Bioresource Technology 198 (2015) 867–875

Contents lists available at ScienceDirect

Bioresource Technology

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

Improved fermentation performance in an expanded ectopic fermentation system inoculated with thermophilic bacteria

Hui Guo [a,1], Changxiong Zhu [b,1], Bing Geng [b], Xue Liu [b], Jing Ye [b], Yunlong Tian [b], Xiawei Peng [a,*]

[a] College of Biological Sciences and Technology, Beijing Forestry University, Beijing, China
[b] Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, Beijing, China

HIGHLIGHTS

- An expanded EFS to deal with large quantities of cow wastewater was investigated.
- Effects of the thermophilic bacterial consortium on EFS were studied.
- Physicochemical and biological characteristics explained the efficient fermentation.
- The relationship between environmental factors and dominant bacteria was revealed.

ARTICLE INFO

Article history:
Received 26 July 2015
Received in revised form 11 September 2015
Accepted 30 September 2015
Available online 9 October 2015

Keywords:
Cow wastewater
Ectopic fermentation system
Thermophilic bacterial consortium
T-RFLP

ABSTRACT

Previous research showed that ectopic fermentation system (EFS) inoculated with thermophilic bacteria is an excellent alternative for cow wastewater treatment. In this study, the effects of thermophilic bacterial consortium on the efficiency and quality of the fermentation process in EFS were evaluated by measuring physicochemical and environmental factors and the changes in organic matter composition. In parallel, the microbial communities correlated with fermentation performance were identified. Inoculation of EFS with thermophilic bacterial consortium led to higher temperatures, increased wastewater requirements for continuous fermentation, and improved quality of the litters in terms of physicochemical factors, security test, functional group analysis, and bacterial community composition. The relationship between the transformation of organic component and the dominant bacteria species indicated that environmental factors contributed to strain growth, which subsequently promoted the fermentation process. The results highlight the great potential of EFS model for wide application in cow wastewater treatment and re-utilization as bio-fertilizer.

1. Introduction

Groundwater pollution is a major threat to residential areas, and unreasonable emission of farm wastewater is a major contributor to this problem (Ruane et al., 2011). The most common method to treat farm wastewater is anaerobic digestion, which is also one of the best techniques for industrial wastewater treatment (Goncalves et al., 2012). However, the use of this method to treat cow wastewater is expensive because biogas residues must be further processed to achieve zero-pollution emissions. Electrocoagulation can be used to pretreat wastewater in order to reduce decolorization and chemical oxygen demand, but this method is effective only for a single treatment, and other techniques must be used as well (Yetilmezsoy et al., 2009). Several studies have suggested that wastewater could be treated using an anaerobic sludge blanket reactor, which would reduce possible environment hazards (Farhadian et al., 2007; Chavez et al., 2005). However, the sludge still has potential safety hazards due to the presence of heavy metals.

An ectopic fermentation system (EFS) has been proposed to overcome these limitations. Previously, EFS was developed as a novel system for wastewater treatment by using a complex microbial preparation of functional thermophilic microbes mixed with straw prior to fermentation (Guo et al., 2013). In this process, cow wastewater was first added to an impounding reservoir mixed with padding comprising maize straw, rape stalk, rice straw, mushroom residue, and sawdust. This system was an *ex situ* experimental setup that worked dynamically by the inoculation of complex microbial agents and successive supplementation of the collected cow wastewater. This EFS was more advanced than common static

* Corresponding author at: College of Biological Sciences and Technology, Beijing Forestry University, No 35 Tsinghua East Road, Haidian District, Beijing 100083, China. Tel./fax: +86 10 62336013.
E-mail address: bjfu220@163.com (X. Peng).
[1] These two authors contributed equally to this paper.

http://dx.doi.org/10.1016/j.biortech.2015.09.105
0960-8524/

fermentation systems because the continuous fermentation was relatively stable and not easily influenced by environmental conditions. One of the main benefits of this method is that it can settle cow wastewater matter and avoid straw burning in rural areas, which damages the air environment. Additionally, the cow wastewater is completely absorbed by the padding materials during fermentation and leaves adequate residues, which can have huge economic value for agronomic applications as bio-fertilizer. For these reasons, this system is beneficial to both environmental protection and rural economic development.

