Effects of Different Proportions of Pig Manures and Sugarcane Leaves on Cu and Zn Forms in Dry Fermentation

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Abstract: To explore the influence of different proportions of pig manures and sugarcane leaves on Cu and Zn in pig manures, pig manures and sugarcane leaves were used as raw materials to determine the changes of Cu and Zn effectiveness in pig manures under different proportions of pig manures and sugarcane leaves. The results show that under different proportions of pig manures and sugarcane leaves, (1) when the proportion of sugarcane leaves and pig manures was 2:1, it was adverse against the bio-availability of Cu. When the proportion was 1:2, bio-availability form (EX and RED) of Cu decreased significantly. (2) The bio-effective form content is ranked as below in Zn: C (1:2) >B (1:1)>A (2:1). When the proportion of sugarcane leaves and pig manures was 1:2, bio-availability form (EX and RED) of Zn was the minimum, only 22.72%, and the passivation effect was good.

1. Introduction

In intensive livestock and poultry breeding, all kinds of high-content heavy metal additives are generally added into feeds to promote livestock and poultry growth and prevent various diseases. The additive amount of Cu and Zn in pig feeds reaches 20-40 times of normal physiological needs of pigs. At present, the content of Cu, Zn, Cd, As and other heavy metals in pig manures is significantly higher than that in chicken manures, duck manures and cow dungs. According to the limit standards of livestock and poultry manures in vegetable fields in the guidelines for safe use of livestock and poultry manures, the over-limit rates of Cu, Zn and As in pig manures are 74.59%, 78.69% and 9.84% respectively, where Cu and Zn severely exceed the limits[1]. Most heavy metals cannot be absorbed by animals, and these heavy metals will be excreted along with pig manures and urines. If heavy metals in livestock and poultry manures exceed the limits, fail to be strictly treated and are used as fertilizers to plant fruits and vegetables, human health will be severely impaired[2-3]. Although Cu and Zn have been included in the feed limit standard again in China, merchants still add them in quantity for economic benefits. If heavy metals in livestock and poultry manures exceed the limits, fail to be strictly treated and are used as fertilizers to plant fruits and vegetables, human health will be severely impaired, and this is also adverse against long-term economic development.

As the understanding of heavy metals is deepened, it is found that the total amount of heavy metals in pollutants cannot well represent the environmental effect after heavy metals enter soil. For example, the total amount of heavy metals does not present a simple linear relation with the degree of damages to plants. According to the extent to which pollutants are utilized in biological transport in water
environment, people put forward the concept of bio-availability, and later it extended to soil, deposit sediment and other solid environments. With BCR sequential extraction method, the existence forms of heavy metals in deposit sediments include four forms: acid soluble form (EX), reducible form (RED), oxidizable form (OXI), and residual form (RES). Among them, acid soluble form and reducible form have strong bioactivity and belong to bio-effective form. The content of bio-effective form has a direct effect on soil and crop absorption, while stable form refers to the sum of oxidizable form and residual form[4-7].

In this study, pig manure was chosen as the object. BCR sequential extraction method was adopted to investigate the morphologic changes of Cu and Zn which were 2 heavy metals with the highest content in pig manures in the anaerobic fermentation process of sugarcane leaves and pig manures with different proportions and to provide theoretical basis for seeking effective heavy metal passivation methods.

2. Experimental Materials and Methods

2.1. Experimental materials
Sugarcane leaves: The discarded sugarcane leaves of South Subtropical Crop Research Institute, China Academy of Tropical Agricultural Sciences in Zhanjiang City, China were collected by the bander. The moisture content was 13%, and the sugarcane leaves were smashed to less than 5cm for standby application.

Pig manures: Pig manures were fresh pig manures taken from an individual pig farm in Mazhang District, Zhanjiang City, and the moisture content was 26.78%.

Inoculant: The fermentation inoculant was the residue of dry anaerobic fermentation of pig manures from Biogas Production Base of Institute of Agricultural Machinery Research, Chinese Academy of Tropical Agricultural Sciences.

| Material       | Cu   | Zn    |
|----------------|------|-------|
| Pig manure     | 128.63 | 824.26 |
| Inoculant      | 27.3  | 43.25 |

2.2. Testing apparatus
A 1L conical flask was used as the anaerobic fermentation flask, and the gas generated in the anaerobic process was collected by the 1L wide-mouth bottle. The 1L conical flask was used as the water collector. The device was connected by a corrosion-resistant latex tube, and the flask neck was sealed strictly by structural adhesive to form a set of airtight anaerobic digester, as shown in Fig.1. The electric-heated thermostatic water bath was used for heating and thermal insulation of the testing apparatus.

![Fig.1 Fermentation apparatus of dry anaerobic methane](image)

(1. Thermostatic water bath; 2. Anaerobic fermentation flask; 3. Air duct; 4. Gas taking mouth; 5. Gas collection flask; 6. Water manifold; 7. Gas collection flask)
2.3. Experimental method

The raw materials were grouped according to dry matter ratio, mixture and fermentation, including Group A (sugarcane leaves: pig manures 2:1), Group B (sugarcane leaves: pig manures 1:1) and Group C (sugarcane leaves: pig manures 1:2). 3 repetitions were set for each group of experiments, and TS (total solid) was adjusted to 20% through adding a proper amount of water. Other fermentation conditions: temperature 38℃, inoculant 30%, pH value 7, and fermentation period 30d.

