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Thermochimica Acta 420 (2004) 119–125
thermochimica acta
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Evaluating microbial activity in composts using microcalorimetry

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Received 12 September 2003; accepted 14 January 2004
Available online 30 July 2004

Abstract

Microcalorimetric isothermal monitoring was carried out for compost and compost-containing growing medium, to provide fingerprints of compost microbial activity at different conditions. Microbial activity is a key property of composts used in agricultural practice. Two aspects are addressed in this study: (1) microcalorimetric evaluation of compost response to glucose as a model stimulating agent; (2) examination of the impact of compost pre-drying and re-wetting on its biological activity.

Addition of glucose solution to the compost involved a strong increase in microbial activity, which was associated with a significant heat evolution without a lag period. In certain cases, this heat evolution was of complicated shape thus manifesting the heterogeneity of microbial populations in composts. Relation between cumulative energy evolved and the amount of added glucose was found to be helpful in distinguishing between aerobic and anaerobic regime of compost microbial activity. As distinct from non-dried compost or growing mixture, glucose addition to samples pre-dried at 65 °C resulted in delayed heat response with initial exponential-like heat evolution. This delay in heat evolution suggests that biological activity was significantly suppressed upon compost drying. Such a temperature-induced inactivation process might also result in dominance of a relatively homogeneous microbial population which survived the heating, thus involving a smooth, exponential-like initial step of the heat evolution. Noteworthy, addition of water (without glucose) to pre-dried compost results in an outburst of activity as compared with non-dried compost. This heat evolution outburst was also characterized by a lag period and an initial exponential-like phase. The heat evolution obtained with pre-dried compost upon water addition was not related to the compost wetting energy but rather reflected the formation of new carbon source due to the changes in compost organic matter upon heating or to the dead biomass of those microbial populations that did not resist the compost heating/drying.

Keywords: Microcalorimetry; Heat evolution; Compost; Microbial activity; Compost drying

1. Introduction

Compost is a material derived from aerobic decomposition of recycled plant wastes, biosolids or other organic materials. Manures and composts have been used as means to increase soil fertility and crop production all along the history of farming. Such organic materials are, in essence, slow release fertilizers, supplying nutrients through microbially induced mineralization processes [1,2]. In contrast to chemical fertilization, composts used for fertilization require a proper level of biological activity in order to exploit their nutrient sources. This aspect is particularly considered in organic farming where compost becomes an important nutrition source, mainly through N mineralization [3,4]. Composts and their associated microbial activity may also improve soil hydraulic properties. It is believed that addition of organic substrate available to microorganisms induces intensive production of soil stabilizing agents such as polysaccharides which improve soil aggregation [5]. It was also found that mature compost can suppress various soil-borne diseases [6,7]. Therefore, it is clear that high attention should be given to the microbial characteristics of composts, as these biologically active organic mixtures may substantially affect soil fertility and health.

Compost is a dynamic organic mixture, undergoing substantial physical, chemical and biological changes during the composting process until it becomes relatively stable [8–10]. Stabilization of some general parameters such as pile tem-

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0040-6031/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.tca.2004.01.038

perature, CO_2 production, oxygen uptake, C/N ratio, and organic matter may be achieved faster than stabilization of microbial populations [8]. In addition to the commonly used microbial assays (mainly respirometric measurements), microcalorimetry may become a very sensitive and powerful tool for assessing the microbial status of compost. It enables quantitative and non-invasive monitoring of the energetic response of compost microorganisms to the presence of various substrates, inhibitors and to the variation of environmental conditions. In addition, microcalorimetry has a potential to assess microbial processes not followed by complete substrate mineralization.

Microcalorimetric measurements were applied for estimating microbial activities of soils in several studies [e.g. 11-14]. Focus was made on comparison of microbial activity in different soils [15,16], on evaluation of the effect of soil water content [17,18], and of other physico-chemical parameters such as temperature, pH and C/N ratio [18]. Microcalorimetry was used also to assess inhibitory effect or biodegradation of soil pesticides [19,20]. Calorimetric manifestations of microbial activity were correlated with data obtained using common microbiological methods, such as respirometric methods [21], microbial counting [22], and microbial biomass measurements [16]. In most of the above studies, soil microbial activity was stimulated by the addition of glucose as a readily available carbon and energy source [e.g. 13,18,21,23], such that an exponential phase of microbial growth was reported on the basis of heat output curves. These studies revealed the potential of calorimetric methods for assessing microbial activity of environmental samples under various controlled conditions.

