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Soil properties and enzyme activities as affected by biogas slurry irrigation in the Three Gorges Reservoir areas of China

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Abstract

Biogas slurry, as a quality organic fertilizer, is widely used on large scale livestock farmland in Southwest China. In the present study, slurry collected from anaerobic tank of dairy farm was used to irrigate farmland having typical purple soil in Chongquing, China. The study revealed that irrigation with biogas slurry increased soil ammonium nitrogen and soil nitrate by 47.8 and 19 % respectively as compared to control check. The average soil available phosphorus and soil phosphorus absorption co-efficient changed slightly. Relative enzyme activities of N and P transformation were indicated by catalase, urease, invertase and phosphatase activity. Irrigation period and irrigation quantity were selected as variable factor. Catalase, invertase and urease activity was highest when irrigation period and irrigation quantitiy was 4 days and 500 ml; whereas highest phosphatase activity increased significantly in purple irrigated by biogas slurry. The result of the present study is helpful in finding optimum irrigation conditions required for enzyme activity within defined range. It further reveals that biogas slurry enriches soil with various nutrients by enhancing N, P content and enzyme activities as well as it also deals with large number of biogas slurry for protecting the environment.

Key words

Biogas Slurry Irrigation, Soil Properties, Enzyme Activities

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Introduction

Anaerobic digestion of livestock waste is a process widely used for waste stabilization, pollution control and improvement of manure quality. The amount of livestock and poultry manure reached 4.5 billion tons and was about 85.7 million tons in the southwest of China. Majority of researches indicate that people's interest in biogas production has increased, since biogas is an environmentally friendly energy. The previous researches have revealed that biogas slurry is also one kind of quality organic fertilizer which contain necessary organic matter and various ions to help improve the quality of soil. According to a survey conducted, the contribution rate of biogas slurry manure for farmland to the nitrogen is 10% to 30% and phosphorus is 3% (Weiland, 2006; Department of Agricultural Technology, 2009). However, effective disposal and comprehensive utilization of livestock wastes, and protection of ecological environment from increasing amount of slurry from anaerobic digesters, is an urgent issues. Further, it may also result in several environmental issues such as soil erosion, nutrient loss and non-point source pollution. Though numerous full-scale biogas irrigation works have been carried out, it is still difficult to define the danger of overloading the system input with high-energetic substrates.

As renewable energy sources, anaerobic co-digestion is becoming more important and provides a way of utiliziting of organic wastes, food wastes, animal manure and organic fraction from municipal solid waste. Over the last 10 years, researchers have focused on finding soil properties that best reflects the change in soil quality. Nitrogen and phosphorus are not only important elements of soil but also the most significant ecological factors in biogeochemical cycle.

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Soil is a vital natural resource, but absence of comprehensive standards of quality and several other factors make it inherently variable. Soil enzyme investigation is helpful in better understanding of relationship between microorganisms and nutrients in soil. Research showed that conservational agricultural management significantly increased total N and available P stock in the surface layer of soil, the contents of soil total nitrogen and soil available phosphorus in the soil ammonium nitrogend fractions, and promoted all the enzyme activities in soil (Norris, 2008 and Environmental Protection Administration of China, 2002). Gil-Sotres *et al.* (2005), Bandick and Dick (1999) reported that biochemical properties related to bio-cycle of elements (C, N, P and S) can be used to diagnose soil quality. Since enzymatic activity is highly sensitive to external agents, and easy to determine, numerous enzymes have been widely used in recent years to study the effect of various soil, used in different processes, such as cycling of C, N and P. Soil enzymes have been suggested as potential indicator of soil quality because of their relationship to soil biology, ease of measurement, and rapid response to changes in soil management. Enzymes catalyze all biochemical reactions and are an integral part of nutrient cycling in soil (Dick, 1994). Wallenius (2011) showed that within-plot variation of soil microbiological characteristics could be best explained by variation in soil organic matter. In the present study, soil enzyme activities were used as indicator of soil potential for biodegradation of organic molecules. Microorganisms play a key role in nutrient cycling and ecosystem, but Macdonald (2009) suggested the need to learn more about the specific effect of different land-use practices on soil microbial communities.

However, according to Trasar-Cepeda *et al.* (2008) and Gao *et al.* (2010) research, the effect on enzyme activity varies depending on the type of land use or management and the type of enzyme. It is evident that changes in soil quality caused by different types of land use must first be quantified in order to establish the most sustainable types of use and management, which also render least disturbance to soil (Acosta, 2008). Enzyme activities can be used to describe changes in soil quality due to land use or management, and to understand soil ecosystem. The research indicated that the activities of these enzymes respond mostly to soil management (Gil-Sotres *et al.,* 2005). In the present study, input of biogas slurry during irrigation was as an activity which adds to soil organic matter. Few attempts were made on the efficiency of enzymes for the nitrogen cycle in soil irrigated by high concentration of biogas slurry over a long period of time.

