The research on effect of purple soil enzyme activities by biogas slurry recirculation at large-scale farms

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**Abstract.** Soil enzyme plays an important role in converting soil nutrient, improving soil fertility and enhancing agricultural products quality. In this paper, the effect of purple soil enzyme activities was investigated by different biogas slurry (BS) recirculation load and period at large-scale farm in southwest China. The results indicated that the value of soil enzyme activities all increased by different BS recirculation load and period comparing to control check group. Its value was at high level by low BS recirculation load and long BS recirculation period. Soil urease and invertase activity reached highest value by T₄ (7 m³·hm⁻²) and T₈d (8 d), respectively. The value of soil catalase activity was highest by T₄ (40 m³·hm⁻²) and T₁₀d (10 d). In addition, soil phosphatase activity got peak value by T₄ (7 m³·hm⁻²) and T₄d (4 d). The effect of purple soil enzyme activities by BS recirculation at large-scale farms was explored to provide the theoretical basis for utilizing biogas slurry rationally.

1. **Introduction**

“Raise-Biogas-Recirculation” ecological pattern is applied to relieve the pressure on biogas slurry advanced treatment at large-scale farms at present. Biogas slurry recirculation can improve the structure of soil microbial community and agricultural products quality [1], maintain soil fertility and reduce the amount of pesticides and fertilizers application [2-5]. The research of Ding [6] et al. showed that the yield of tomato and rape increased significantly after biogas slurry recirculation. The high grade rate of tomato and rape seed was up 35% and 36% respectively. Soil microbes and enzymes play a vital role in transformation of material and energy. Soil fertility is largely dependent upon soil enzyme activities which are metabolic kinetics of soil organisms [7-8]. Correlation between soil enzyme activities and soil fertility was significant according to He [9] et al. It was also suggested that urease, invertase and alkaline phosphatase activity of soil could be used as an indicator for soil fertility. As demonstrated by Cao [10] et al, catalase and polyphenol oxidase activity of soil was increased by 66.7% and 76.7% respectively compared to control check group after BS recirculation. Hao [11] et al. revealed that soil invertase, phosphatase and protease activity was increased by BS recirculation, as well. In the present study, to identify the effect of soil enzyme activities by different BS recirculation load and period, experiments were conducted to investigate urease, invertase, catalase and phosphatase activities of purple soil surrounding a large-scale farm in southwest China.
2. Materials and methods

2.1. Samples

2.1.1. Biogas slurry. Biogas slurry samples were collected from anaerobic tank of wastewater treatment station, with pH 7.2 and average hydraulic retention time (HRT) 15 d. Concentrations of pollutants and heavy metals are given in table 1.

Table 1. Biogas slurry quality and standard (average concentration).

| Indicator | COD (mg·L⁻¹) | BOD₅ (mg·L⁻¹) | SS (mg·L⁻¹) | NH₄⁺-N (mg·L⁻¹) | NO₃⁻-N (mg·L⁻¹) | PO₄³⁻-P (mg·L⁻¹) |
|-----------|--------------|----------------|-------------|------------------|------------------|-------------------|
| Content   | 639          | 260            | 246         | 201              | 5.5              | 5.89              |
| Standard  | 100          | 40             | 60          | -                | -                | -                 |

*According to The Use of Urban Reclaimed Water—Quality of Farmland Recirculation Water GB20922-2007.

2.1.2. Soil. Soil samples, obtained from a large-scale farm in southwest China, were collected in three depths (0-5 cm, 5-10 cm and 10-20 cm) and stored at 4 °C in laboratory for further analysis at each month during trial period. Following this, they were air dried at room temperature (25 °C). After crushing, the dried samples were filtered through 2 mm, 1 mm or 0.25 mm sieve for determination of soil enzyme activities and physicochemical properties.