Microbial activity in composts may behave in a more complicated manner than in many soils. This is because composts are generally much more chemically and physically heterogeneous as compared with organic matter in many soils, have very high concentration of active biomass, and their organic matter is less stable. This heterogeneity may indeed be responsible to the inconsistent effect of compost in field applications [6,24].

To the best of our knowledge, there are currently no studies that extensively explored the potential of calorimetric technique to monitor microbial activity in composts, and to relate such properties with the role of composts in agronomical practice. This study aims at exploring microcalorimetry as a quantitative as well as descriptive tool to assess compost activity and microbial dynamics. Potential microbial activity in composts may be evident by testing the utilization of a model energy source. This utilization should be affected by environmental factors such as compost drying/hydration which are of evident importance for agricultural practice and, in particular, for microbial expression. Hence, in the present work two aspects are addressed: (1): microcalorimetric evaluation of compost response to glucose selected as a model stimulating agent; (2) examination of the impact of compost drying on compost biological activity.

2. Materials and methods

2.1. Materials

2.1.1. Sde-Eliyahu (commercial) compost

A compost sample (about 0.5 kg) was taken from a batch pile, which was purchased from Sde-Eliyahu composting facility, Israel. This compost is made from cow manure (80–90%), chicken manure (10–15%) and plants residues. A compost subsample was homogenized for microcalorimetric measurements by gently crushing it with a mortar and pestle and removing large fragments using a forceps. The crushed material was frozen to serve as a stable "stock" material for the whole experimental period.

2.1.2. Peat/compost growing substrate mixture

The growing mixture was prepared from a white peat (Klasmann-Deilmann, GmbH) and compost at a ratio of 80:20 peat:compost (v/v). The compost used in this mixture was prepared at the composting facility in Newe-Ya'ar under controlled conditions [25]. It was made from cow manure (40%) and peppers plant residues (60%) as raw materials. A sample of this mixture was homogenized (as described above) and stored at 4 °C.

2.1.3. Bentonite

Fischer Na-montmorillonite (CEC = 90 meq/100 g), was used as an inert material (biologically non-active) for exemplifying the magnitude of hydration energies.

Table 1 presents selected properties of the materials used in this study. Water content was determined after drying the sample overnight at 105 °C and is expressed in Table 1 as weight percent on a wet basis. Organic matter content was determined from the weight loss after ignition at 550 °C for 5 h. Total N (for C/N ratio) was determined by acid digestion.

2.1.4. Sample pre-drying

To study the effect of pre-drying, subsamples were maintained at 65 °C for the desired period of time and then re-wetted with deionized water or glucose solution just before calorimetric measurements. In comparisons between dried and non-dried material, the amount of water or glucose solution added was adjusted to account for moisture con-

Table 1
Selected properties of the materials used

Material	Property		
	Initial water content (%, w/w)	Organic matter content (%)	C/N ratio (w/w)
Sde-Eliyahu compost	23	40	12.0
Peat/compost growing medium	74	74	28.2
Bentonite	9.1	n.d.[a]	n.a.[b]

[a] Not determined.
[b] Not applicable.

tent of non-dried samples such that the final water content during the calorimetric measurement was identical in both dried and non-dried samples. Control sterilized dried compost was prepared by autoclaving the compost for 20 min, following the same drying procedure as described above.

2.2. Microcalorimetric measurements

Microcalorimetric measurements were made on a CSC 4100 Multi-cell Differential Scanning Calorimeter, using four 1.43 ml Hasteloy removable ampoules closed with viton gaskets. The DSC instrument was operated under isothermal mode at 30 °C. The stability of the baseline was tested either without ampoules, with empty ampoules or with ampoules filled with water or inert solid matrix (air-dry bentonite or glass beads) over a period of up to 55 h. During that period the observed drift of the baseline did not exceed 6 μW.

