Bioresource Technology 241 (2017) 134–141

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Impact of phosphate-solubilizing bacteria inoculation methods on phosphorus transformation and long-term utilization in composting

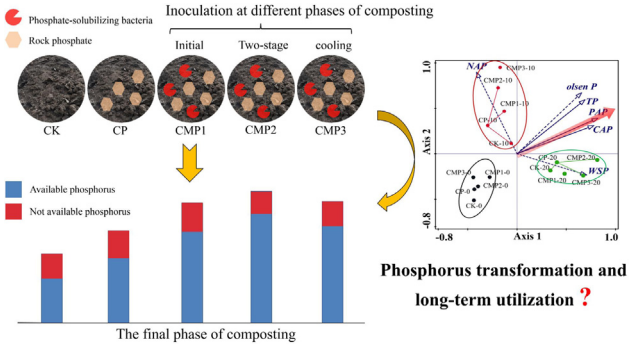
Yuquan Wei [a], Yue Zhao [a], Yuying Fan [a], Qian Lu [a], Mingxiao Li [b], Qingbin Wei [c], Yi Zhao [a], Zhenyu Cao [a], Zimin Wei [a,*]

[a] College of Life Science, Northeast Agricultural University, Harbin 150030, China
[b] State Key Laboratory of Environment Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing 100012, China
[c] Heilongjiang Province Environmental Monitoring Centre, Harbin 150056, China

HIGHLIGHTS

- PSB was inoculated at different phases to solubilize RP during composting.
- Composting bacterial community was compared in different inoculation groups.
- The biotransformation of P was described by potential available P in all treatments.
- A suitable method of PSB inoculation was stated to enhance the efficiency of P use.
- Three methods were suggested to improve P transformation and long-term utilization.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 31 March 2017
Received in revised form 12 May 2017
Accepted 16 May 2017
Available online 19 May 2017

Keywords:
Composting
Different stages inoculation
Phosphate-solubilizing bacteria (PSB)
Denaturing gradient gel electrophoresis (DGGE)
Potential available phosphorus (PAP)

ABSTRACT

This study aimed to assess the effect of phosphate-solubilizing bacteria (PSB) application and inoculation methods on rock phosphate (RP) solubilization and bacterial community during composting. The results showed that PSB inoculation in different stages of composting, especially both in the beginning and cooling stages, not only improved the diversity and abundance of PSB and bacterial community, but also distinctly increased the content of potential available phosphorus. Redundancy analysis indicated that the combined inoculation of PSB in the initial stage with higher inoculation amount and in the cooling stage with lower inoculation amount was the best way to improve the inoculation effect and increase the solubilization and utilization of RP during composting. Besides, we suggested three methods to improve phosphorus transformation and long-term utilization efficiency in composts based on biological fixation of phosphates by humic substance and phosphate-accumulating organisms.

1. Introduction

In China, the improvement of living standards as a result of its rapid economic development and population expansion means the growing output of municipal solid waste (MSW), which affects the urban environment (Wang et al., 2015a). To achieve the goals of resource conservation and reducing environmental and health impacts, composting is a widely used method to effectively manage MSW though a biological stabilization process (Wei et al., 2007). Microbes play key roles in all the events related to the biotransformation of substrates during composting (Zhao et al.,

* Corresponding author.
E-mail addresses: weizm691120@163.com, weizimin@neau.edu.cn (Z. Wei).

http://dx.doi.org/10.1016/j.biortech.2017.05.099
0960-8524/

2016a). Insufficient quantity or limited biological activities of the indigenous microbial communities could lead to undesirable composting efficiency. Numerous studies have shown that inoculation or strengthening indigenous microorganisms may improve the composting process (Wei et al., 2016a; Xi et al., 2015).

Phosphorus (P) is an essential element for plant growth and is widely applied as inorganic fertilizer for agricultural purposes (Wei et al., 2015). Rock phosphate (RP) is the main base raw material from which inorganic fertilizers can be made in large quantities (Owen et al., 2015). However, considering that the reserves of RP are relatively finite, it is crucial to enhance the availability of P and avoid potential risk for P loss in runoff and leachate. A number of studies showed that most of the organic wastes composting from different sources could influence the content and distrib ution of P fractions (Wei et al., 2015; Hashimoto et al., 2014; Ngo et al., 2013), thus, some organic wastes with low P content are always used for composting amended with RP for increasing the quantity of organic matter and available P. Phosphate-solubilizing bacteria (PSB) have fundamental roles in P cycling in natural and agricultural ecosystems (Wei et al., 2016a). Given that composting ecosystem exhibited rich bacterial diversity with different enzymatic capabilities but the complicated environmental conditions, e.g., temperature fluctuations and limited nutrients, could inhibit microbial activities, numerous studies have been conducted to apply inoculants for adjusting the correlation between microbial communities with different biochemical functions, such as, enhancing degradation of cellulose, improving humification and reducing ammonia emission (Zhao et al., 2016b; Zhang et al., 2016; Xi et al., 2015; Hachicha et al., 2012), while there is little information about the relationship among the inoculation period of PSB inoculants, indigenous bacteria and P-solubilization efficiency.

