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# Characteristics of the soil-like substrates produced with a novel technique combining aerobic fermentation and earthworm treatment

Wenli Kang, Wenting He, Leyuan Li, Hong Liu\*

Laboratory of Environmental Biology and Life Support Technology, School of Biological Science and Medical Engineering, Beihang University, Beijing 100191, China

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#### Abstract

The soil-like substrate (SLS) technique is key for improving the closure of bioregenerative life support system (BLSS) by recycling the inedible biomass of higher plants. In this study, a novel SLS technique (NSLST) was proposed: aerobic fermentations at 35 °C for 1 day, then 60 °C for 6 days, finally 30 °C for 3 days, followed by earthworm treatment for 70 days. Comparing with the original SLS technique (OSLST), its process cycle was 13 days shorter, and the dry weight loss rate (81.1%) was improved by 24.77%. The cellulose and lignin degradation rates were 96.6% and 94.6%. The concentrations of available N, P and K in mature SLS were respectively 776.1 mg/L, 348.0 mg/L and 7943.0 mg/L. Low CH<sub>4</sub> and NH<sub>3</sub> production was observed, but no accumulation. According to the seed germination test, the SLSs were feasible for plant growth. This investigation will provide a preliminary foundation for BLSS design. © 2012 COSPAR. Published by Elsevier Ltd. All rights reserved.

Keywords: Soil-like substrate (SLS); Bioregenerative life support system (BLSS); Wheat-rice straw; Degradation rate; Earthworm

## 1. Introduction

Bioregenerative life support system (BLSS) is crucial for long-term and far-distance manned space missions (Czupalla et al., 2005; Hu et al., 2010; Liu et al., 2008). One of the key problems for improving the closure of BLSS is to dispose and recycle the inedible biomass of higher plants. In recent years, soil-like substrate (SLS) technique has received extensive attention because of its efficiency on the recycle of the inedible biomass of higher plants and the increase of BLSS closure (Liu et al., 2008; Manukovsky et al., 2001; Tikhomirov et al., 2003).

Researchers from Russia have carried out long-time tests of planting wheat and radish on the SLS, and the continuous cultivation of wheat and radish for multiple generations has been achieved (Manukovsky et al., 1997). The traditional SLS technique was successive conversion of rice or wheat straws with mushrooms and worms during a period of 123 days (Manukovsky et al., 1997; Yu et al., 2008). Tikhomirov et al. (2003) also indicated that SLS worked very well in BLSS, and the system closure was improved from 66.19% to 72.13%. When mushrooms and earthworms were introduced to prepare SLS, the straw conversion was as high as 77.31% and the mushroom production was 46.67 g/100 g straw (Kang et al., 2010; Yu et al., 2008). In addition, Nesterenko et al. (2009) have indicated that SLS had obvious inhibiting effects on wheat root rot fungus (Bipolaris sorokiniana), and the inhibiting effects were better with more earthworms. In our previous work, by comparing three SLS production techniques, we got an optimal technique (it will be called "the original SLS technique, OSLST" in the following parts of the paper). As is mentioned in He et al. (2010), the OSLST was as follows: aerobic fermentation at 60 °C for 1 day, followed by fermentation at 45 °C for 2 days, then by

<sup>\*</sup> Corresponding author. Tel./fax: +86 10 82339837.

E-mail address: LH64@buaa.edu.cn (H. Liu).

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earthworm treatment for 90 days. Compared with the traditional technique, the straw degradation rate of OSLST was higher and its cycle (93 days) was 30 days shorter. Nevertheless, in order to apply SLS technique in BLSS with higher system closure, the cycle of the technique is still too long and the straw degradation rate is not high enough. Hence, the OSLST needs further improvement.

In order to shorten the SLS preparation cycle and enhance the degradation rates of cellulose and lignin, a novel SLS technique (NSLST) was developed by simulating the process of composting (Magalhães et al., 1993; Strom, 1985) and combining it with earthworm treatment in this study. The characteristics of SLS prepared with NSLST, including the respiration, the release of trace gases, the degradation of cellulose and lignin and seed germination rate were observed.

#### 2. Materials and methods

#### 2.1. Materials

The spring wheat straw (*Triticum aestivum*) and the upland rice straw (*Oryza sativa*) chosen for this experiment were obtained from Chinese Academy of Agricultural Sciences (CAAS).