Calorimetric procedure involved initial 1–2 h stabilization of the calorimetric output of empty DSC cells, at 30 °C. Different amounts of compost or growing substrates (up to 100 mg wet based) were placed into three removable ampoules. In certain experiments, aqueous solution of glucose (100 μL) serving as an energy source was added. A 100 μL of deionized water without glucose was also added to samples served as control for background activity. The water or glucose solution were added dropwise using a micropipette, directly onto the sample in the ampoule. By using this procedure, samples looked homogeneously wetted. The reference ampoule was filled with deionized water at equivalent mass as the sample ampoules. In contrast to Barja and Núñez [23], use of compost not amended by energy source addition as the reference material, was not applicable due apparently to inherent microbial activity of the original compost samples. Then, three sample ampoules and the reference ampoule were simultaneously lowered into their proper numbered cell positions. Time interval between the addition of solution to the samples in the ampoules and starting of data acquisition was typically less than 10 min.

Microcalorimetric curves were obtained by continuous recording the power versus time for each sample ampoule versus the reference ampoule. Measurements were generally made in triplicates, i.e. the three sample ampoules were used for three subsamples of identical treatment. The average output was calculated for each data point after correcting the three curves to produce a single final baseline as shown in Fig. 1. Differences between the three subsamples were substantially minimized by using this baseline correction procedure. In many experiments, it was not possible to obtain an initial stable baseline due to immediate response to glucose addition. As such, final baseline was used for comparing different calorimetric curves and calculating the areas corresponding to the heat evolved.

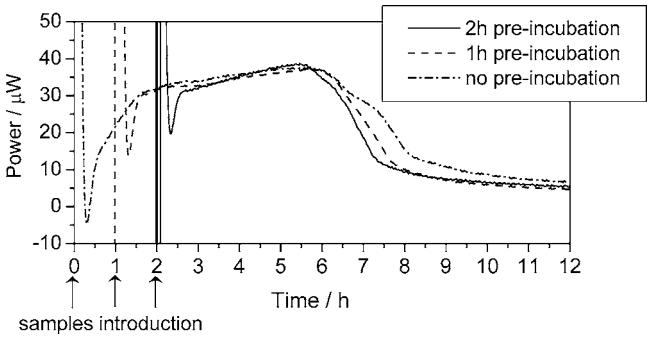

Fig. 2. Heat output stabilization for differently incubated samples of Sde-Eliyahu compost (100 mg wet; upon addition of 0.1 mg glucose in 100 μL solution).

Due to the time needed for instrument stabilization (30–40 min), a certain area under the heat evolution curves was lost in calculating cumulative energies. Impact of this non-accounted portion of the area was neglectable for the calculated cumulative energies. This was proven by the sample pre-incubation experiment (Fig. 2). In this experiment, the three compost sample ampoules were prepared with time interval of 1 h such that glucose solution was added to the first sample ampoule, and the ampoule was pre-incubated outside the calorimeter. After 1 h, glucose solution was added to the second ampoule, and the ampoule was also incubated outside the calorimeter. After

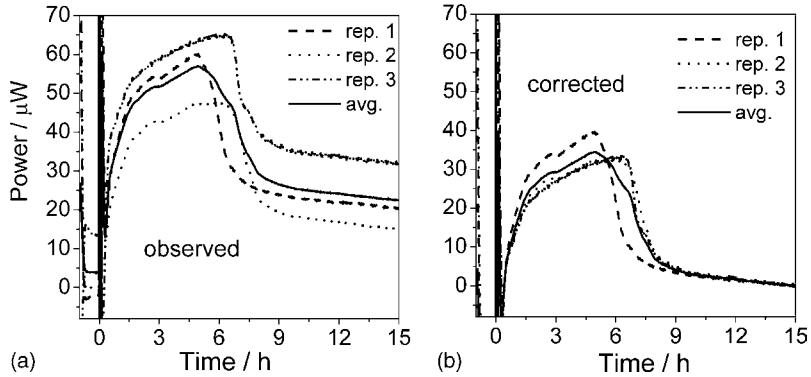

Fig. 1. Treatment of heat evolution curves. Triplicate curves for Sde-Eliyahu compost (100 mg wet) upon addition of 0.1 mg glucose (100 μL solution) (a) were corrected to produce a single final baseline (b).

another hour, the third ampoule was prepared and the three ampoules (plus the reference ampoule) were introduced together into the calorimeter. The time scale in Fig. 2 is shifted according to these pre-incubation periods. It is seen that the heat output was stabilized after 30–40 min, as evident from the coincidence of the three curves. Note that in the various microcalorimetric experiments performed in this study, the maximal heat output level after 30–40 min did not exceed 30 μW and in most cases was much lower. Considering also possible small drift of the baseline (i.e. 6 μW during 55 h), it is clear that the not unaccounted area under heat evolution curve corresponding to the first 30–40 min after solution addition to the sample never exceeded 0.07 J (30 μW × 40 min × 60 s) and in most cases was much less, and therefore, had no significant effect on comparison of much larger cumulative energies.