The soil was neutral purple layer, with pH 7.0-7.5 and calcium carbonate content less than 30 g·kg⁻¹. The physicochemical properties of soil such as porosity, bulk density, volume weight, soil organic carbon (SOC), and potassium are 54.7%, 1.2 g·cm⁻³, 2.65 g·cm⁻³, 130 mg·kg⁻¹ and 90 mg·kg⁻¹, respectively. Additionally, the soil fabric is uniform with general soil fertility, low soil organic matter (SOM) content and great water storage property. Soil total nitrogen (TN), total phosphorus (TP), total potassium (TK), available nitrogen, available phosphorus and available potassium were investigated based on The Chemistry Analysis Methods of Soil Agriculture [12]. More detailed physicochemical properties on purple soil are provided in table 2.

Table 2. Physicochemical properties of purple soil.

| Depth (cm) | Particles composition (%) | Total nitrogen | Total phosphorus | Total potassium |
|------------|---------------------------|----------------|------------------|----------------|
| 0-20       | 12.96                     | 21.16          | 42.64            | 23.12          |
| 0.2-0.02   | 0.01                      | 2.0            | 0.1              | 0.2            |
| 0.02-0.002 | 0.01                      | 0.01           | 0.01             | 0.01           |
| <0.002     | 0.01                      | 0.01           | 0.01             | 0.01           |

| Depth (cm) | Available nitrogen (mg·kg⁻¹) | Available phosphorus (mg·kg⁻¹) | Available potassium (mg·kg⁻¹) | Cation exchange capacity (me·100g⁻¹) | Fe (mg·kg⁻¹) | Mn (mg·kg⁻¹) |
|------------|-----------------------------|-------------------------------|-----------------------------|--------------------------------------|--------------|--------------|
| 0-20       | 69                          | 5                             | 64                          | 26.9                                 | 6.9          | 22.5         |

2.2. Experiment design

Soil samples were evenly packed in eleven sets of trial device (0.625 m × 0.4 m × 0.5 m) with three replications. BS recirculation load was 40 m³·hm⁻² (original BS), 20 m³·hm⁻², 10 m³·hm⁻² and 7 m³·hm⁻² (T₁, T₂, T₃ and T₄) and period was 2 d, 4 d, 6 d, 8 d and 10 d (T₁d, T₂d, T₃d, T₄d and T₁₀d). Besides, two control check groups were set for comparison respectively. Traditional soil enzymology was applied to explore variation of soil enzyme activities by four BS recirculation loads and five BS
recirculation periods. Variation of soil urease, invertase, catalase and phosphatase activities were investigated during 5 months BS recirculation (Aug. to Dec.) in same total application.

2.3. Soil enzyme activities analysis

The assay methods are as follows. The urease activity was determined by the amount of ammonia after hydrolyzing urea and NH$_4^+$ mass (µg) per g soil and 3 hours. The invertase activity was determined by the amount of hexose after hydrolyzing sucrose and 0.1 mol·L$^{-1}$ Na$_2$S$_2$O$_3$ volume (mL) per g soil. The catalase activity was determined by the remaining amount of hydrogen peroxide after titrating enzymatic reaction and 0.1 mol·L$^{-1}$ KMnO$_4$ volume (mL) per g soil. The phosphatase activity was determined by the amount of phenol hydrolyzed by matrix (phosphoric acid ester et al.) and phenol mass (µg) per g soil and 3 hours.

2.4. Statistical analysis

All statistical analyses were performed using SPSS19.0 statistical software. Statistical significance was accepted at p<0.05. The data (mean ± SE) of soil properties were analyzed using ANOVA analysis and compared by LSD test at p<0.05. Repetitive experiments comparison used relative standard deviation (RSD). Data plotting was processed using OriginPro 9.0.0.

3. Results and discussions

3.1. Soil urease activity

Urease, one of the amide enzymes, can promote hydrolysis of peptide linkage in organic molecule. It hydrolyzes urea to CO$_2$ and NH$_3$ as hydrolytic enzyme, widely used in soil quality evaluation. Urease exists in most bacteria, fungi and higher plants. Jia [14] et al. found that the urease activity had significant positive correlation with activities of microorganism, SOM, TN and available nitrogen content. Dindar [15] et al. reported that the urease activity can be served as an indicator of soil quality.