The earthworms (*Eisenia foetida Savigny*) used in this study were bought from Deyi earthworm and organic fertilizer company of Beijing.

The seeds of lettuce sativa (*Green Oak*) used for germination were also bought from CAAS.

### 2.2. SLS production procedures

For shortening the treatment time and enhancing the conversion efficiency, the procedure of NSLST was proposed by simulating the composting process and combining it with earthworm treatment, shown as follows:

First, with the rice and wheat straw ground to 1 mm and blended in the proportion of 1:1, the raw material was obtained. Then, they were treated by a series of aerobic fermentations:  $35 \,^{\circ}$ C for 1 day, then  $60 \,^{\circ}$ C for 6 days, finally  $30 \,^{\circ}$ C for 3 days. The products were named as fermented material (F-SLS). Then, F-SLS were converted to mature SLS (M-SLS) by cultivating earthworms (200 earthworms per kilogram biomass) in a dark and quiet environment with good ventilation for 70 days. The temperature and relative humidity were maintained within 20–25 °C and 70–80%, respectively.

# 2.3. Determination of the physical and chemical properties

Dry weight loss was calculated by the subtraction of the dry weights before and after a preparation. The concentrations of available N, P and K were measured based on ASI (Ye et al., 2006). Van Soest analysis method was used to determine the concentrations of cellulose and lignin (Van Soest and Wine, 1968).

#### 2.4. Measurement of the SLS respiration

The respiration of SLS was measured with a respiration measuring apparatus (RP1LP, Qubit Systems Inc., Canada).

 $O_2$  consumption rate (OCR) of microorganisms was measured using a closed cylindrical device whose volume was 220 mL. 2–3 g SLS was put in the device each time through a sample entrance at the end of the device sealed with a silicone pad. The air within was sampled once per hour for 8 h.  $O_2$  was measured with the gas chromatograph (GC 7890 II Techcomp International Corp, China).

### 2.5. Determination of $CH_4$ and $NH_3$

To determine the safety of SLS during both the production process and storage period, the concentrations of trace gases (NH<sub>3</sub> and CH<sub>4</sub>) were measured. During the SLS process, we determined the trace gas accumulation once per phase for a period of 22 h each time with the reactor closed by sampling them at a frequency of once per two hours, each time 10 min at a flow rate of 0.8 L/min. NH<sub>3</sub> and CH<sub>4</sub> were sampled twice a day for 210 h during the SLS storage period starting from the moment when the M-SLS was produced and put in the storage.

The trace gases sampling apparatus was shown in Fig. 1, in which the control was a container with atmosphere inside. The air sampler and the valved air inlets were both linked to the SLS container with  $NH_3$  and  $H_2O$  filters. Open the valve when sampling for keeping a stable air pressure and the earthworms and microbes alive.

 $NH_3$  was measured with Nessler's reagent colorimetric method (Carneiro et al., 2000). The  $NH_3$  sampling system consisted of trapping tubes, absorbing flasks, flow meters and pumps.  $H_2SO_4$  was used as the absorption liquid.

CH<sub>4</sub> was measured with gas chromatogram (FID detector, 120 °C, high-purity  $N_2$  as carrier gas, the flow rate 20 mL/min, the minimum detectable limit 0.5 ppm). 1 mL of air sample was analyzed each time.

In order to provide  $O_2$  for earthworms and microbes in the reactor, the sampling time of  $NH_3$  was extended with the air sampler linked to the atmosphere. The compensatory calculations of  $NH_3$  and  $CH_4$  were shown as Eq. (1) and (2). For Eq. (1), the  $NH_3$  should be no less than 30 mL. There was no  $NH_3$  but  $CH_4$  in the reactor because of the  $NH_3$  filter.

The compensatory calculation of NH<sub>3</sub> was given as

$$c_i = b_i + \frac{v_1 \times \sum_{k=1}^{i-1} c_k}{v}$$
(1)

where  $c_i$  is the actual concentration of NH<sub>3</sub> sampled at the *i*th time;  $b_i$  is the detectable concentration of NH<sub>3</sub> sampled at the *i*th time;  $v_1$  is the lost gas volume, 8 L; v is the gas volume in the reactor.

The compensatory calculation of CH<sub>4</sub> was given as

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