3. Results and discussion

3.1. Heat evolution as manifestation of compost microbial activity

Initially, it was proved that the heat output produced in compost by addition of water or aqueous solution is mostly related to compost biological activity and does not involve remarkable contribution from sample wetting. For this, sterile (autoclaved) compost sample was dried at 65 °C for 22 h (residual moisture 2.5%), and then monitored after re-wetting (Fig. 3). It is clear that at the absence of microbial activity, there is no remarkable heat evolution upon wetting of dried compost. This result was also supported by wetting air-dry bentonite, as a biologically inert material, well capable of water–sorbent interactions. In this case also, wetting effect did not reveal a significant heat output.

On the basis of these results, it was concluded that most of the detectable energy related to wetting of pre-dried or wet compost materials must be released during the first short period of instrument stabilization. As such, while considering long-term heat evolution processes in composts, the heat evolved from compost–water interactions may be neglected, and the entire heat output can be assigned to biological activity of the medium.

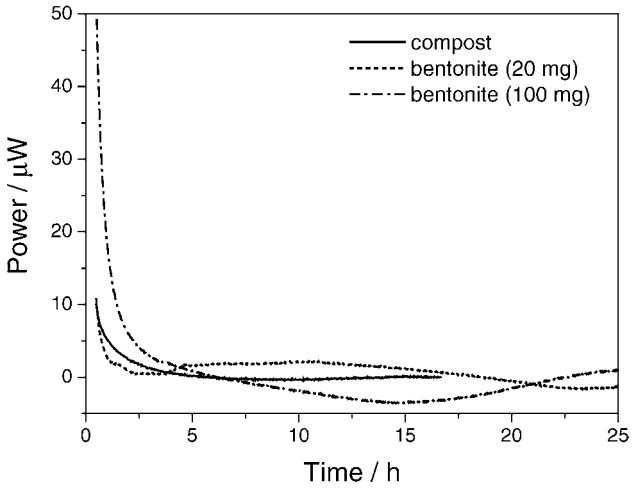

Fig. 3. Heat evolution upon wetting: autoclaved-dried Sde-Eliyahu compost (75 mg dry) was wetted by 125 μL of deionized water; air-dry Fisher bentonite (20 and 100 mg), was wetted by 100 μL of deionized water.

3.2. Heat evolution in compost and growing medium

Monitoring the heat output in Sde-Eliyahu compost not amended by glucose revealed a certain (not very substantial) background activity, which was mostly completed after 3–5 h (Fig. 4a). Addition of glucose resulted in immediate increase of heat evolution. As explained in Section 3.1, heat production upon glucose addition was not related to the energy involved in compost wetting.

The heat evolution curves obtained upon glucose addition were of quite complicated shape. Unless some important kinetic features were missed during instrument equilibration period (first 30–40 min), a lag phase was not observed in these curves. In addition, no evident exponential-like phase was seen, in contrast to the exponential microbial growth-like behavior typically reported in calorimetric monitoring of soils [13,15,23]. Lack of an exponential-like step is demonstrated in Fig. 4b where the first derivative of the heat output is plotted against time. As it is seen from Fig. 4b, when 0.05 mg glucose was added, the first derivative decreases almost continuously during the first 5 h. For 0.1 mg glucose, there is only a short period of increase (between 1 and 2.5 h), thus indicating the absence of