![Figure 1. Soil urease changes by different BS recirculation load over time.](image1)

![Figure 2. Soil urease changes by different BS recirculation period over time.](image2)

Variation in urease activity by different BS recirculation load is illustrated in figure 1. The value of urease activity was increased by 84.5% in Sep. by T$_1$ and 27% in Dec. by T$_2$. Its value was 2.24 and 2.46 times than Aug. and Nov. by T$_3$, respectively. As for T$_4$, it was up 52.2% in Nov. Albeit the highest change rate over months by different BS recirculation load was different, the urease activity all improved in various degrees, reaching highest value in Jul. Variation trend, decreasing in Aug., then increasing in Sep. and decreasing in Oct. again, can be obtained. On the whole, the value of urease activity during the first three months (Jul. to Sep.) after BS recirculation was higher. Figure 2 showed
that the urease activity reached peak value in Sep. by different BS recirculation period in brief. The value of urease activity was increased by 89.7% by T2 and 61.5% by T10d in Sep. What’s more, its value was increased by 54% by T4d, 40.6% by T6d and 248% by T8d in Nov. Generally, variation of urease activity was obvious by longer BS recirculation period.

Figure 1 and 2 indicated that the urease activity changed significantly after BS recirculation. The urease activity varied along with the change of BS recirculation load, consistent with the conclusion of Feng et al [1]. Mean value of soil urease activity by T3 was higher. In earlier stage, soil urease activity greatly increased as lots of NH4+-N addition by BS recirculation. Concentration of NH4+-N was too high with adding BS recirculation continuously, so excessive accumulation effect may depress urease activity. Qin [16] et al. demonstrated that the urease activity determined by the ammonium production rate was underestimated by 7.38%-15.97% because of nitrification in soil, depressing urease activity instead. Despite the value of soil urease activity improved in later stage, it was still lower than its value in Jul. Accumulation of NH4+-N was slow especially by long BS recirculation period, so the inhibition of soil urease activity was not effective. Therefore, soil urease activity enhanced by different BS recirculation period in general.

3.2. Soil invertase activity

As a kind of hydrolytic enzyme, the invertase hydrolyzes sucrose to glucose and fructose. Related studies revealed that the invertase activity had positive correlation with SOM, clay content, maturity and microorganism of soil. Thus, it was commonly used as indicator of soil maturity and fertility [3].

As shown in figure 3, soil invertase activity varied greatly by different BS recirculation load. Soil invertase activity reached highest value in Oct. by T1, in Sep. by T2 and T3 and in Dec. by T4. Furthermore, the activation time of invertase activity was delayed with reducing recirculation load. The value of invertase activity was increased by 64.7% in Nov. by T1. Its value was 4.39 times by T2 than Sep., 2.58 times by T3 and 5.17 times by T4 than Nov., respectively. Briefly, the invertase activity was activated earlier by high BS recirculation load. The value of activated invertase activity was higher by low BS recirculation load in spite of being activated later. Soil invertase activity also changed significantly by different BS recirculation period over time by the fact in figure 4. The invertase activity reached highest value by T10d while getting highest value in Sep. Its value was 2.49 times by T2d and 2.53 times by T6d than Nov., respectively. Compared with Aug., it was increased by 40.9% by T4d, 87.1% by T8d and 132.9% by T10d. In general, the value of invertase activity was all high by different BS recirculation period in Sep. and Oct.
As depicted in figure 3 and 4, the change of soil invertase activity was basically same by different BS recirculation load and period. Moreover, its value was at low level in Aug. and increased in Sep. and Oct.

3.3. Soil catalase activity
Soil catalase activity, related with soil respiration and microbial activity, reflects the intensity of soil microbes activity to some degree. As a kind of oxidoreductase, the catalase catalyzes hydrogen peroxide to \( \text{O}_2 \) and \( \text{H}_2\text{O} \) and oxidizes hydroxide to various kinds of organic compounds, so it would avoid the accumulation of hydrogen peroxide from doing harm to organisms [17]. Soil catalase activity is dependent on organic compounds activity and respiration intensity of soil. SOM content changed for inputting organic compounds by BS recirculation, thus catalase can be used as an indicator of soil microorganism activity intensity.

