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## New insights into biodrying mechanism associated with tryptophan and tyrosine degradations during sewage sludge biodrying



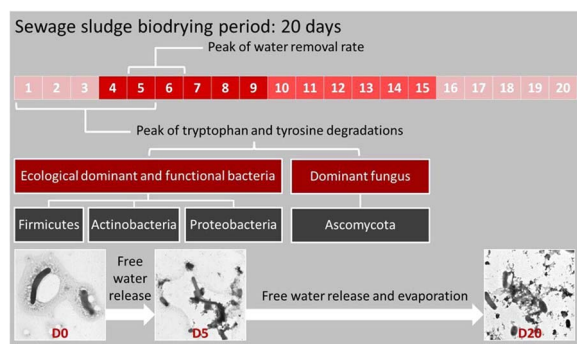
Lu Cai<sup>a,b,\*</sup>, Thomas Krafft<sup>b</sup>, Tong-Bin Chen<sup>c</sup>, Wen-Zhou Lv<sup>a</sup>, Ding Gao<sup>c</sup>, Han-Yan Zhang<sup>a</sup>

<sup>a</sup> Faculty of Architectural, Civil Engineering and Environment, Ningbo University, Ningbo 315211, China

<sup>b</sup> Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht 6200 MD, The Netherlands

<sup>c</sup> Center for Environmental Remediation, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China

### GRAPHICAL ABSTRACT



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### ABSTRACT

Sewage sludge biodrying is a treatment that uses bio-heat generated from organic degradation to remove water from sewage sludge. Dewatering is still limited during biodrying, due to the presence of extracellular polymeric substances (EPS) in sludge. To study the biodrying mechanism associated with EPS compositions tryptophan and tyrosine degradations, this study investigated the microbial function in sludge biodrying material. This study conducted a taxonomic analysis of biodrying material; determined the most abundant genetic functions; analyzed the functional microorganisms involved in the degradations of tryptophan and tyrosine; and summarized the metabolic pathways. The results indicated efficient degradations of tryptophan and tyrosine were observed during the initial thermophilic phase; functional microorganisms were mainly from the phyla Firmicutes, Actinobacteria, and Proteobacteria, enriched with genes involved in amino acid transport and metabolism. These findings highlight the potentially important microorganisms and typical pathways that may help improve dewaterability during biodegradation.

### 1. Introduction

Activated sludge processes are widely used in municipal wastewater treatment plants. These processes generate and discharge a large amount of waste sewage sludge (SS) (Shannon et al., 2008; Ju and

Zhang, 2015). Although the condensed SS is mechanically dehydrated, its moisture content (MC) is still as high as 80–85%. This complicates subsequent treatment and accounts for a major portion of treatment cost. Given these factors, a more environmental-friendly and affordable SS treatment is needed.

\* Corresponding author at: Faculty of Architectural, Civil Engineering and Environment, Ningbo University, 818 Fenghua Road, Ningbo 315211, China.  
 E-mail address: [cailu@nbu.edu.cn](mailto:cailu@nbu.edu.cn) (L. Cai).

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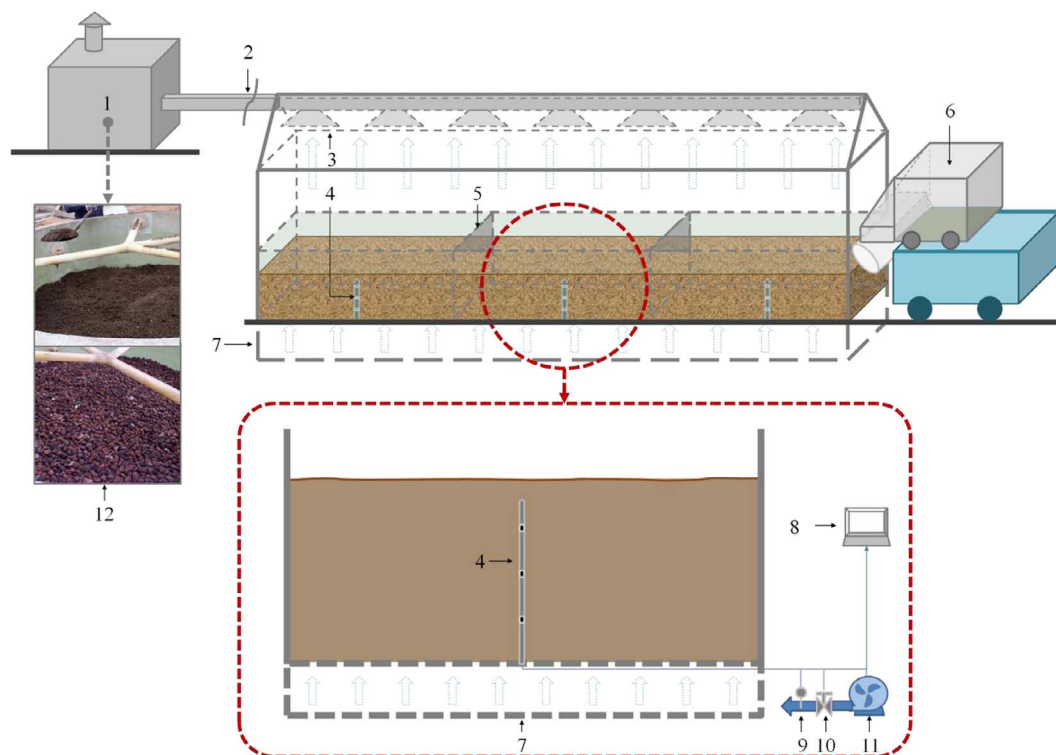


