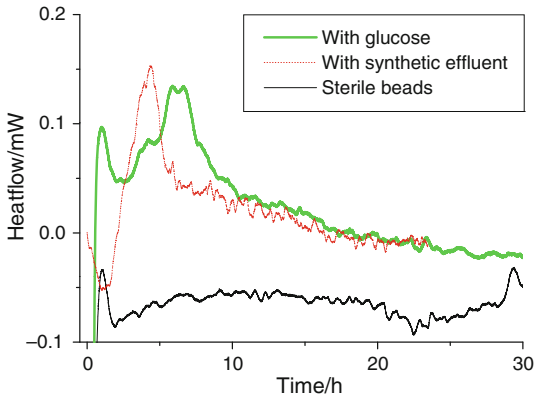

Fig. 7 Power-time curves of calorimetric response of 1-week-old glass beads on different substrates showing microbial growth reaction recorded from 2 mL of glass beads samples amended with 1 mL of a solution containing 1 mg glucose or a synthetic solution

Conclusions

This preliminary study shows that microcalorimetric technique is a suitable tool to study microbial activity in porous media irrigated with sewage. An experimental procedure has been defined to analyze this kind of media by calorimetric technique. Indeed synthetic solution has been successfully used for microbial activity recordings on calorimeter. Experiments have to be carried out to enhance understanding of metabolism involved in synthetic solution aerobic oxidation compared to glucose one. The results show that environmental media of biofilm growth has an effect on microbial activity. Indeed microbial response to glucose amendment is faster and bigger in the case of biofilm grown up on plastic beads compared to glass beads. The type of substrate used for calorimetric measurements has an influence on the microbial response too. Calorimetric experiments carried out with two glass beads and associated biofilm samples show that microbial response to substrate amendment is totally different if glucose or synthetic solution is amended.

This is promising for calorimetric study of microbial activity in wastewater treatment processes like CWs.

Future calorimetric experiments should be carried out in inert atmosphere to register specifically anoxic measurements because this metabolic pathway is involved in nitrogen removal. These results correlated to a biomass quantification technique should improve our understanding of biodegradation in CW as a function of porous media and type of metabolism involved in biodegradation.

References

1. Kadlec RH. The inadequacy of first-order treatment wetland models. Ecol Eng. 2000;15(1–2):105–19.
2. Prochaska CA, Zouboulis AI, Eskridge KM. Performance of pilot-scale vertical-flow constructed wetlands, as affected by season, substrate, hydraulic load and frequency of application of simulated urban sewage. Ecol Eng. 2007;31:57–66.
3. Molle P, Liénard A, Grasmick A, Iwema A. Effect of reeds and feeding operations on hydraulic behaviour of vertical flow constructed wetlands under hydraulic overloads. Water Res. 2006;40(3): 606–12.
4. Kadlec RH. Overview: surface flow constructed wetlands: review article. Water Sci Technol. 1995;32(3):1–12.
5. Kadlec RH, Werner TM. Wetland residence time distribution modeling. Ecol Eng. 2000;15(1–2):77–90.
6. Vymazal J. The use of sub-surface constructed wetlands for wastewater treatment in the Czech Republic: 10 years experience. Ecol Eng. 2002;18(5):633–46.
7. Vymazal J. Horizontal sub-surface flow and hybrid constructed wetlands systems for wastewater treatment. Ecol Eng. 2005; 25(5):478–90.
8. Vymazal J, Kröpfelová L. Removal of organics in constructed wetlands with horizontal sub-surface flow: a review of the field experience. Sci Total Environ. 2009;407:3912–22.
9. Hadad HR, Maine MA, Bonetto CA. Macrophyte growth in a pilot-scale constructed wetland for industrial wastewater treatment. Chemosphere. 2006;63(10):1744–53.
10. Chazarenc F, Merlin G, Gonthier Y. Hydrodynamics of horizontal subsurface flow constructed wetlands. Ecol Eng. 2003; 21(2–3):165–73.
11. Zhao L, Zhu W, Tong W. Clogging processes caused by biofilm growth and organic particle accumulation in lab-scale vertical flow constructed wetlands. J Environ Sci. 2009;21:750–7.
12. Nguyen LM. Organic matter composition, microbial biomass and microbial activity in gravel-bed constructed wetlands treating farm dairy wastewaters. Ecol Eng. 2000;16:199–221.
13. Akratos CS, Tsihrintzis VA. Effect of temperature, HRT, vegetation and porous media on removal efficiency of pilot-scale horizontal subsurface flow constructed wetlands. Ecol Eng. 2007; 29(2):173–91.
14. Calvet É, Prat H. Microcalorimétrie: Applications physico-chimiques et biologiques. Paris: Masson & Cie; 1956.
15. Battley EH. The thermodynamics of microbial growth. In: Kemp RB, editor. Handbook of thermal analysis and calorimetry. vol 4: from macromolecules to man. Amsterdam: Elsevier Science; 1999. p. 219–66.
16. Larsson C, Gustafsson L. Calorimetry of microbial processes. In: Kemp RB, editor. Handbook of thermal analysis and calorimetry. vol 4: from macromolecules to man. Amsterdam: Elsevier Science; 1999. p. 367–403.
17. von Stockar U, Vojinović V, Maskow T, Liu J. Can microbial growth yield be estimated using simple thermodynamic analogies to technical processes? Chem Eng Process Process Intensif. 2008;47(6):980–90.
18. Nunez-Regueira L, Nunez-Fernandez O, Rodriguez Anon JA, Proupin Catineiras J. The influence of some physicochemical parameters on the microbial growth in soils. Thermochimica Acta. 2002;394:123–31.
19. Barros N, Gallego M, Feijóo S. Sensitivity of calorimetric indicators of soil microbial activity. Thermochimica Acta. 2007;458(1–2): 18–22.
20. Barros N, Salgado J, Feijóo S. Calorimetry and soil. Thermochimica Acta. 2007;458(1–2):11–7.
21. Barros N, Feijoó S, Balsa R. Comparative study of the microbial activity in different soils by the microcalorimetric method. Thermochimica Acta. 1997;296(1–2):53–8.
22. Barros N, Feijoo S, Simoni JA, Airoldi C, Ramajo B, Espina A, Garcia JR. A mass and energy balance to provide microbial growth yield efficiency in soil. J Therm Anal Calorim. 2008; 93–2:657–65.
23. Wadsö I. Characterization of microbial activity in soil by use of isothermal microcalorimetry. J Therm Anal Calorim. 2009; 95–3:843–50.