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Use of sonication for measuring acid phosphatase activity in soil

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This article is dedicated to the memory of our much missed friend and colleague Professor Giacomo Randazzo.

Abstract

Extracellular enzymes in soil often occur in immobilised forms, a state that may alter their interactions with substrates in comparison with enzymes in the solution phase. Sonication was evaluated for its usefulness in studying immobilised acid phosphatase by dispersing soil aggregates. Factors affecting soil dispersion during ultrasound application were soil extraction ratio, total applied energy and power output ml^{-1} of sonicated soil slurry. For the clay loam soil used, optimal values for these variables were, respectively, 1:6 (w/v) and, at least, 1800 J ml^{-1} and 15 W ml^{-1}. At the optimal sonication conditions for soil dispersion a substantial increase in phosphatase activity (up to 156% greater than the non-sonicated control) was induced by sonication. This increase in activity with sonication, which coincided with a release of soil chromophores, might be related to the exposure or release from aggregates of the extracellular enzyme fraction immobilised on humic colloids. Analysis of multiple regression between the phosphatase activity (dependent variable) and chromophore solubilisation and ATP release (independent variables) suggested the increased activity was from complexed enzymes that were released and not due to cell lysis. Soil treatment with sonication appeared to have liberated a large dormant portion of acid phosphatase activity. Coefficients of variation of the activity decreased greatly (from 20% in control soil to 4% as an average after sonication).

Keywords: Soil acid phosphatase; Soil enzyme assay; Sonication

1. Introduction

Sonication is used in soil science for a number of purposes, such as fractionation of mineral particles and organic matter (Edwards and Bremner, 1967; Gregorich et al., 1988), determination of particle-size distribution and aggregate stability (Fuller and Goh, 1992), bacterial detachment from particle surfaces (Lindahl and Bakken, 1995; Ramsay, 1984), and estimation of ATP content (Contin et al., 1995; Webster et al., 1984).

Ultrasonic waves are generated by vibrations that act through expansion and compression cycles on bubbles preexisting in aqueous solutions. These cycles can lead to an implosive collapse of such cavities resulting in increase in the temperature and pressure at microsites (Mamba and Kratochvil, 1995; Watson, 1971). Occasionally, solvent or solute molecular fragmentation into reactive free radicals (such as hydrogen atoms and $OH^{\cdot}$ radicals from water vapour) may occur during sonication (Ku et al., 1997). These effects, however, do not significantly alter the chemical properties of soil mineral particles (Eivazi and Tabatabai, 1977; Hunter and Busacca, 1989) or native organic

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matter (Watson, 1971), unlike when dispersing reagents are used (Ahmed and Oades, 1984; Edwards and Bremner, 1965; Protz and St. Arnaud, 1964). Therefore, sonication disrupts aggregates both by formation of collapsing bubbles and friction between and within aggregates. These forces can also lyse microbial cells, thereby releasing enzymes and other intracellular components into soil solution (Frizzel, 1988).

Soil enzymes rarely occur free in soil solution, but are more frequently associated with proliferating or dormant cells, whole dead cells, or cell debris, or as immobilised molecules (Burns, 1982; Skujins, 1976) bound to clay minerals or coupled with humic colloid polyphenols (Burns, 1986). Overcoming the physicochemical complexation of enzymes is a challenge for biochemists wishing to extract and purify enzymes from soil or determine the location of enzyme activity, i.e. cellular vs complexed enzymes (Burns, 1986). Because of the location of enzymes on and within soil particles and aggregates, the substrate concentrations generally used in soil enzyme assays to ensure saturation are orders of magnitude higher than those used in soil-free systems (Ramírez-Martínez and McLaren, 1966). Moreover, enzyme assays often last hours or days instead of minutes (Beyer et al., 1993; Frankenberger and Johanson, 1982), often with substantially different soil conditions between the beginning and the end of the assay. This is particularly true if microbial proliferation, or chemical modification as a consequence of microbial metabolism, takes place. If this occurs, it is unlikely that the amount or the activity of the enzyme will remain constant during the assay. Garzillo et al. (1996) recognised this problem and described a method that reduced the assay of acid phosphatase in soil from 1 h to 10 min.