Activities of enzymes such as invertase, cellulase and urease were generally higher in continuous grass fields than in cultivated fields (Bandick and Dick, 1999). Urease, phosphatase, invertase and catalase were selected for their critical role in production of low molecular weight sugars which serve as an important energy source for microorganisms. The microbiological properties change depending on the practice and biotic conditions indicated by different enzyme activities. In the present study, soil enzymes were used as indicators of soil nutrients changed by biogas slurry irrigation. Assuming that biogas slurry will increase the organic matter and then promote nutrient cycling over long-term slurry irrigation, thereby stimulating soil microbial community. It was expected that the slurry can best increase enzyme activities within defined range of carrying capacity (Huang, 2010). Although enzymes undoubtedly perform functions critical to cycling of nutrients in soil, the optimal irrigation condition is not clear. They may be most useful for predicting variation trends in soil properties over time.

Furthermore, reported on the effect of biogas slurry irrigation on special purple soils is meagre. The mechanism of nutrient (N, P) change due to biogas slurry input, as indicated by enzymes, is still unknown. In view of the above, the present study was carried out to provide more data on this effect due to biogas slurry irrigation behavior. The overall objective of our present research was to obtain change in properties of purple soil by biogas irrigation, and to find optimal irrigation conditions for enzymes within defined range.

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Materials and Methods

Experiment site : The present study was conducted in a dairy farm in the southwest of China. The experiment station is located in Jiangbei district, Chongqing (29°61'N, 106°75'E) which experiences a tropical monsoon climate. The annual mean temperature, precipitation and frost-free period are 18.1 ºC, 1150 mm and 296 days respectively. The main soil type is Regosol, which is derived from purple mudstone and soil ammonium nitrogendstone of Jurassic age. The trial began in June 2010 and ended in August 2011.

Experiment design : The experimental design was as follows: Wastewater of biogas slurry from the farm sewage station and clean water (control check) were used for irrigation. Clean water was normal irrigation water from city water supply system. The experimental purple soil was classified as a Pup-Orthic Entisol in the Chinese Soil Taxonomy and as an Entisol in U.S. Soil Taxonomy (Gong *et al.,* 2007). The thickness of purple soil was less than 50 cm. A completely random block design was used, consisting of 3 block experiments. Visually uniform plot areas of 1.0m×1.0m of soil were selected, avoiding any distinct environmental gradients. Firstly, after continuous irrigation by biogas slurryand control check the changing properties of soil for each nutrient were examined. The quantities of irrigation were 500ml each day during first part of research. The second part of trial constituted of comparing the properties of soil between biogas slurry and control check irrigation to investigate whether biogas slurry irrigation was valuable to enrich soil nutrient or not. In third block, appropriate irrigation period and quantity as two factors to investigate enzyme activities and obtain optimal conditions. The experiment was designed in a randomized complete block with three replications.

Test material : Biogas slurry was quantified in terms of wastewater ammonium nitrogen, wastewater phosphate, wastewater nitrogen, wastewater oxidize carbon and wastewater pH. It was found to be 200.9 mg l^{-1}, 6.09 mg l^{-1}, 5.31 mg l^{-1} and 7.2, respectively. The soil was neutral purple layer, with calcium carbonate content less than 30 g kg^{-1} and pH 7.5. The physico-chemical properties of purple soil such as soil porosity, volume weight, soil organic carbon (SOC), potassium and bulk density were 47%, 1.2 g cm^{-3}, 130 mg kg^{-1}, 90 mg kg^{-1} and 1.33 g cm^{-3}, respectively. The fertility level such as nitrogen and phosphorus was insufficient. Raw soil organic matter, soil total nitrogen, soil available phosphorus and pH was 1.7%, 650 mg kg^{-1}, 120 mg kg^{-1} and 7.5, respectively. Change in accumulation of nitrogen and phosphorus in soil had different effect on chemical properties, microbial activity and enzyme activities. The chemicals and reagents used in the preset investigation were of analytical grade with a certified purity of 99.99%. Standard reference material was used to check the accuracy of results and precision of the instrument. Normally the corresponding results matched within ±1.5%.

