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Characterization of bacterial consortium and its application in an ectopic fermentation system

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ABSTRACT

This study aimed to develop an ectopic fermentation system (EFS) to reduce the pollution of cow wastewater and to provide a basis for the production of biofertilizer with fermentation residues. Six thermophilic strains, three of which have efficient cellulose-degrading abilities and the other have good ammonia-N utilizing abilities, were chosen as the microbial inocula. The results showed that EFS inoculated with microbial consortium brought higher temperature and more wastewater was needed to ensure continuous fermentation. The pH values decreased in the early stage of fermentation, and then increased during the process. It caused increases in total Kjeldahl nitrogen, total phosphorous, and total potassium content. Decreases in organic matter content and *C*/*N* ratio were also observed. The high level of nutrients indicated the suitability of the paddings after fermentation for agronomic uses. It firstly attempted to combine cow wastewater treatment and bio-organic fertilizer production by EFS with mixed microbial culture.

1. Introduction

Groundwater pollution is a major threat to human living environments, and the unreasonable emission of farm wastewater is a major source of this problem. Rising population levels, improved standards of living, and changing dietary patterns have increased the demand for dairy food products (Ruane et al., 2011). The expansion of breeding scales from place to place and the more intense production in dairy farms are possible contributors to the increasing volumes of wastewater. Thus, effective measures are required to address this urgent concern.

The most common method to treat wastewater is anaerobic digestion, which is the natural degradation pathway of organic materials into methane, carbon dioxide, and fertilizer. This method is one of the bests for treating industrial wastewater (Goncalves et al., 2012). But there are some biogas residues and biogas slurry produced from anaerobic digestion, and so the using of this way to treat cow wastewater is expensive because the biogas residues were still needed to be further processed to achieve the aim of zero-pollution emission, and the transportation of biogas slurry also needs more cost. Electrocoagulation technique is used to pretreat wastewater in order to reduce decolorization and chemical oxygen demand (Yetilmezsoy et al., 2009), but it only has a single treatment effect and other techniques must be added. Several studies suggested that the wastewater could be treated by anaerobic sludge blanket (UAFB) reactor to reduce possible environment hazards (Farhadian et al., 2007; Chavez et al., 2005). However, the sludge still have potential safety hazard because of the existence of heavy metal.

Functional microbes can be made into complex microbial preparation and mixed with straws for fermentation, i.e., cow wastewater diverted into an impounding reservoir mixed with paddings (including maize straw, rape stalk, rice straw, mushroom residue, sawdust and so on) to ferment using a mixed culture of thermophilic microbes. On one hand, this method can settle the matter of cow wastewater because it can avoid the burning of straws in rural areas, which damages the air environment. On the other hand, the cow wastewater was absorbed by the padding materials during the fermentation, leaving no surplus and the fermentation residues have a huge economic value because of their application in agronomic aspects. Hence, this measure is beneficial to both environmental protection and economic development. Several studies have introduced the transformations and decomposition in the pig-on-litter system (Tam and Vrijmoed, 1993), with reference to the deep litter housing system for beef cattle (Deininger et al., 2007; Westerath et al., 2009). However, these studies were

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Table 1
Experimental design.

Tests	Litter combination	Treatment 1	Treatment 2
Test 1	Maize straw: mushroom residue: sawdust = 55:35:10	W1-1: control (no inoculated strains)	W1-2: microbial consortium (2‰ inocula)
Test 2	Rape stalk: mushroom residue: sawdust = 55:35:10	W2-1: control (no inoculated strains)	W2-2: microbial consortium (2‰ inocula)
Test 3	Rice straw: mushroom residue: sawdust = 55:35:10	W3-1: control (no inoculated strains)	W3-2: microbial consortium (2‰ inocula)

Table 2
Physicochemical properties of mixed materials.