Considering the complex nature of soil and the presence of many enzymes in an immobilised form, ultrasonication may facilitate both the disruption and the homogenization of soil aggregates, thereby rendering a larger amount of enzyme available for reaction with substrates. As a preliminary assessment of the effectiveness of ultrasonication in treating soil samples prior to enzyme assays, we have used acid phosphatase as a model enzyme. Acid phosphatase is important in P transformations in soils and aquatic systems (Pierzynski et al., 1995) and in the bioremediation of soils contaminated with organophosphate pesticides (Nannipieri and Bollag, 1991).

2. Materials and methods

2.1. Soil

Clay loam forest soil (USDA classification: Typic Hapludand) under *Fagus sylvatica* L. was collected after discarding the litter from the top 10 cm. Pertinent characteristics of the soil include: sand 355 g kg^{-1}, silt 459 g kg^{-1}, clay 186 g kg^{-1}, organic C 25.9 g kg^{-1}, total N 1.7 g kg^{-1}, total carbonates 17.5 g kg^{-1}, cation exchange capacity 21.1 cmol (+) kg^{-1} soil, pH in water 5.2, pH in KCl 4.33. Soil was sieved (<2 mm) and stored at 5°C at least for 4 months. Before each experiment, soil was conditioned at 25°C for 5 days in the dark with the water potential adjusted to pF 2.2 in order to restore microbial activity.

2.2. Sonication procedure

The reliability of the sonicator was tested by determining temperature increases over 25.6 ± 0.3°C (room temperature) of 10 ml of deionized water sonicated at different power outputs (0, 5, 10, 15, 20, 25, 30 W ml^{-1}) and after a fixed time of sonication (2 min). Nominal total applied energies were derived from the relationship:

$$\text{applied energy (J ml}^{-1}) = \text{power output (W ml}^{-1}) \times t_s$$

where t_s is the time in seconds.

The optimal soil extraction ratio for sonication was determined by extracting portions of fresh soil corresponding to 1000, 667, 500, and 400 mg of dry weight with 4 ml of 0.5 M potassium acetate buffer, pH 5.0 (final soil/water ratios of 1:4, 1:6, 1:8 and 1:10, respectively) in flat-bottomed cylindrical 10 ml glass vessels. The extraction ratio chosen for all subsequent experiments was 1:6 (w/v). All soil samples were stirred for 10 s and left to equilibrate for 3 min at 25°C before sonication. Ultrasonication was performed using a 20 kHz/400 W Sonics and Materials Vibra Cell VCX 400 apparatus (Connecticut, USA); the titanium probe tip (3 mm dia) was placed at 1/3 of the suspension height. Ultrasound application to soil slurries was pulsed (4 s of burst and 2 s of resting) and performed in an ice bath to prevent sample heating, enzyme denaturation and foaming. The average temperature of the extracts was measured at the end of sonication and never exceeded 17°C (data not shown). No loss in weight of soil suspension was detected at the end of the sonication treatment. Each sonication procedure was carried out in duplicate.

2.3. Evaluation of soil dispersion

For evaluation of soil dispersion by sonication, the release of chromophores from soil was measured. Soil slurries were sonicated at different conditions by varying power outputs (from 0 to 30 W ml^{-1}), time of sonication (from 0 to 6 min) and extraction ratios (from 1:4 to 1:10, w/v). Soil suspensions were kept at 37°C for 10 min under gentle agitation, centrifuged at

4000 rev min^{-1} for 10 min, and the absorbance of the supernatant fractions was determined at 410 nm.

