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Full Paper

High-throughput Procedure for the Peat Soil Reference Sample Stabilization Using Microwave Radiation

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Abstract: Reference materials used in quality control laboratories need to maintain homogeneity and stability that can be affected by temperature, humidity and microbial activity. This research aimed to investigate a rapid, cheap and high-throughput procedure, using microwave radiation for the inhibition of microbial growth in peat reference sample, in order to increase its stability. Different exposure times (60, 120 and 180 seconds) of the material to radiation were evaluated, and the microbiological tests were conducted to assess the percentage reduction in CFUs for fungi and bacteria. The results showed 100% and >90% of reduction in growth for bacterial and fungi, respectively, over 90 days of monitoring the material, using 120 to 180 seconds of microwave radiation exposure times. The results demonstrate that this method is economical and efficient at stabilizing peat reference materials.

Keywords: microwave; microbiological stabilization; peat; reference materials

1. INTRODUCTION

The evolution of good agricultural practice and precision agriculture has been increased investments in soil quality control including fertilizers and soil correctives. Organic fertilizers and preparations based on peat soil are widely used in agriculture, because peat is a very rich source in organic matter and biologically active substances [1]. 50-95% of organic substances constitute the peat soils [2], highlighting that this type of soil is formed by the accumulation and partial decomposition of plant biomass in marshes, including vast peatlands in boreal, temperate and tropical regions [3].

Therefore, the soil quality control laboratories have searching to ensure the reliability and quality of the analytical methods measurements. In this sense, the use of reference materials for all soil types is becoming increasingly necessary. These materials must show essential characteristics of homogeneity and stability [4] and their stability must be evaluated at specific periods of time and temperature, since the material may undergo degradation by the action of temperature, humidity and microbial activity [5].

Sterilization methods should not cause significant changes in the physical or chemical properties of the soil sample (i.e.), only provide the completely elimination of the all viable organisms [6]. The autoclaving is the sterilization method generally used for sterilizing soil samples, because this equipment normally is available in most research and teaching laboratories [7]. However, this sterilization technique shows a problem due to spore forming microorganisms contaminant remains in the carrier after autoclaving process [8]. Since it's first applications, microwaves have been used for many purposes, including industrial processes [9], environmental treatments [10], and samples preparations [11].

The microwave sterilization method has the advantage to have a greater sterilization time control, in comparing to sterilization method using autoclave

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[12]. Different studies have shown that sterilization or disinfecting using microwaves is viable, such as inactivation of *Clostridium difficile* spores in aqueous suspension [13], in the disinfection of maxillary complete dentures on the treatment of *Candida* related denture stomatitis [14] and the destruction of fecal coliforms from biosolids [15]. Both thermal and non-thermal effects have been suggested to play parts in antimicrobial activity [16-18]. In addition to being an effective method of sterilization and disinfection, presents a low cost, high speed, simplicity to use [19].

Although the gamma irradiation is a technique used for sterilizing different materials by the National Institute of Standards and Technology (NIST), this method has a high operating cost, making its use feasible only on a small scale [20]. Thus, in this research we investigated the use of microwave radiation to inhibit microbial growth on peat to increase its stability, in order to use this method for the stabilization of reference materials, for inter-laboratory comparisons studies.

2. MATERIAL AND METHODS

2.1. Samples and Reagents

The peat sample was obtained from Agrinobre Company (www.agrinobre.com.br) located in Sinimbu, RS, Brazil. This peat sample, named NTGOLD, is a turface soil enriched with expanded vermiculite, dolomitic limestone, gypsum, fertilizer (nitrogen, phosphorus and potassium) and micronutrients (http://www.agrinobre.com.br/produtos/tn-gold/). The NTGOLD physical properties are pH 5.00, electrical conductivity (mS/cm): 0.4 - 0.6, density: 140 kg/m^3 and water retention capacity (w/w) 150%.

Distilled and deionized water was used to prepare all standard solutions and reagents (resistivity > 18 MΩ·cm). The culture medium used for microbial growth was nutrient agar (Difco™, São Paulo, Brazil) and dextrose agar (SDA) (Difco™, São Paulo, Brazil) pH 4.5 for the cultivation of fungi and yeasts.

2.2. Procedure

Ten kilograms peat sample were submitted to drying in an oven with air circulation (model MA037, Marconi) at 65°C (to a constant weight) to reduce the natural moisture, and were ground using a grinder (model Osterizer, Blender). The dry sample remaining (5 kg, moisture < 5%) was homogenized in a V-type homogenizer (model MA 200, Marconi), quartered using a splitter (model DR 100, Retsch) and placed in appropriate bags for microwave treatment, containing approximately 50 grams.