Previous studies have demonstrated that a microbial consortium of six thermophilic bacteria can be used in an EFS to effectively process cow wastewater by aerobic fermentation. This EFS model with a microbial consortium provides continuous fermentation with supplementation of large wastewater quantities from dairy farm operations (Guo et al., 2013). However, the effect of the microbial consortium on the EFS as well as the relationship between the microbial consortium and fermentation indicators in the reaction system are largely unknown. It was the goal of the current study to evaluate physicochemical characteristics during the fermentation process and to measure changes in the thermophilic bacterial consortium structure in relation to environmental factors. Security of the fermentation materials was evaluated by determining the germination index (GI), ascaris egg mortality, and fecal coliform counts. Changes in characteristic functional groups and overall structure of organic matter during the fermentation process were detected by FTIR spectroscopy and nuclear magnetic resonance (CPMAS ^{13}C NMR). The bacterial community structure was studied using terminal restriction fragment length polymorphism (T-RFLP) analysis, and the effect of environmental factors on bacterial community composition was comprehensively analyzed by canonical correlation analysis (CCA) and litter environmental factor analysis. This approach allowed us to evaluate the effects of different members of the thermophilic bacterial consortium and provide a theoretical basis for EFS improvements and applications in the future.

2. Methods

2.1. Experimental design

The experimental design included a control group (without microbial inocula; CK) and treatment group (with microbial inocula; Sample 1). Six bacterial strains screened from the previous study (G21, G14, G4-1, CR-3, CR-14, and CR-15) were inoculated on LB medium (10 g peptone, 5 g yeast extract, and 10 g NaCl in 1 L distilled water) and cultured until OD_{600} reached 0.8. The microbial inoculum contained these six microorganism cultures (G21:G14:G4-1:CR-3:CR-14:CR-15 = 1:1:1:1:1:1, v/v). Strains G21, G4-1, and CR-15 were *Bacillus subtilis*, CR-3 was *Bacillus methylotrophicus*, CR-14 was *Bacillus licheniformis*, and G14 was *Paenibacillus lactis* (Guo et al., 2013). To create an EFS model, padding comprising maize straw, mushroom residue, and sawdust (55:35:10) was added to fermentation vessels ($200 \times 100 \times 110$ cm). The litter height was 70 cm, and to this, 20% cow dung wastewater was added. Table 1 lists the physicochemical properties of the starting materials. After the cow wastewater was poured into the vessels, the litter was stirred properly to maintain an initial moisture content of approximately 50–60%. The fermentation process was conducted with manual stirring every 3 days, and additional wastewater was added as needed. At the end of the first fermentation, new material was added to the initial litter height, and the weight of the new material was recorded. After addition of an equal amount of wastewater, a second fermentation was performed. Samples were collected by mixing three subsamples from different sites of the fermentation substrate and then stored at low temperature prior to analysis (Guo et al., 2013, 2014).

2.2. Physicochemical analysis of the fermentation process

The temperature of the fermentation heap and environment were measured daily after the start of the compost. The measurement sites were selected from multiple depth points in the vessels. During the 71 days of the composting process, sampling occurred every 7 days to measure pH and moisture content. Available P, available K, NH_4^+—N, NO_3^-—N, and the C/N ratio were determined on days 0, 14, 28, 35, 49, and 71 during the fermentation process using previously described methods (Guo et al., 2013; Zhang et al., 2013; Zhang and Sun, 2014).

2.3. FTIR spectroscopy and NMR analysis

FTIR spectroscopy and CPMAS ^{13}C NMR analysis of samples was performed based on published methods (Caricasole et al., 2010; Zhou et al., 2014).

2.4. Toxicity tests

Seed germination index, ascaris egg mortality, and fecal coliform counts were determined at the initial and final points of ectopic fermentation using previously described methods (Awasthi et al., 2014; Zhang and Sun, 2014).

2.5. DNA extraction and PCR amplification

Total DNA was extracted from frozen samples using the CTAB method and then was stored at −80 °C until use (Wetzel et al., 2014). For bacterial community analysis, PCR was used to amplify the 16S rRNA (de la Fuente et al., 2014; Székely et al., 2009).

2.6. Terminal restriction fragment length polymorphism (T-RFLP) analysis

Bacterial community analysis was accomplished using T-RFLP analysis. Labeled PCR products (10 μL) were digested using Msp I (TAKARA, Japan). Terminal fragments were analyzed using time-resolved fluorescence at the Center of Forecasting and Analysis, Chinese Academy of Agricultural Sciences (Beijing, China). The digestion products were separated on a 3730XL genetic analyzer, and the results were evaluated by short tandem repeat technology. T-RFLP profile analysis was performed using GelComparII 3.0 software (Applied Maths, Kortrijk, Belgium) directly from chromatogram files (de la Fuente et al., 2014; Székely et al., 2009).

2.7. Statistical analysis

One-way ANOVA was used to detect differences between treatments. Tukey's test was also performed to evaluate the distribution of the data sets. The SPSS statistical package (Window Version 13.0) was used for data analysis. All results of statistical analysis in this study were deemed significant at $P < 0.05$.

3. Results and discussion

3.1. Analysis of changes in physicochemical characteristics during fermentation

Temperature, pH, water content, available P, available K, NH_4^+—N, NO_3^-—N, and the C/N ratio were the key indicators of fermentation that were monitored. These factors determine the rate of