2.4. Acid phosphatase assay

To test possible inactivation of acid phosphatase by sonication, 100 µl of a commercial potato acid phosphatase solution corresponding to 133 mU was diluted in 3.9 ml of 0.5 M K-acetate buffer, pH 5.0, and sonicated at several power outputs from 0 to 30 W ml^{-1} for 2, 4 or 6 min. In a parallel set of experiments, the same amount of enzyme was added to aliquots of fresh soil corresponding to 667 mg of dry soil suspended in 3.9 ml of the K-acetate buffer (1:6 w/v extraction ratio), mixed for 30 s and then kept for 15 min at 25°C before sonication.

Acid phosphatase activity in sonicated and non-sonicated soil samples was determined by a modified (Garzillo et al., 1996) method of Tabatabai and Bremner (1969). *p*-Nitrophenyl phosphate (pNPP) 845 µl, 200 mM in 0.5 M K-acetate buffer, pH 5.0, and 155 µl of the same buffer was added to buffered soil suspensions from sonicated or non-sonicated samples (final pNPP concentration = 33.8 mM) and vortexed for 10 s. After incubation at 37°C for 10 min under agitation, the reaction mixture was cooled in an ice bath, 5.0 ml of 5% (w/v) K_2CO_3 added, and stirred and centrifuged at 4000 rev min^{-1} for 10 min. Two controls were carried out: the first to reduce the contribution of soil chromophores extracted during the sonication procedure and the second to take into account any non-enzymatic hydrolysis of substrate. The absorbance of supernatant fractions was measured at 410 nm. Potassium acetate buffer and K_2CO_3 were chosen for acid phosphatase assays (Garzillo et al., 1996) instead of Modified Universal Buffer, $CaCl_2$ and NaOH (Tabatabai and Bremner, 1969), to reduce clay dispersion or organic matter extraction which might interfere with *p*-nitrophenate detection. Acid phosphatase activity was calculated as I.U. g^{-1} dry soil (ϵ_{410} of *p*-nitrophenate = 18.5 mM^{-1} cm^{-1}).

2.5. ATP content

Soil ATP was determined according to published methods (Ciardi and Nannipieri, 1990; Webster et al., 1984), with some modifications. The fresh soil equivalent of 667 mg (dry weight equiv.) was suspended in 4 ml of extracting solution (1:6 w/v) consisting of 670 mM H_3PO_4, 2 M urea, 20% DMSO, 1.8 mg l^{-1} adenosine, 20 mM EDTA and 20 µl Antifoam A emulsion (Sigma). After stirring, the samples were sonicated at several power outputs from 0 to 30 W ml^{-1} for 2 or 4 min. Soil suspensions were then shaken for 30 min in an ice bath and centrifuged at 4100 rev min^{-1} for 10 min at 5°C. Aliquots of 0.5 ml were taken from the supernatant fractions and were transferred into polystyrene tubes, mixed with 4.5 ml of EDTA–Tris acetate buffer (0.1 M Tris, 2 mM EDTA), pH 7.8, and vortexed for 10 s. Aliquots of 100 µl were tested for ATP by adding them to a buffered (EDTA–Tris) luciferin–luciferase (Bio-Orbit, Turku, Finland) solution. The radiation emission of the mixtures was measured with a LKB 1250 Luminometer after solution stabilisation. Internal ATP standard was added to correct the influence of soil extracts on the light output. The estimate of ATP content of soil was carried out in triplicate.

2.6. Statistics

The results were analysed through single-classification (the presence or absence of sonication) analysis of variance (ANOVA), and least significant differences (LSDs) were calculated at $P < 0.05$ (Sokal and Rohlf, 1995).

3. Results

3.1. Sonicator reliability

The application of ultrasounds to water at increasing energies induced a very significant linear temperature enhancement ($P < 0.0001$). The relationship between temperature increases and sonication energies was:

$$y = (0.0156 \pm 0.0009)x + (23.99 \pm 0.82); \quad r^2 = 0.978, \quad n = 7$$

where y is the measured temperature (C) and x the applied energy (J ml^{-1}).