To generate random numbers for selecting samples, the Excel® software data analysis was used. Three bags of samples containing approximately 50 grams each were used to microwave treatment; one sample for each reference times (60, 120 and 180 seconds), by using a domestic microwave oven (model NN-S60BK, Panasonic), with a power of 690±32 watts (n=7).

For this, seven hundred grams of distilled and deionized water were heated at maximum power for 180 seconds, and the output power of the microwave oven was evaluated from the general ratio, where the output power (P), in watts was calculated according to equation (1):

$$P = \frac{k.Cp.m.\Delta T}{t} \qquad (1)$$

where k is the conversion factor (from thermal chemical calories s^{-1} to watts, 4.184 J cal^{-1}), Cp is the heat capacity (or thermal capacity, $ºC^{-1}$), m is the mass of the sample (g), ΔT is the temperature variation (°C) after microwave heating, and t is the irradiation time (s) [21].

After microwave radiation treatment, the samples were stored in polystyrene box at room temperature in the dark, and the microbial growth was monitored up to 90 days. For microbiological analysis, two grams of each sample were dissolved in 150 mL of 0.85% sodium chloride solution. After this, the material was subjected to 160 rpm shaking for 30 minutes to obtain the initial suspension. From this suspension, successive dilutions up to 10^{-7} were prepared to obtain the best dilution for the experiments.

The experiment was conducted according to surface scattering technique, where the inoculation was performed plating 0.1 mL of the sample in their respective dilutions, on the surface of solidified culture medium, and with the supported of a spreader glass, the inoculum was spread for all the entire surface of the culture medium [22]. The microbiological count was performed 3 days after incubation. The plating was performed in triplicate for each sample and its respective exposure time to microwave radiation.

The culture medium used for microbial growth was nutrient agar at pH 6.8 for the cultivation of bacteria in an incubator at 30 °C and SDA at pH 4.5 for

the cultivation of fungi and yeasts, incubated at a controlled temperature of 25 °C. Count of colony forming units (CFU) for bacteria and fungi were assessed after incubation. The inoculation procedures were conducted in a laminar flow hood, and the material used for the handling and dilution of the samples were adequately prepared and sterilized following the standard NBR 15313 [23].

The results obtained through the developed procedure were compared with the results obtained by the control sample using the Kruskal-Wallis non-parametric test (GraphPad Instat® software, version 3.00).

3. RESULTS AND DISCUSSION

The dilution rate was established at 10^{-2}, because it was the lowest dilution rate that allowed the correct count of CFUs. Initial tests were conducted using 60, 120 and 180 seconds the radiation treatment. However, the time of 60 seconds was not effective in inhibiting bacterial colony growth when compared to 120 and 180 seconds. Therefore, samples treated for 120 and 180 seconds were used in further tests to assess the efficiency of microwave radiation in reducing microbiological growth in peat material

Subsequent tests were run on the 1st, 7th, 17th and 90th days after treatment of the sample by radiation. The results obtained at 120 and 180 seconds of exposure to electromagnetic radiation, showed that these treatments were highly efficient in reducing bacteria, considering that sterilization was completely achieved (Figure 1). Similar results were presented by [24] using a microwave oven in eliminating bacteria in cotton seed hulls, with 150 seconds of exposure to electromagnetic radiation.

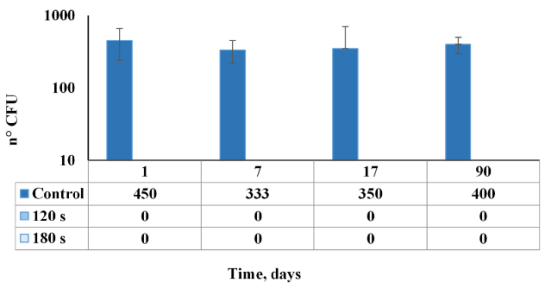

Figure 1. Count of colony forming units (CFU) for bacteria. Number of CFU of bacteria (n = 3) at different exposure times (seconds - s) of the sample to electromagnetic radiation on the 1st, 7th, 17th and 90th days after treatment.

Microwave radiation was applied by [5] to elimination of mycobacteria in peat used as a supplement for pigs, and good results were obtained using littler samples mass (up to 5 g), and 5 minutes of exposure in 700 watts power. However, the authors don´t monitored the microbial growth after microwave treatment. According to [26], microwave radiation is direct, efficient and requires less time when compared to conventional sterilization techniques due to the strong penetrating effect of the waves.