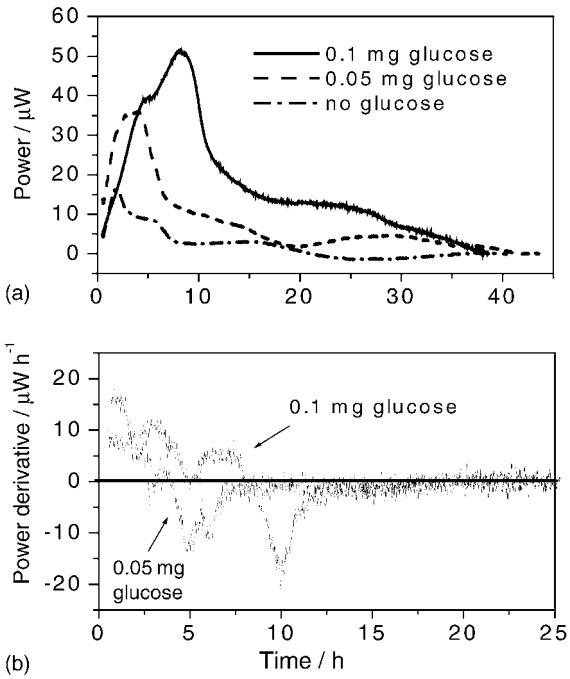

Fig. 4. (a) Heat evolution from Sde-Eliyahu compost (100 mg wet) upon addition of 100 μL glucose solution. (b) First derivative of the heat output plotted against time.

marked exponential-like behavior during considerable time intervals.

Lack of an exponential phase on the heat evolution curve of compost (Fig. 4b) may indicate a more complicated microbial activity on composts as compared with some soils. It may be related to a greater heterogeneity and dynamics of microbial populations in composts, such that there is no dominant growing population in the compost that could be considered alone to be responsible for major heat release at the beginning step. The amounts of glucose added could also be too low to support microbial growth, considering the higher content of active biomass in compost as compared with most soils. The cumulative energy (i.e. overall heat released by compost to a certain time upon glucose addition) was calculated from the heat evolution curves shown in Fig. 4a and plotted against time in Fig. 5a.

The cumulative curves in Fig. 5a level off after 25–30 h, indicating completion of the heat-evolving processes. The horizontal dashed lines at 0.8 and 1.6 J represent the level of energy needed to completely oxidize the corresponding amount of glucose (as calculated using the heat of glucose oxidation, 2816 kJ/mol [26]). It is evident from Fig. 5a that the overall cumulative energy exceeded expected energy level, presumably due to certain background activity related to the presence of compost-derived sources of energy. In such a case, the cumulative energy corresponding to the background activity was subtracted from the data shown in Fig. 5a. The remaining energy which is presumably related to glucose degradation only was normalized per mol of glucose added and plotted against time (Fig. 5b). The dashed line in Fig. 5b shows the energy needed to oxidize one mol of glucose. It is seen that this resulting cumulative energy became fairly comparable to glucose oxidation energy for both glucose amounts applied. These results are suggestive also that microbial activity is essentially aerobic, which is expectable since the amount of oxygen in the closed ampoule was sufficient for full glucose burning. At the same time, it is recognized that part of the energy of glucose oxidation should be utilized also in chemical and biochemical processes in the compost.

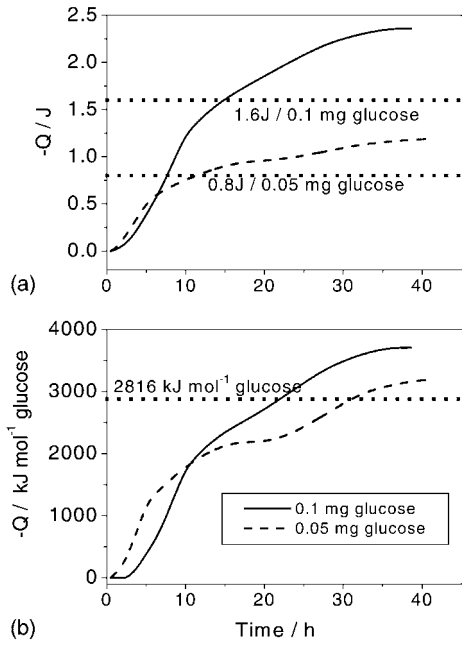

Fig. 5. (a) Cumulative energy released by Sde-Eliyahu compost upon addition of 100 μL solution containing 0.05 and 0.1 mg of glucose plotted against time. Dashed lines show the energy level corresponding to full oxidation of the glucose amount applied (0.8 and 1.6 J for 0.05 mg and 0.1 mg glucose, respectively). (b) Cumulative energy released by Sde-Eliyahu compost normalized per mol of glucose plotted against time. Dashed line corresponds to the oxidation energy for 1 mol of glucose. Background activity of the compost (upon addition of 100 μL deionized water) was subtracted from the cumulative energies obtained at glucose application.