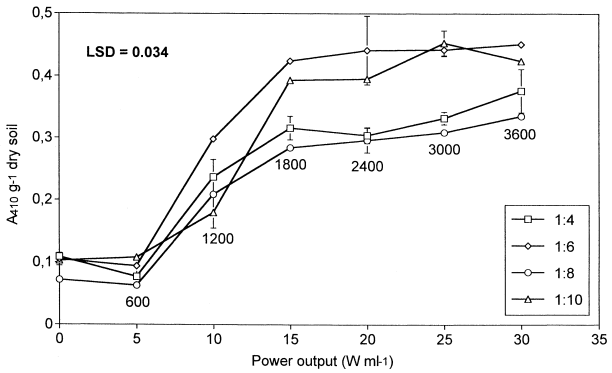

Fig. 1. Absorbance at 410 nm of supernatant fractions from soil slurries after 2 min sonication at several extraction ratios (w/v, in the inset) as a function of power outputs. Data are means of duplicates. Bars are standard deviations. Total applied energies (J ml^{-1}) are shown within the graph. LSD was calculated for $P = 0.05$.

3.2. Soil disaggregation

The importance of soil extraction ratio, total energy applied and power output per unit volume are shown in Figs. 1 and 2. The absorbance of supernatant fractions from sonicated soil extracts was always higher ($P < 0.05$) than, or at least equal to, non-sonicated soil extracts (controls). The 1:6 (w/v) extraction ratio exhibited the highest absorbance values for all energy levels (Fig. 1), and was therefore considered to be the optimal ratio. A total energy of 600 J ml^{-1} applied at 5 W ml^{-1}, with 2 min of actual sonication, was not effective in releasing chromophores at any extraction ratio (Fig. 1), whereas, the same total energy applied for 1 min (10 W ml^{-1}) induced a much higher release of chromophores than observed with non-sonicated soil (Fig. 2). The relationship of chromophore absorbance g^{-1} of dry soil with power outputs (Fig. 1) followed a sigmoidal trend for all extraction ratios with a plateau at 15 W ml^{-1}. At the optimal extraction ratio a plateau of absorbance was reached at 1800 J ml^{-1} in 2 min. However, with the 1:10 (w/v) extraction ratio the absorbance was significantly lower than the 1:6 ratio in two of six cases only (Fig. 1).

When the soil was extracted at the optimal extraction ratio and sonicated for different times at 10 W ml^{-1} and 20 W ml^{-1} soil slurry, the chromophore absorbance followed a hyperbolic trend with a plateau >2 min (Fig. 2). At any time interval, 20 W ml^{-1} power output generated greater absorbance values than 10 W ml^{-1}. Once again, under the same amount of total applied energy (e.g. 2400 J ml^{-1} supplied as 20 W ml^{-1} for 2 min or 10 W ml^{-1} for 4 min), the higher the power output, the greater the chromophore release. It was confirmed that values of total energy lower than 1800 J ml^{-1} did not totally disperse soil, even at power outputs higher than 15 W ml^{-1} (Fig. 2). Although using the optimal extraction ratio and total applied energies much higher than 1800 J ml^{-1}, the maximal absorbance value was approximated very slowly with a power output lower than 15 W ml^{-1}, but higher than 5 W ml^{-1} (Fig. 2). Thus, only when both variables were over a threshold value (1800 J ml^{-1} and 15 W ml^{-1}, respectively), was the plateau absorbance reached. Therefore, extraction ratio, total applied energy and power output per unit of volume are important for maximal release of chromophores from soil. For the clay loam soil used, optimal values for these variables were, respectively, 1:6 (w/v) and, at least, 1800 J ml^{-1} and 15 W ml^{-1}.

Stirring soil suspensions during sonication did not significantly ($P < 0.05$) change the above results (data not shown). No significant pH change was detected in soil samples at different extraction ratios after sonication up to 30 W ml^{-1} for 2 min (data not shown). Thus, the ionic strength of 0.5 M acetate buffer counteracted any release of H^+ or OH^- during sonication.