2.4. Detection method

TS was determined by gravimetric method. The temperature was automatically controlled at 38℃ through the thermostatic water bath. The gas production quantity was measured by daily drainage method. Methane content was determined by the biogas analyzer. Heavy metal form and content: BCR extraction method was used for analysis and determination. The content of Cu and Zn was determined by ICP method, and they were digested by aqua regia - perchloric acid. BCR tri-state continuous extraction method proposed by European Communities Bureau of Reference was used for morphological analysis of Cu and Zn.

3. Results and Analysis

3.1. Effects of different proportions of pig manures and sugarcane leaves on gas yield and methane content of dry fermentation volume

As shown in Fig.2, each group could achieve gas production in a short time. The gas yield was low in the first 4 days and reached the peak on the 5th - 8th day. Later, the sharp downtrend appeared, and then the stationary phase of gas production emerged. After the fermentation for 20d, the gas yield was very small. In the fermentation process, the wax coat of sugarcane leaves was destroyed with mass anaerobe propagation and under the action of bio-enzymes so that celluloses and hemicelluloses were degraded to form polysaccharide for microorganism. The gas yield in Group C reached the peak first (1.58L/d), and the gas production period was the longest. The gas yield of Group B was only second to that of Group C. In each fermentation group, methane content presented the process of first rising and then descending. According to Fig.3, average methane content was C (57.38%) >B (56.66%) >A (55.62%). Methane content of Group C could reach 63.4% at most, followed by Group B (61.7%) and Group A (58.5%).

Fig.2 Daily gas production of anaerobic fermentation under different proportions of raw materials

Fig.3 Average methane content of anaerobic fermentation under different proportions of raw materials
3.2. Effects of different proportions of pig manures and sugarcane leaves on Cu and Zn forms in dry fermentation

As shown in Fig. 4, the effects of different proportions of pig manures and sugarcane leaves on Cu content in the biogas residues differ a lot. In Group A (sugarcane leaves: pig manures 2:1), the content of bio-availability form (EX and RED) of Cu rose, while the content of stable form (OXI and RES) of Cu decreased, without the expected passivation effect. Based on the analysis of the fermentation process, the possible reason is that, in this proportion, the addition proportion of straw raw materials was large, and the carbon-nitrogen ratio was high. As a result, acidification appeared, making the heavy metal form transforming to the unstable form. In Group B (sugarcane leaves: pig manures 1:1), the content of various forms of Cu changed a little. The content of EX and RED showed a descending trend, while the content of OXI and RES presented a rising trend. In Group C (sugarcane leaves: pig manures 1:2), the content of bio-availability form (EX and RED) of Cu declined significantly, with a good effect of heavy metal passivation.

![Fig.4 Effects of different proportions of pig manures and sugarcane leaves on Cu forms before and after dry anaerobic fermentation](image)

As shown in Fig. 5, the effective form content of Zn in biogas residues under different proportions of pig manures and sugarcane leaves decreases with the rise of pig manures, and the decreasing amplitude of bio-effective form content is ranked as below: C (10.3%) > B (3.7%) > A (1.3%). In Group A (sugarcane leaves: pig manures 2:1), the content of bio-availability form (EX and RED) of Zn basically remained unchanged, without the increase. In Group B (sugarcane leaves: pig manures 1:1), the content of EX and RED of Zn decreased by 3.7%. In Group C (sugarcane leaves: pig manures 1:2), the content of bio-availability form (EX and RED) of Zn was the minimum, only 22.72%, and the passivation effect was good.

![Fig.5 Effects of different proportions of pig manures and sugarcane leaves on Zn forms before and after dry anaerobic fermentation](image)
4. Conclusions
In this study, pig manures and sugarcane leaves were chosen as the objects, and BCR sequential extraction method was adopted to investigate the morphologic changes of Cu and Zn which were two heavy metals with the highest content in pig manures in the anaerobic fermentation process of sugarcane leaves and pig manures with different proportions. The following conclusions were obtained:

(1) The effects of different proportions of pig manures and sugarcane leaves on Cu content in the biogas residues differ a lot. Different proportions of raw materials are sensitive to the transformation of heavy metal forms. When the proportion of sugarcane leaves and pig manures was 2:1, it was adverse against the bio-availability of Cu. When the proportion was 1:2, bio-availability form (EX and RED) of Cu decreased significantly, with a good effect of heavy metal passivation.

(2) The effective form content of Zn in biogas residues under different proportions of pig manures and sugarcane leaves decreases with the rise of pig manures, and the decreasing amplitude of bio-effective form content is ranked as below: C (10.3%) >B (3.7%)> A (1.3%). When the proportion of sugarcane leaves and pig manures was 1:2, bio-availability form (EX and RED) of Zn was the minimum, only 22.72%, and the passivation effect was good.

(3) The highest addition proportion of pig manures which can lower biological effective form content of heavy metals Cu and Zn in the dry anaerobic fermentation is not found in the research results. Besides, other key fermentation conditions influencing morphologic changes of heavy metals still need to be explored deeply.

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