The response of microbial activity to glucose addition was measured also for the peat/compost growing medium (Fig. 6a). Using this medium, the effect of transition from aerobic to anaerobic conditions was tested by increasing glucose amounts. It is seen that heat evolution at 0.05 mg glucose added was highly similar to that obtained for Sde-Eliyahu compost also amended with the same amount of glucose. Similarly to the activity observed in Sde-Eliyahu compost, a distinct increase of the heat evolution was observed upon further glucose addition. The cumulative energies calculated for different glucose applications are reported as a function of time in Fig. 6b. It is seen that the heat-evolving process was mostly completed in less than 20 h. The dashed lines at 0.8, 3.2 and 8 J correspond to the level of energy needed to completely oxidize the applied amount of 0.05, 0.1 and 0.5 mg glucose, respectively. It may be concluded from the curves presented in Fig. 6b that cumulative energy associated with application

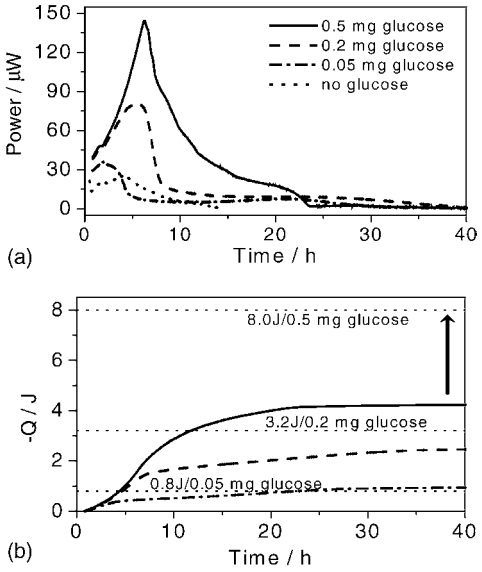

Fig. 6. Heat evolution from peat/compost growing substrate (100 mg wet) upon addition of 100 μL glucose solution. (a) Heat output plotted against time. (b) Cumulative energy plotted against time. Dashed lines show the energy level corresponding to full oxidation of the glucose amounts applied (0.8, 3.2, and 8 J for 0.05, 0.2, and 0.5 mg, respectively).

of 0.05 mg of glucose corresponds tightly to the expectable glucose oxidation level. However, when 0.2 mg of glucose was applied, less energy was released as compared with glucose oxidation energy. This difference between overall released energy and glucose oxidation energy became very distinctive when 0.5 mg of glucose was applied. Simple calculation shows that 34% of the oxygen content in the closed ampoule is consumed by fully oxidizing 0.1 mg glucose. Hence, it is clear that at applications of greater amounts of glucose, microbial activity would occur at oxygen deficit, thus involving anaerobic conditions. These results reveal the potential of microcalorimetric measurements to monitor microbial activity during transition from aerobic to anaerobic conditions.

3.3. Pre-drying effect on heat evolution and microbial activity

Effect of pre-drying (65 °C) on sample activity is demonstrated in Fig. 7, where heat output is shown for Sde-Eliyahu compost (a) and peat/compost growing substrate (b) upon glucose addition. It is evident that in both cases drying and re-wetting resulted in delayed heat response as compared with non-dried samples. Additionally, the initial step of the heat evolution curve in pre-dried samples corresponds with an exponential-like phase. Interestingly, the overall heat released and the shape of curves are comparable for Sde-Eliyahu compost and peat/compost growing substrate, despite the amount of compost (as the main active material) which was about 15 times lower in the growing substrate as compared with the compost sample (estimation based on the content of organic matter in the composts and the initial moisture of the two media; Table 1).

To extend the examination of the pre-drying effect, heat evolution of Sde-Eliyahu compost was monitored after different drying periods followed by re-wetting with deionized water (Fig. 8a). It is shown that the heat evolution profile was very similar both for 41 and 68 h drying period but differed drastically from profile obtained for non-dried compost. From Fig. 8a it is evident that the addition of water (without glucose) to the pre-dried compost results in an outburst of activity as compared with non-dried compost. This heat evolution outburst shown in Fig. 8a is associated with the appearance of a lag period and an initial exponential-like phase as observed also in Fig. 7.