3.3. Determination of acid phosphatase activity

To determine the effect of sonication on the enzyme, ultrasonic irradiation was applied to commercial grade potato acid phosphatase at several power outputs and sonication times. Nominal applied sonication energies up to 10800 J ml^{-1} (30 W ml^{-1} for 6 min) did not induce any significant ($P < 0.05$) effect on the activity of the commercial enzyme (partial data shown in Fig. 5).

Sonication of soil samples at various power outputs for 2 min and different extraction ratios was also tested for its effect on acid phosphatase activity. The enzyme activity increased as a function of power output ml^{-1} of soil slurry at all the extraction ratios tested, and showed a sigmoidal trend similar to that of chromophore release (Fig. 3). Plateau conditions were

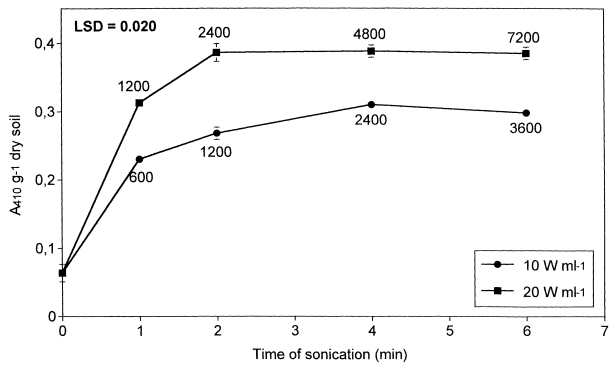

Fig. 2. Absorbance at 410 nm of supernatants of soil slurries sonicated at the extraction ratio of 1:6 (w/v) and 10 W ml^{-1} or 20 W ml^{-1} as a function of sonification time. Data are means of duplicates. Bars are standard deviations. Total applied energies (J ml^{-1}) are shown within the graph. LSD was calculated for $P = 0.05$.

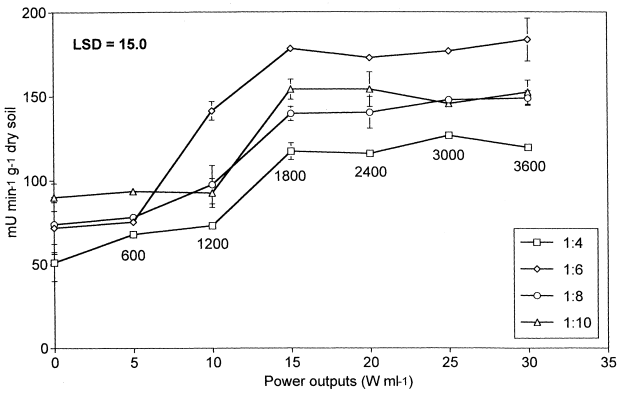

Fig. 3. Acid phosphatase acidity of soil slurries sonicated for 2 min at several extraction ratios (w/v, in the inset) as a function of power outputs. Data are means of duplicates. Bars are standard deviations. Total applied energies (J ml^{-1}) are shown within the graph. LSD was calculated for $P = 0.05$.

reached at 15 W ml^{-1}, and the 1:6 (w/v) extraction ratio yielded the most effective sonication-mediated release of enzyme activity. Sonication within each extraction ratio increased acid phosphatase activity from 71% (1:10) to 156% (1:6). The coefficients of variation (CVs) of acid phosphatase activity significantly decreased in sonicated soils (4% as an average) compared to non-sonicated controls (20%), showing sonication to be more reproducible than non-sonicated treatments. The lowest CV occurred for the 1:6 (w/v) extraction ratio at 20 W ml^{-1}, and 25 W ml^{-1} with CVs of 0.8% and 0.6%, respectively.