Mixtures	*C*/*N* ratio	Organic matter (%)	Total *N* (%)	Total *P* (%)	Total *K* (%)
Test 1	89.22	99.98	0.65	0.31	0.38
Test 2	78.55	96.15	0.71	0.39	0.49
Test 3	60.96	87.23	0.83	0.40	0.42

only related to livestock that mainly produces solid waste. The problem of large volumes of wastewater from dairy farms cannot be solved using the same method. Thus, it was proposed to solve this kind of pollution via an ectopic fermentation system (EFS). The EFS was developed as a novel system for wastewater treatment. It is an ex-situ experimental setup filled with paddings, which dynamically works by the inoculation of complex microbial agent and successive supplementation of the collected cow wastewater. It is advanced than common static fermentation that this continuous fermentation system is relatively stable and not easy to be influenced by the environmental conditions. In this study, cellulose-degrading microorganisms were isolated, identified, and applied to efficiently degrade cellulose in padding materials that mainly contain cellulose, hemicellulose, and lignin. The microorganisms for ammonia-N utilizing were also screened and cultured to deal with the ammonia-N composition in cow wastewater. It maintains the continuous fermentation by the supplementation of wastewater from the dairy farm, and some of the biological and chemical properties were analyzed.

2. Methods

2.1. Isolation and identification of thermophilic cellulose-degrading bacteria and thermophilic ammonia-N utilizing strains

Samples were collected from dairy manure that was composted with straw in a farm in Beijing, China, and then stored at 4 °C in the Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences. Then, thermophilic cellulose-degrading bacteria were isolated and cultivated by CMC medium (carboxymethyl cellulose medium: CMC-Na 15.0 g/L, NH_4NO_3 1.0 g/L, yeast extract 1.0 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, KH_2PO_4 1.0 g/L, and agar 15.0 g/L) (Rastogi et al., 2009; Sizova et al., 2011). The cellulose-degrading abilities of all the bacterial isolates that survived under high temperature were determined (Ponnambalam et al., 2011). The bacterial strains which could survive at 50 °C and utilize ammonia-N were screened (Fan and Lin, 2010). After that, the thermophilic cellulose-degrading bacteria and ammonia-N utilizing strains with high biological activities were selected for identification according to literature (Song et al., 2011).

2.2. Antagonistic effects

Efficient cellulose-degrading strains were tested for their mutual antagonistic activities in order not to influence the inoculation. Two different strains cross-streaked but did not intersect on a Luria–Bertani (10 g peptone, 5 g yeast extract, 10 g NaCl, and 20 g agar in 1 L distilled water) plate. The antagonistic phenomena were observed after incubation at 30 °C for 48 h.

2.3. Ectopic fermentation

The padding containing maize straw, rape stalk, rice straw, mushroom residue, and sawdust, respectively, was added into fermentation vessels (47.5 × 37.0 × 43.0 cm) to create a small model for understanding the possibilities and suitable conditions of an ectopic fermentation system (EFS). Next, cow wastewater mixed with the microbial agents was poured into the vessels. The padding was stirred properly to maintain the moisture content and then the fermentation was carried out. The vessels were stirred manually every three days.

Three kinds of mixing padding in nine vessels (10 kg each) were used in this experiment. Each kind was processed by two treatments as shown in the Table 1.

The microbial agent contains the mixed microorganism culture (G21:G14:G4-1:CR-3:CR-14:CR-15 = 1:1:1:1:1:1, v/v). The cow wastewater was added into these vessels on days 0, 4, 8, 12, and 18, successively.

The mixing paddings were obtained separately from farms in the cities of Beijing and Chaohu. Select characteristics of the materials are shown in Table 2.

2.4. Parameter analysis of the fermentation process

The fermentation heap and environment temperatures were measured every day after the start of the compost. The measurement sites were taken from multipoint depths in vessels. During the 27 days of the composting process, sampling occurred on days 0, 1, 3, 4, 6, 8, 12, 16, 18, 21, 24, and 27, to measure the pH value and test the moisture content.

Total Kjeldahl nitrogen (TKN), total phosphorous (TP), total potassium (TK), organic matter content, and *C*/*N* ratio were measured at the initial and final points of ectopic fermentation by using the methods according to literature (Jackson, 1975; Crosland et al., 1995; Kalembasa and Jenkinson, 1973).

3. Results and discussion

3.1. Isolation of thermophilic cellulose-degrading bacteria and ammonia-N removing strains

The padding materials, including maize straw, rape stalk, rice straw, mushroom residue, and sawdust, mainly contained cellulose and hemicellulose. During fermentation, the temperature will be high and the ammonia-N composition of cow wastewater will produce NH_3. Thus, screening for thermophilic cellulose-degrading