However, higher P-solubilization may lead to an increased potential risk for P loss in runoff and leachate and be possibly linked to the activity of PSB with a negative feedback (Wei et al., 2015; Mander et al., 2012). So, to improve the long-term fertility of composts with RP, it is inevitable to discuss how to stabilize redundant labile P or control P-solubilization process and P forms. Composting is also a controlled biological process for rapid stabilization and humification of organic matter as an increase of humic substances (Zhao et al., 2017; Wei et al., 2007). It is known that humic matter can affect the solubility of different P-compounds in soils through its chelation capacity or metal bridging, which is considered as a form of biological fixation of phosphates (Borggaard et al., 2005). If considered together, the period of PSB inoculation, P-solubilization process, and changes of indigenous microbial community as well as the formation of humic substances may provide powerful new insights into the transformation of P fractions during composting based on biological and physicochemical method, which would truly enhance the efficiency of P use.

In this work, five MSW composting experiments were conducted with different PSB or RP-addition disposals. The principal goals of this study were to (1) analyze the microbial biomass and bacterial structure in different composting treatments, (2) compare the influence of different inoculation methods on PSB activity and P fractions, (3) characterize the interactions between inoculants, indigenous bacteria, P forms and treatments, and (4) present a biological and physicochemical method for future studies to better control the P transformation and utilization efficiency in composts.

2. Materials and methods

2.1. Composting experimental design

Composting materials were mainly residual MSWs with inorganic materials such as metals, plastics, and glasses removed before composting, which were collected from Northeast Agricultural University (Harbin, China). Straw chopped into small pieces (20–30 mm) was used to adjust the initial C/N ratio of composting materials. The basic chemical characterization of the raw materials were shown in Table 1. The ratio of water content is approximately 60% and C/N ratio is about 25:1. Composting materials were put in the special small cylinder compost reactor, which referenced to Zhao et al. (2016b). The changes of reactor temperature see supplementary Fig. S1. The actual temperature of compost was basically the same as reactor. During composting, moisture was maintained at 50–60% and the ventilation rate was 0.5 L min^{-1}. The initial and final conditions of composting were shown in the Table S1.

Five groups of composting experiments were carried out and all of the treatments were replicated three times. MSW was chosen as the major raw material because it has a relatively low P content (Wei et al., 2015) and therefore allowed the effects of the PSB and RP on composting to be readily observed. The first group is blank control group (CK). Nothing is added during composting. For the other four groups, RP was added with 5%, which was obtained from KaiLin company, Guizhou, with 28.27% P_2O_5, 3.55% Olsen P and 5.21% soluble P in 2% citric acid. The group with only RP addition was prepared as negative control (CP). There are three PSB treatment groups, which are inoculated in the initial stage (0d) as CMP1, in the cooling stage (8d) as CMP3 and in the above two stages as CMP2, respectively. The inoculant of composting in this study was a kind of compound PSB agent, which was described in a separate paper (Wei et al., 2016b). Inoculant in each group was at the level of 1.5% in dry weight with the same inoculation amount, and the concentrations of the composite PSB strains were 1×10^8 CFU mL^{-1}. The whole time of the experiment in this work is 20 days and the samples collected every two days. Some samples were stored at −20 °C for DNA extraction and others were used for physical-chemical analysis and P content, while those for microbial quantification were freshly processed.

2.2. Microbiological enumeration and molecular biological analyses

The cultivable bacteria and PSB were estimated using a standard dilution-plating procedure as described by Wei et al. (2016a). Bacteria were cultured in standard Nutrient Agar (Cultimed, Spain) for 48 h at 30 °C. PSB was cultivated in National Botanical Research Institute's phosphate growth medium devoid of yeast extract (NBRIY) medium supplemented with 1.5% Bactoagar (Difco Laboratories, Detroit, MI, USA).

DNA was isolated for dominant microorganism using soil DNA kit (Omega Biotek, Inc.). To analyze the DNA of the bacterial community, 16S rRNA genes were amplified using the prokaryotic primers 341F/534R (Xi et al., 2015). A GC clamp was attached to forward primers to prevent complete separation of the strands during DGGE. Polymerase chain reaction (PCR) conditions for each 50-μL reaction mixture and touch down PCR protocol were performed as described by Wei et al. (2016a). Each PCR concoction was set in a polyacrylamide (8%) gels at a 35–60% denaturant gradient. A Gene Mutation Detection System (Bio-Rad Laboratories, Inc.) was run at 150 V for 4 h at 60 °C to separate the fragments. After electrophoresis, gels were stained in 3 × GelRed™ nuclear acid gel stain (Biotium, USA) and photographed with a UVP Imaging System (UVP Inc., USA). This procedure was performed in triplicate for each sample with different gels. Representatives of bands that were clear and had high intensity were excised from DGGE gels and transferred to 30-μL Milli-Q water (Millipore, USA), incubated overnight for elution of DNA at 4 °C. Then, PCR was performed to re-amplify the DNA using primers 534R and 341F without a GC-clamp. After sequencing, the results were compared with the GenBank from the National Center of Biotechnology Information (NCBI) using BLAST.