At the optimal extraction ratio (1:6 w/v), soil slurries were sonicated by varying both power outputs (10 W ml^{-1} and 20 W ml^{-1}, Fig. 3) and sonication periods (from 0 to 6 min). The highest enzyme activities were observed at 4 min sonication at both power outputs (2400–4800 J ml^{-1} in total), with increases of 108 and 118%, respectively (Fig. 4). These values are not significantly different from each other, which indicated the activity was on a maximal plateau. However, the rate of change before maximal activity was different: a constant linear increase was observed at 10 W ml^{-1}, whereas a steeper increase occurred at 20 W ml^{-1} during the first min, followed by a less pronounced slope. The activity values measured at 10 W ml^{-1} were usually lower than those obtained for treatments that had the same total energy but shorter times of exposure (e.g. 10 W ml^{-1} for 2 min vs 20 W ml^{-1} for 1 min, i.e. 1200 J ml^{-1}), except for 10 W ml^{-1} for 4 min compared to 20 W ml^{-1} for 2 min (Fig. 4). A similar trend was observed for chromophore release (Fig. 2).

To simulate the fate in the soil of intracellular acid phosphatase released during sonication, soil samples were spiked with a known amount of potato acid phosphatase. In the absence of sonication, adding pure enzyme to the soil slurry and measuring activity after 15 min did not increase phosphatase activity. Based on the amount of enzyme added, activity should have increased by 27%, i.e. soil plus added phosphatase. Actually, sonication released some of the added enzyme, as the measured activity was only 8% lower than expected (Fig. 5).

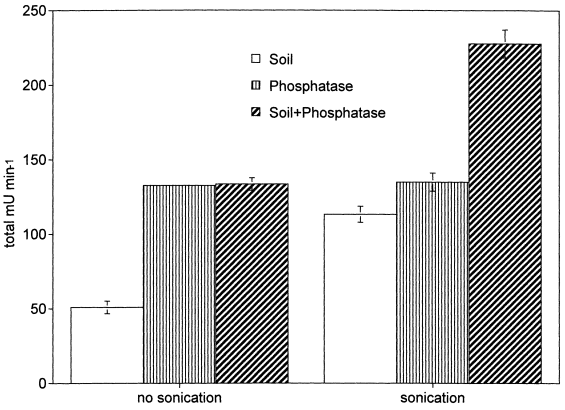

Fig. 5. Effect of sonication (20 W ml^{-1} for 4 min) on free commercial acid phoshatase activity and after addition to soil with or without sonication. Data are means of duplicates. Bars are standard deviations. LSD was calculated for $P = 0.05$.

3.4. ATP content

Sonication conditions similar to those inducing increases of acid phosphatase activity in soil supernatant fractions (i.e. 10 W ml^{-1} and 20 W ml^{-1} up to 4 min) were tested for their effectiveness to release intracellular ATP (Fig. 6). Sonication enhanced the recovery of ATP, with a maximum after 2 min sonication at both power outputs. The ATP recovered at

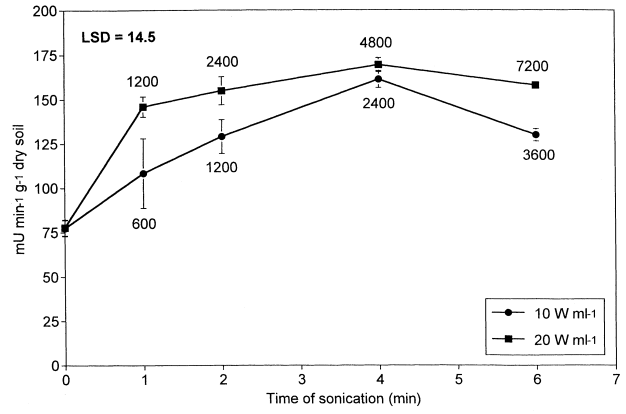

Fig. 4. Acid phosphatase activity of soil slurries sonicated at the extraction ratio of 1:6 (w/v) and 10 W ml^{-1} or 20 W ml^{-1} as a function of sonication time. Data are means of duplicates. Bars are standard deviations. Total applied energies (J ml^{-1}) are shown within the graph. LSD was calculated for $P = 0.05$.