![Figure 5: Soil catalase changes by different BS recirculation load over time.](image1)

![Figure 6: Soil catalase changes by different BS recirculation period over time.](image2)

According to figure 5, the catalase activity all reached highest value in Oct. Its value was increased by 16.5%, 13.1%, 13.5% and 14.1% in Oct. by four BS recirculation loads, respectively. Still, its activity stayed relatively stable around Oct. Variation of catalase activity of which value was about 5.6 mL KMnO\(_4\)·g\(^{-1}\) was little during trial period. The catalase activity also got peak value in Oct. by different BS recirculation period as plotted in figure 6. The mean value of catalase activity was 4.92 mL KMnO\(_4\)·g\(^{-1}\) from Aug. to Dec. Furthermore, its value was increased by 18.2%, 11.2%, 13.6%, 14.4% and 17.2% by five BS recirculation periods in Sep., respectively.

It is noteworthy from figure 5 and 6 that different BS recirculation load and period had little effect on soil catalase activity, of which value was all high from Aug. to Dec. The catalase activity all reached highest value in Oct. (i.e., after three months BS recirculation). This may be due to the addition of SOM by BS recirculation.

3.4. Soil phosphatase activity
Phosphatase hydrolyzes phosphate to phosphoric acid and other compounds. Phosphatase activity is an indicator of soil fertility, especially phosphorus state [18].

Figure 7 illustrated that the phosphatase activity all reached peak value by different BS recirculation load in Oct. Besides, its value was highest by T\(_{4d}\). Having biggest promotion in Sep., the value of phosphatase activity was 3.78 times by T\(_1\), 4.21 times by T\(_2\) and 3.56 times by T\(_4\) than Aug., respectively. During trial period, its value was at low level in Jul., Aug. and Dec., at high level in Sep., Oct. and Nov. and got highest value in Oct. In addition, the value of phosphatase activity maintained at high level by T\(_4\) in later stage. Variation of phosphatase activity by different recirculation period is
shown in figure 8. Compared with Oct., the value of phosphatase activity was increased by 17.5% by T_{4d} and 19.96% by T_{6d}. Moreover, its value was increased by 23.6% by T_{8d} in Oct. and 20% by T_{8d} in Sep. and 28.1% by T_{10d} in Oct. The phosphatase activity was activated earlier by long BS recirculation period. Its value was all high and had no significant change by same BS recirculation load and different BS recirculation period. And it was at correspondingly high level by T_{4d} and T_{6d}.

4. Conclusions
Biogas slurry recirculation on purple soil at large-scale farms could promote soil urease, invertase, catalase and phosphatase activity by different recirculation load and period in general. Soil urease reached highest value of 24.1 μg NH_{4\+}-N·g^{-1}·3h^{-1}, 61.7 μg NH_{4\+}-N·g^{-1}·3h^{-1} by T_{4} (7 m^{3}·hm^{-2}) and T_{10d} (10 d). Soil invertase activity got peak value of 0.326 mL Na_{2}S_{2}O_{3}·g^{-1}, 0.362 mL Na_{2}S_{2}O_{3}·g^{-1} by T_{4} (7 m^{3}·hm^{-2}) and T_{8d} (8 d), respectively. In addition, different BS recirculation load and period has little effect on soil catalase activity because its value was all high. Soil catalase activity got peak value of 6.25 mL KMnO_{4}·g^{-1} and 5.71 mL KMnO_{4}·g^{-1} by T_{1} (40 m^{3}·hm^{-2}) and T_{10d} (10 d), respectively. Soil phosphatase activity reached highest value of 19.5 μg phenol·g^{-1}·3h^{-1} and 60.1 μg phenol·g^{-1}·3h^{-1} by T_{4} (7 m^{3}·hm^{-2}) and T_{4d} (4 d), respectively.

Biogas slurry recirculation can save water resources and make full utilization of various kinds of organic compounds. It can also prevent manure and urine produced by livestock and poultry from polluting environment. Biogas slurry recirculation plays a considerable role in improving soil fertility and physical and chemical properties as high-quality organic fertilizer. Therefore, it is indispensable to change BS recirculation load and period according to different soil properties and crops, avoiding soil pollutions by irrigating excessive biogas slurry.

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