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Fermentation performance optimization in an ectopic fermentation system

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ABSTRACT

Ectopic fermentation systems (EFSs) were developed for wastewater treatment. Previous studies have investigated the ability of thermophilic bacteria to improve fermentation performance in EFS. Continuing this research, we evaluated EFS performance using principle component analysis and investigated the addition of different proportions of cow dung. Viable bacteria communities were clustered and identified using BOX-AIR-based repetitive extragenic palindromic-PCR and 16S rDNA analysis. The results revealed optimal conditions for the padding were maize straw inoculated with thermophilic bacteria. Adding 20% cow dung yielded the best pH values (6.94–8.56), higher temperatures, increased wastewater absorption, improved litter quality, and greater microbial quantities. The viable bacteria groups were enriched by the addition of thermophilic consortium, and exogenous strains G21, G14, G4-1, and CR-15 were detected in fermentation process. The proportion of *Bacillus* species in treatment groups reached 70.37% after fermentation, demonstrating that thermophilic bacteria, especially *Bacillus*, have an important role in EFS, supporting previous predictions.

1. Introduction

Problems associated with groundwater pollution caused by excessive emissions of farm wastewater present a severe threat to human living environments (Ruane et al., 2011). Anaerobic digestion is the most common method to treat farm wastewater and also one of the best techniques for industrial wastewater treatment (Gonçalves et al., 2012). However, it is a costly approach for treating farm wastewater because biogas residues and biogas slurry require further processing to achieve zero-pollution emissions. Several additional approaches can be used to treat wastewater, such as electrocoagulation and anaerobic sludge blankets. However, electrocoagulation techniques only have a single treatment effect, and other techniques must be used for complete treatment. Meanwhile, the sludge from anaerobic sludge blankets presents a potential safety hazard due to the presence of heavy metals (Anfruns-Estrada et al., 2017; Chen et al., 2017; Shi et al., 2017; Tejedor-Sanz et al., 2017).

Ectopic fermentation system (EFS), composed of complex microbial communities that include functional thermophilic microbes mixed with litter, have been developed to overcome the limitations of existing wastewater treatments (Guo et al., 2013). As an *ex situ* experimental setup, EFS works dynamically based on the inoculation of complex microbial agents and successive supplementation of collected cow wastewater. This method is more advanced than common static fermentation because of the relative stability and lower influence of the external environment. In the previous study, six thermophilic bacteria strains were identified and chosen as the microbial inocula, and the EFS inoculated with them brought higher temperature and more wastewater was needed to ensure continuous fermentation (Guo et al., 2013). Further research showed that the expanded EFS inoculated with thermophilic bacteria provided a model of continuous fermentation with the supplementation of wastewater from a dairy farm. The effects of the thermophilic bacterial consortium on EFS were studied and the relationship between environmental factors and dominant bacteria was revealed. It showed that various environmental factors and the dominant bacterial species, mainly *Bacillus*, synergistically accelerate the fermentation rate for improved and greater wastewater treatment (Guo et al., 2015). Moreover, EFSs can settle solid matter from cow wastewater and prevent straw burning in rural areas, and the post-fermentation padding materials have substantial economic value as a bio-fertiliser. Changes in the thermophilic bacterial consortium structure have been measured using terminal restriction fragment length polymorphism (T-RFLP) analysis, revealing *Bacillus* and *Proteobacteria* as the dominant bacterial flora (Guo et al., 2015). However, T-RFLP analysis cannot confirm whether exogenous thermophilic bacteria, in particular *Bacillus* species, remain alive during fermentation (Giles et al., 2017; Jernberg et al., 2007). Besides, the comprehensive impacts of the bacteria, padding materials, and addition of cow dung and wastewater on EFSs are unknown, and require further investigation.

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Therefore, in the present study, we evaluated the effects of different bacterial species, padding materials, and the addition of cow dung on EFS performance in a small-scale fermentation bed. The selected consortium and litter were analysed synthetically by principle component analysis (PCA). The pH was regulated by the addition of different proportions of cow dung and then the compost indicators were measured. Based on the evaluation results of a small-scale test, a pilot-scale EFS was tested under optimum fermentation conditions to explore the change in culturable bacteria during the fermentation process. The viable bacteria were recorded using BOX-AIR-based repetitive extragenic palindromic-PCR (BOX-PCR) analysis and the clustered species were identified based on 16S rDNA analysis. These results completed the research, and provided a theoretical basis for large-scale popularization and application of EFS.

2. Materials and methods

2.1. Experimental design

The EFS was designed and controlled with reference to the literature (Guo et al., 2013; Guo et al., 2015). Nine small-scale EFSs (vessel size: 47.5 × 37.0 × 43.0 cm) and one pilot-scale EFS (vessel size: 200 × 100 × 110 cm) were used in this experiment. The small-scale systems were processed under three treatments, as shown in Table 1. A lab-made microbial consortium contained 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*. A purchased microbial consortium contained photosynthetic bacteria, lactic acid bacteria, yeast, gram-positive actinomycetes, and filamentous flora. Padding was obtained from cattle farms in Beijing and Chaohu, China. Cow wastewater was added successively into the reaction vessels on days 0, 4, 8, 12, and 18, which were stirred regularly to maintain the moisture content and ensure continuous fermentation. Measurements were taken from multipoint depths in the systems. The fermentation heap, environment temperatures, pH, moisture content, total nitrogen (TN), total phosphorous (TP), total potassium (TK), organic matter content, and carbon-to-nitrogen (C/N) ratio were measured according to previous studies (Guo et al., 2013).