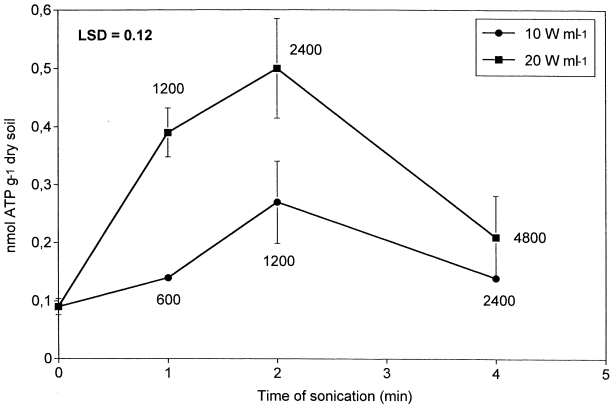

Fig. 6. ATP content of soil sonicated at the extraction ratio of 1:6 (w/v) and 10 W ml^{-1} or 20 W ml^{-1} as a function of sonication time. Data are means of triplicates. Bars are standard deviations. Total applied energies (J ml^{-1}) are shown within the graph. LSD was calculated for $P = 0.05$.

20 W ml^{-1} was generally greater than at 10 W ml^{-1}. Thus ATP release due to sonication was similar to enzyme and chromophore release.

4. Discussion

Mechanical methods for soil dispersion, such as gentle shaking of slurries are simple techniques. However, they do not enable quantification of the amount of applied energy, and require long periods to disperse aggregates. Conversely, ultrasonication for 2–6 min can provide high, measurable and reproducible dispersion energies to soil slurries, possibly reducing metabolic changes in soil during the dispersion phase.

Physical fractionation of soil by ultrasound should disrupt the bonds responsible for primary particle aggregation. Power outputs usually applied to soil slurries range from 60 to 600 W, with corresponding energies of 90 to about 5000 J ml^{-1}. According to Christensen (1992) and Eriksen et al. (1995), complete soil dispersion only occurs when at least 1500–1800 J ml^{-1} is applied. Conversely, Gregorich et al. (1988) found that for some soils, a complete disruption and dispersion occurred at 300–500 J ml^{-1}. In the present work, the applied energies ranged from 600 to 7200 J ml^{-1}, and are in accordance with the previous studies.

It has been reported that formation of cavitation bubbles during sonication is inversely related to the soil slurry concentration (Atchley and Crum, 1988); thus the expected optimal extraction ratio might be 1:10 (w/v). Our data do not reveal why the clay loam soil used was optimally dispersed at 1:6 (w/v) ratio. The suitability of this ratio for optimally dispersing other soils must be tested.

Sonication induces disintegration of soil aggregates, and thus solubilizes spectrophotometrically active organic compounds; therefore, a direct relationship between an increase in absorbance and soil dispersion can be assumed. In the soil we studied there were two maximal plateaux for chromophore release and acid phosphatase activity. The profile of the chromophore absorbance plateau may be linked to the complete release of fulvic acid compounds that were desorbed from soil colloids by sonication. A similar pattern for enzyme activity curve indicates that sonication may have partially released the extracellular fraction of acid phosphatase immobilized onto humic colloids. Soil enzymatic activity at 10 W ml^{-1} for 6 min (3600 J ml^{-1}, Fig. 4) decreased, whereas the activity of pure enzyme was unaffected for energies up to 10,800 J ml^{-1}. These results suggest that sonication did not cause enzyme denaturation; it is more likely that sonication released enzyme inhibitors from soil constituents. Many authors have reported that fulvic acid compounds from soil organic matter inhibit acid phosphatase activity (Butler and Ladd, 1971; Vuorinen and Saharinen, 1996).

Our results indicated that sonication did not induce chromophore release or extra-enzyme activity if 5 W ml^{-1} were applied, regardless of extraction ratio, whereas above 5 W ml^{-1} chromophore levels and enzyme activity increased. A threshold value is likely to be specific to soil types. Therefore, our results demonstrate that when defining the optimal conditions for soil dispersion through sonication, it is not sufficient to indicate total energy; the power output applied per unit of volume must also be reported.