Estimation of soil properties : Fresh sample characterizes the natural state of soil in field, so estimation of soil ammonium nitrogen and soil nitrogen were sampled from fresh soil. The upper part of soil was collected, and transported in isothermal bags to the laboratory and stored at 4ºC for further analysis. Following this, they were air dried at room temperature for analyzing the general properties. After crushing, the dried samples were filtered through 2 mm, 1 mm or 0.25 mm sieve for determination of effective nutrients, pH, enzyme activity and physical properties of soil. A variety of physical, chemical and biochemical properties were analyzed which are as follows: Soil ammonium nitrogen was tested by dichromate digestion. Soil total nitrogen by semi-micro Kjeldahl method. Soil available phosphorus was estimated by Olsen method (1982). Soil ammonium nitrogen by Nessler reagent colorimetry and soil nitrogen was detected by Ultraviolet Spectrophotometry. Soil phosphorus absorption coefficient was measured by diammonium hydrogen phosphate colorimetry (Lu, 2000; Lin, 2010; Qi, 2009). Nortcliff (2002) pointed out that evaluation of soil quality has little or no value if the indicators are not selected rigorously. Considering the aim of the present research, N, P nutrients were measured and enzyme activity of urease, catalase, invertase and phosphatase were determined. The test methods followed were indophenol blue colorimetry, 3,5-dinitrosalicylic acid colorimetry, titering process, alkaline phosphatase respectively. Wastewater ammonium nitrogen, wastewater phosphate and wastewater nitrogen were analyzed by the methods of Nessler reagent, Ultraviolet and Mo-Sb Anti spectrophotometry (using the HACH DR4000 spectrophotometer equipment) respectively. Software Design-expert was used to obtain optimum conditions. It had two-level factorial screening designs: Identifying vital factors that affects process or products that make significant improvements. It could determine the best combination of categorical factors and find optimal process settings to achieve peak performance.

Statistical analysis : Multiple-response permutation procedure analysis was used to test the enzymes by biogas slurry irrigation was statistically different $P<0.05$. In the present study, statistical analyses were carried out using descriptive statistics, data transformation and correlation analysis. The main statistical software used was SPSS (v.14). The data of soil properties were analyzed using ANOVA. Data (mean ± SE) were compared by LSD test at $P< 0.05$.

Results and Discussion

Accumulation of N and P in soil varied affecting the chemical properties, microbial activities and enzyme activities. However, Piotrowska and Wilczewski (2012) reported that enzyme activities are more sensitive to the presence of nitrogen fertilization rates than chemical properties. Fig.1(a) present the difference of soil ammonium nitrogen content between biogas slurry and control check group irrigation. Control check test simply relied on the soil organic matter mineralization supply, with no additional nitrogen input. As a result, soil content ammonium nitrogen was low in control check test. From day 3 to day 10, soil organic matter consumption the supply became weaker. Therefore, soil ammonium nitrogen content also reduced continuously. The average content of soil ammonium nitrogen by biogas slurry irrigation was 47.8% higher than control check irrigation. Soil ammonium nitrogen content and total applied biogas slurry were significantly positively related. Due to microbial enzyme and excessive input of organic matter and organic nitrogen directly affected enzyme actually. Biogas slurry irrigation improved the soil quality which can be proved by average soil organic matter and available nitrogen increasing to 0.49 g·kg $^{-1}$, 14.7 mg·kg $^{-1}$ respectively. Soil nitrate was prone to nitrification and denitrification. soil nitrogen content was in dynamic equilibrium as which the conversion was active and the negative charge of NO_3^- was easily removed by leaching and movement. Claudio's (2015) tried to mitigate soil nitrate leaching by using animal manure. The type of soil determined the basic content of soil nitrogen . Meanwhile, fertilizer and nitrogen supplied by output were primary factors affecting soil nitrogen content. Fig.1(b) shows that average content of soil nitrogen by biogas slurry irrigation was 19% higher than the control check group. Soil nitrogen and total biogas slurry applied were positively related. The content of soil nitrogen increased slowly and unsteadily. Osvaldo's *et al.* (2014) stated that nitrate moved through the vadose zone. Also Bécel *et al*. (2015) studied soil nitrate leaching in Europe. It presented the fluctuant and slightly increasing tendency. The changes of soil nitrogen directly reflected the input content of NO_3-N by irrigation. The reason was due to unstable properties of NO_3-N and liquidity. The average content of soil nitrogen by control check group showed early declining and then raising in later period. That was also due to soil