Fig. 1. Schematic diagram of sewage sludge biodrying pile. 1. Biofilter for exhaust gas treatment; 2. Gas-collection pipe; 3. Gas-collection hood; 4. Probe with triplicate temperature sensors; 5. Plastic partition; 6. Turning machine; 7. Air chamber; 8. Computer; 9. Thermal flowmeter; 10. Electric valve; 11. Roots blower; 12. Filter media: biodrying product and volcanics.

Biodrying is a technique that maximizes water removal from biomass waste, using the microbial heat generated from organic matter degradation. This process disinfects and partially bio-stabilizes the biomass waste (Rada et al., 2007; Velis et al., 2009). Within the biodrying reactor, the degradable organic matter in sewage sludge biodrying material (SSBM) decomposes, releasing thermal energy (Navae-Ardeh et al., 2010; Zhang et al., 2015). Water evaporates from SSBM through air convection and air diffusion; this evaporation is promoted by biological heat and mechanical operations (Zhao et al., 2010; Cai et al., 2013). Biodrying is featured for its efficiency and flexibility. It can be used to pretreat compost, coupled with a maturing or curing period after the biodrying, or it can be used as a treatment to make biofuel or landfill cover soil (Winkler et al., 2013; Rada and Ragazzi, 2014). Biodrying has been widely used to treat SS (Huiliñir and Villegas, 2015; Tom et al., 2016).

One of the main concerns during SS biodrying is the limitation on dewatering. Cai et al. (2013) observed that during the initial 3–4 days of biodrying, the dewatering was limited, even with an excessive forced aeration, which may be related to the abundant bound water in the initial SSBM. Cai et al. (2016a) used dielectric constant measurement to confirm that when biodrying pile temperature exceeded 40 °C, the greatest amount of free water was released, and the dewaterability increased.

Recent studies have shown that sludge dewaterability is correlated well with the proteins in the extracellular polymeric substances (EPS) in SS (Zhang et al., 2016). Based on previous studies, proteins and polysaccharides are dominant EPS compositions (Xu et al., 2013; Guo et al., 2016); of the proteins, a large proportion are composed of tyrosine and tryptophan substances (Li et al., 2014; Li et al., 2015). Zeng et al. (2016) found that the tyrosine and tryptophan strongly depend on microbial community and activity, and Zhang et al. (2016) reported that destroying EPS compositions results in the release of trapped water. Cai et al. (2016a) found that the tryptophan and tyrosine were degraded when the biodrying process entered the thermophilic phase,

accompanying with the free water release. Given that the correlation between EPS composition degradation and dewaterability, it is interesting to find biodrying mechanisms associated with tryptophan and tyrosine degradations. However, no systematic research has identified the corresponding degradation mechanism.

This study investigated the biodrying mechanism related to the degradations of EPS compositions tryptophan and tyrosine. First, the contents of total protein, tryptophan and tyrosine were determined. Next, high-throughput sequencing was performed to examine the SSBM collected during different biodrying phases in the fungal Internal Transcribed Spacer (ITS) region, and a complete metagenomic study of SSBM during the initial thermophilic phase was also performed. Then, microbial function analyses were conducted to get a better understanding of the mechanisms. This provided information about the microbial composition of SSBM, and revealed critical genetic functions related to biodrying. The study results provide valuable microbiota information that may inform the control of biodrying.

## 2. Materials and methods

### 2.1. Materials

Mechanically dewatered SS cake and biodrying product were collected from an SS treatment plant in Shanghai, China. The biodrying product was a cured mixture of SS and bulk agent, biodried before this experiment. Sawdust (SD) was obtained from a timber mill. Biodrying product and SD were used as the combined bulk agent. The SS, biodrying product, and SD were mechanically mixed using an optimized mixer (GreenTech Environmental Engineering Co., Beijing, China). This ensured a homogeneous SSBM mixture, at a mass ratio of 6:3:1 (SS to biodrying product to SD). For the SS, SD, biodrying product, and initial SSBM, MC values were 83.5, 10.0, 48.3, and 66.3%, respectively, and VS contents were 68.3, 98.9, 76.1, and 87.4%, respectively.

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