Burns (1990) proposed the use of sonication for extracting soil enzymes. However, application of ultrasound to soil enzyme studies has been restricted to investigation of the distribution of various enzymes in different particle-size fractions (Ahmed and Oades, 1984; Ross, 1976; Stemmer et al., 1998). In the present work, enzyme activity was measured directly in dispersed soil after sonication, without fractionation, in an attempt to induce a more rapid and even diffusion of the soluble substrate towards enzyme sites. Sonication, under nearly all conditions tested, caused an increase of acid phosphatase activity relative to non-sonicated soil. Moreover, CVs were significantly lowered with sonication (from 20 to 4%). These results were consistent with an enhanced homogenization of soil suspension due to aggregate disruption. Conflicting opinions have been expressed on the need for stirring soil suspension during sonication (Ahmed and Oades, 1984; Edwards and Bremner, 1967; Gregorich et al., 1988; Hunter and Busacca, 1989; Watson, 1971). The experimental data obtained in the present work were not changed by stirring, and it is likely that the small mixture volumes (4 ml) used in our experiments were efficiently mixed by vibration waves by the sonicator probe.

The increase in enzyme activity upon sonication may be attributed to two independent factors: (i) disaggregation of soil particles that causes a release of enzyme molecules entrapped in aggregates or those mobilised on and within organo-mineral complexes (Boyd and Mortland, 1990; Mato et al., 1972; Ross, 1983); and (ii) microbial cell lysis which releases intracellular enzymes into soil. The possibility that our experimental conditions could cause cell lysis was tested by ATP estimation before and after sonication of soil samples. ATP estimation is generally related either to microbial biomass or to microbial activity or both (Nannipieri et al., 1990). Multiple linear regression with acid phosphatase activity as the dependent variable, and soil dispersion and ATP recovery as independent variables showed that enzyme activity was significantly related ($P < 0.01$; $r^2 = 0.83$; $n = 7$) to soil dispersion but not to ATP recovery. This provides evidence that observed increases in enzyme activity upon

sonication were mostly due to released extracellular components, whereas the intracellular contribution is minimal. Similar results were obtained for soil phosphatases when organic compounds such as toluene, dimethyl sulfoxide, ethanol or Triton X-100 were used for cell lysis (Frankenberger and Johanson, 1986). Our hypothesis is also supported by the observation that the ATP content of sonicated soil samples (theoretically related to cell lysis) significantly increased up to 2 min of sonication at 20 W ml^{-1} power outputs, whereas the highest increase of acid phosphatase activity was observed at 4 min sonication. If we accept that the strongly acidic conditions used for extraction totally inactivated ATPases, the observed decrease in ATP content at 4 min sonication may be explained in terms of liberation of new adsorption sites on soil colloids through sonication or release from soil constituents of non-enzymatic catalysts that dephosphorylated ATP. Sonication per se did not affect the pure enzyme activity or disappearance of commercial ATP from solution, even at energies greater than those adopted for dispersing soil (data not shown).

To further clarify whether the increase of acid phosphatase activity upon sonication is due more to the extracellular than to the intracellular enzyme fraction, the fate of soluble acid phosphatase added to soil was followed. The assumption is that it would be comparable to the enzyme eventually released from intracellular compartments during cell lysis. Given that adding enzyme significantly decreased its activity, this suggests the release of enzymes from cell lysis would result in their being inactivated rapidly either by adsorption, protease degradation or inhibitors. The application of ultrasound to soil mixed with potato acid phosphatase resulted in a substantial recovery of the enzyme activity, probably due to desorption of enzyme from clay-organic complexes. Therefore, cell lysis following sonication caused a release of intracellular acid phosphatase, which was partly adsorbed onto organo-mineral colloids and partly combined with the extracellular free enzyme.

In the light of our findings, the concept of assaying potential activity of soil enzymes should be revised because the results are dependent on the amount of aggregate disruption before the assay. Moreover, soil treatment with sonication seems to liberate a significant dormant portion of acid phosphatase activity. The nature, half-life and kinetic properties of this fraction must be investigated since it could have significant implications for soil biochemistry, biological fertility and bioremediation studies.

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