In the growth control of fungi colonies, we obtained a significant ($p < 0.05$) decrease in the number of CFUs (Figure 2). This was observed both for the time 120 seconds and for 180 seconds. The results remained promising 90 days after the application of radiation using the microwave, thus indicating good efficacy of the technique for the reduction of microorganisms, which might interfere with the stability of peat as a reference material. In studies developed by Komarova *et al*. [27] about the effect of microwave radiation on the germination of *Streptomyces xanthochromogen* spores in peat soil, it has been show that exposure to radiation affects the development of microorganisms, from the spore germination phase to the formation stage of microcolonies.

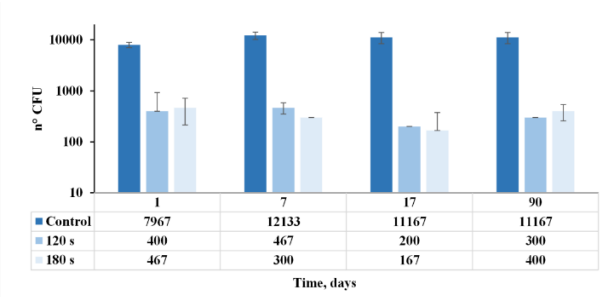

Figure 2. Count of colony forming units (CFU) for fungi. Number of CFU of fungi (n = 3) at different exposure times (seconds - s) of the sample to electromagnetic radiation on the 1st, 7th, 17th and 90th days after treatment.

The time of radiation exposure is dependent on the material to be stabilized [28], demonstrating the efficiency of microwave in the specific resin sterilization, highlighting that the time necessary to inactivate the evaluated microorganisms was of 180 seconds at 650 W. Other study that evaluated the efficiency of microwave in sterilization of orthodontic instruments proved that microwave was efficient requiring 600 seconds for sterilization of all materials

at 800 W [19]. The microwave sterilization also was an effective method in growth control of the bacteria *Aggregatibacter actinomycetemcomitans* [12].

For bacterial growth, 100% reduction in growth was achieved with the application of radiation (Figure 3), both for 120 seconds and for 180 seconds. This reduction was stable up to 90 days after treatment, at which time the microbiological analysis of the material was performed. In the same conditions, for fungi growth, a marked reduction in CFUs was observed (Figure 3), maintaining a reduction percentage >90% over 90 days of monitoring the material.

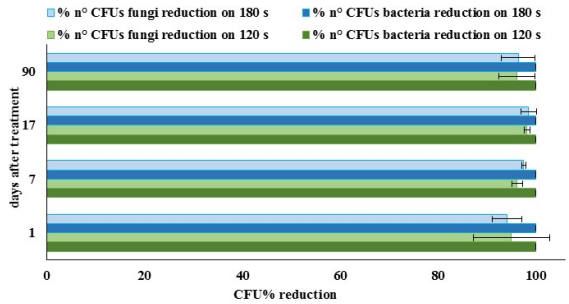

Figure 3. Reduction in growth for bacterial and fungal. Percentage reduction in the growth at 120 and 180 seconds (s) on the 1st, 7th, 17th and 90th days after exposure to radiation.

However, sterility of the material was not achieved, as the growth of fungi colonies continued, but a significant ($p < 0.05$) decline in the number of colonies occurred. This was due to the presence of fungi spores, which are more resistant to sterilization than vegetative cells and in this case requires a longer period of exposure.

4. CONCLUSION

The results of processing the electromagnetic radiation material used in this study proved to be very satisfactory. Both times presented satisfactory results for the reduction of the bacteria and fungi in peat sample. For the bacteria, the inhibition was achieved where the time of 120 seconds has been sufficient for this purpose. The CFUs reduction percentage for fungi exceeded 90%, resulting in an effective disinfectant for vegetative structures and most fungi spore material.

The times of 120 and 180 seconds not presented significant difference for the fungi reduction, so the 120 seconds is an adequate time for sterilization of this type of material. However, for the sterilization of fungi is necessary the application a more time of the electromagnetic radiation exposition. The stabilization was satisfactory up to 90 days after treatment, adequate to be use as reference material in inter-laboratory quality control to organic matter and clay determinations, in the soil fertility investigations. That way, microwaves proved to be an effective alternative for the sterilization and disinfection of work material and was a simple method to operate.

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