2.2. Principal component analysis of the fermentation parameters

PCA is a multivariate statistical method. Based on the principal component scores, it can examine multivariate relationships and explain variance of the data while reducing the number of variables to several groups of individuals (Zhiyuan et al., 2011).

According to the capacity of wastewater absorption and the increase of nutrient content after fermentation, the small-scale EFS were assessed comprehensively, and the evaluation system of indicators was established preliminarily. It is a convenient and reliable method in EFS process analysis.

Using PCA, EFS performance was evaluated based on a number of indicators, including the number of days with a high fermentation temperature (> 50 °C), the number of days with pH values below 7.3, the weight proportions of litter and wastewater, the increase in the proportions of TN, TP, and TK, and the reduction of the proportions of organic matter and C/N after fermentation. The standard range of the Kaiser–Meyer–Olkin (KMO) and Bartlett's test is 0.5–1. The cumulative contribution rate achieving 80% was selected as the main component factor. The comprehensive indicators unrelated to each other were standardized and imported into formulas to obtain F_1, F_2, and F. Then, the principal component comprehensive score and rank were used to evaluate the performance of all EFS treatments (Mather, 1976; Rencher, 2003; Vitale et al., 2017). The formulas to obtain F_1, F_2, and F ($\lambda_1 = 5.684$, $\lambda_2 = 1.177$) are as follows (Gao and Dong, 2007):

$$F_1 = 0.20ZX_1 + 0.274ZX_2 + 0.191ZX_3 - 0.188ZX_4 + 0.271ZX_5 + 0.099ZX_6 + 0.224ZX_7 - 0.181ZX_8$$

$$F_2 = -0.006ZX_1 - 0.141ZX_2 - 0.002ZX_3 + 0.554ZX_4 - 0.147ZX_5 + 0.1ZX_6 - 0.1ZX_7 + 0.551ZX_8$$

Here, ZX_1, ZX_2, ZX_3…, ZXn are the standardised values of the original variable after processing. Then, the principal component synthesis model was calculated:

$$F = (\lambda_1 \times F_1 + \lambda_2 \times F_2)/(\lambda_1 + \lambda_2)$$

2.3. Optimization of the proportion of added cow dung

Padding comprising maize straw, mushroom residue, and sawdust (55:35:10) was added to the small-scale fermentation vessels inoculated with the lab-made microbial consortium. Then, different amounts of cow dung (treatment 1 [0%], 2 [10%], 3 [20%], 4 [30%], 5 [40%], and 6 [50%]) were added to each of the six fermentation vessels. These vessels were operated following the procedure described in Section 2.1.

2.4. Physicochemical property analysis

The fermentation heap and environment temperatures were measured daily after beginning composting. During the 24 days of the composting process, samples were collected on days 0, 2, 5, 7, 10, 12, 14, 16, 19, 22, and 24 to measure the pH, moisture content, TN, TP, TK, organic matter content, and C/N ratio as previously described (Crosland et al., 1995; Jones and Walker, 1963; Kalembasa and Jenkinson, 1973).

2.5. Microbial analysis

Bacteria, actinomycetes, and fungi were isolated from the mixture using the serial dilution method by plating 100 μL of diluted suspension from each medium on beef extract-peptone agar (per litre: 10.0 g peptone, 5.0 g beef extract, 5.0 g NaCl, and 20 g agar; pH 7.2–7.5), actinomycetes culture medium (per litre: 1.0 g KNO_3, 0.01 g $FeSO_4{\cdot}7H_2O$, 0.5 g K_2HPO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.5 g NaCl, and 20 g agar; pH 7.5), and Martin culture medium (per litre: 10.0 g glucose, 5.0 g peptone, 1.0 g K_2HPO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 3.3 mL Rose bengal [1%], and 20 g agar), respectively. The plates were incubated for 3, 5, and 7 days, and the colonies were counted and populations were expressed in terms of colony-forming units (CFUs) (Chandna et al., 2013; Guo et al., 2014).

Table 1
Experimental setup.

Tests	Padding composition	Treatment 1	Treatment 2	Treatment 3
Test 1	Maize Straw: Mushroom Residue: Sawdust = 55:35:10	W1-1: Control (no inoculated strains)	W1-2: Lab-made microbial consortium (2‰ inocula)	W1-3: Purchased microbial consortium (2‰ inocula)
Test 2	Rape Stalk: Mushroom Residue: Sawdust = 55:35:10	W2-1: Control (no inoculated strains)	W2-2: Lab-made microbial consortium (2‰ inocula)	W2-3: Purchased microbial consortium (2‰ inocula)
Test 3	Rice Straw: Mushroom Residue: Sawdust = 55:35:10	W3-1: Control (no inoculated strains)	W3-2: Lab-made microbial consortium (2‰ inocula)	W3-3: Purchased microbial consortium (2‰ inocula)