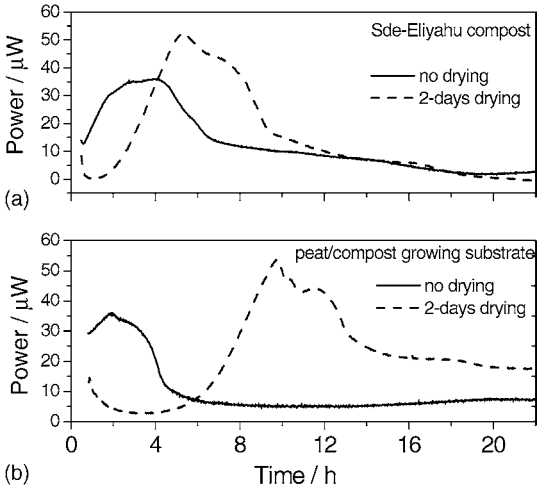

Fig. 7. Effect of pre-drying. Heat evolution profile at 100 mg (wet) medium upon addition of 100 µL solution containing 0.05 mg glucose. (a) Sde-Eliyahu compost. (b) Peat/compost growing substrate.

Importantly, this strong increase of heat evolution on the pre-dried compost upon water addition cannot be related to the energy associated with compost wetting, as proved by testing the heat evolution in sterilized dried compost (Section 3.1, Fig. 3). Two mechanisms may explain the strong increase in heat evolution upon wetting of pre-dried compost. (1) Dead biomass of certain microbial populations that did not resist the heating/drying could provide a new carbon source to those microorganisms that survived the process. (2) Chemical/physical changes in compost organic matter occurring at 65 °C may provide more available energy sources as compared with the original compost.

Distinct differences in the heat evolution profile of dried as compared with non-dried compost were observed also upon addition of glucose solution (Fig. 8b). Again, drying time changed from 19 to 68 h did not have a significant effect on the heat evolution profile. However, as in Figs. 7 and 8a, pre-drying resulted in delayed microbial activity and more smooth exponential-like shape of the initial rise as compared with non-dried sample.

Delay in heat response in pre-dried compost samples suggests that biological activity was significantly reduced upon drying. This inactivation process might also be accompanied

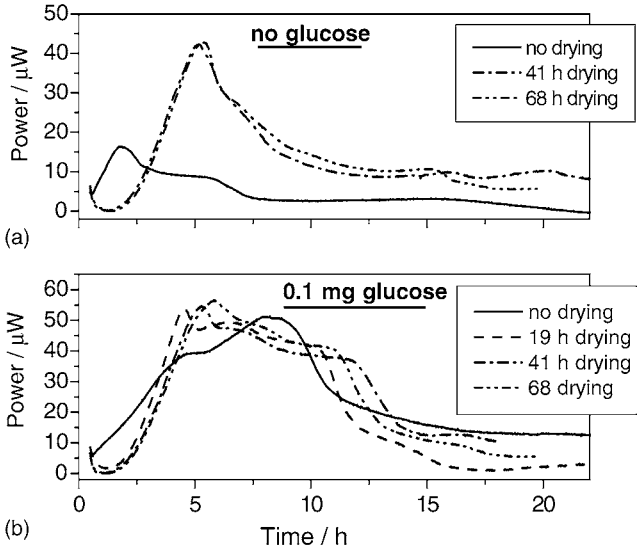

Fig. 8. Effect of different pre-drying periods on heat evolution profile of Sde-Eliyahu compost. (a) 100 mg (wet) non-dried compost with addition of 100 µL deionized water; 75 mg dried compost with addition of 125 µL deionized water. (b) 100 mg (wet) non-dried compost with addition of 100 µL glucose solution; 75 mg dried compost with addition of 125 µL glucose solution.

by the survival of a relatively more homogeneous microbial population, thus involving a smooth, exponential-like initial step of the heat evolution.

It is of interest that overall heat effect corresponding to the area under recorded curve in Fig. 8b seems similar in all experiments of glucose applications in dried and non-dried samples. Hence, the significant heat evolution observed upon water addition to the dried compost sample (Fig. 8a) apparently does not contribute to the heat evolution upon glucose addition. It seems that at the presence of competing energy sourcess provided by glucose and compost organic matter, overall activity does not behave additively. In overall, considering compost moisture as an important factor in compost utilization, it is seen that microcalorimetric monitoring may provide a tool for optimizing moisture hydration regime and governing the microbial activity in wet or differently dried composts.

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