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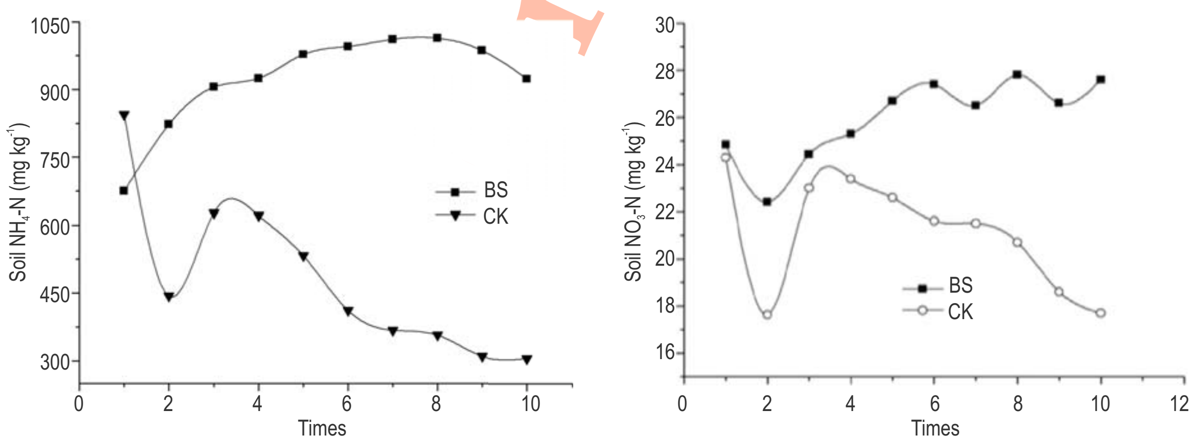
nitrogen leaching accelerated by inputting clean water. Further, soil ammonium nitrogen was transformed into soil nitrogen by nitrification. In the absence of increasing irrigation, the soil nitrogen content decreased.

In the initial stage of biogas slurry irrigation, soil available phosphorus increased rapidly and reached peak value as shown in Fig. 2(a). Following control check group peak, soil available phosphorus reduced back to relatively constant values. Addition of water to control check test weakened the soil phosphorus fixation effect. As a result the curve raised. When it came to peak value of phosphorus release, soil available phosphorus content was relatively stable. As long as pH and organic matter were stable in soil, soil available phosphorus remained unchanged. Soil available phosphorus in the present study was only 5% of active effective phosphorus. Due to biogas slurry irrigation, the content of soil available phosphorus was below control check test during initial period. Biogas slurry irrigation provided organic matter more than control check irrigation. In the beginning, control check irrigation obviously presented higher content of soil available phosphorus in the environment due to soil phosphorus desorption. It was similar to Yang *et al.* (2014) research that organic carbon affected the soil phosphorus desorption. At the same time, soil pH value increased to make iron and aluminum elements forming hydroxide precipitation. It reduced the fixation effect of soil available phosphorus. During later stage, content soil available phosphorus due to biogas slurry irrigation was higher than control check group. The import of phosphorus by biogas slurry was added into the soil. The soil phosphatase also caused decomposition of phosphate. Although phosphorus fixation effect was weakened due to increasing soil organic matter, however phosphorus fixation effect still existed. Characterized by soil available phosphorus content both biogas slurry and control check group increased.

As shown in Fig.2(b), SPAC was above 500 on 3rd day, exhibiting high phosphorus absorption capacity. It slightly decreased after 4 days. After continuous biogas slurry irrigation, pH value reduced. It deteriorated the ability of phosphorus fixation, as reflected in downward trend of the curve, due to existing competition of specific adsorption points between organic acid ions and phosphate solid. So absorption of soil phosphorus was reduced. The tendency of soil phosphorus absorption co-efficient content by biogas slurry and control check irrigation were similar to soil available phosphorus curve. At initial soil phosphorus absorption coefficient (SPAC) curve performance was higher and then stable. It was due to the ability of phosphorus deposit reached saturation. However, biogas slurry irrigation constantly supplement phosphorus could not affect soil to improve their capacity of phosphorus absorption. Whatever biogas slurry irrigation or control check group irrigation, the adsorption and desorption of phosphorus were generate dynamic in balance. Also present the value of soil phosphorus absorption coefficient was characterized stable.

Fig. 3 showed comparison of catalase, urease, phosphatase, invertase activities under biogas slurry and control check irrigation. The catalase activity of biogas slurry group was 21.4% higher than control check test and the average highest value reached 22.6%. The mean activity of invertase by biogas slurry irrigation was 27% higher than control check group. The difference in values of invertase were slightly higher than catalase. Urease was 13% higher than control check group during biogas slurry irrigation period by average value . Relative to catalase and invertase, the difference values were bit small. With changing times, urease during second month appeared more active. Phosphatase activity under biogas slurry irrigation was 19.4% higher than control check test. The effect of biogas slurry irrigation was better than urease, but lower than catalase and invertase. biogas slurry improved the soil enzyme activity because it promoted microbial breeding and soil enzyme activity. Biogas



(a) Soil ammonium nitrogen (b) Soil nitrate nitrogen

Fig.1 : Soil ammonium nitrogen and soil nitrate content under two different irrigation conditions

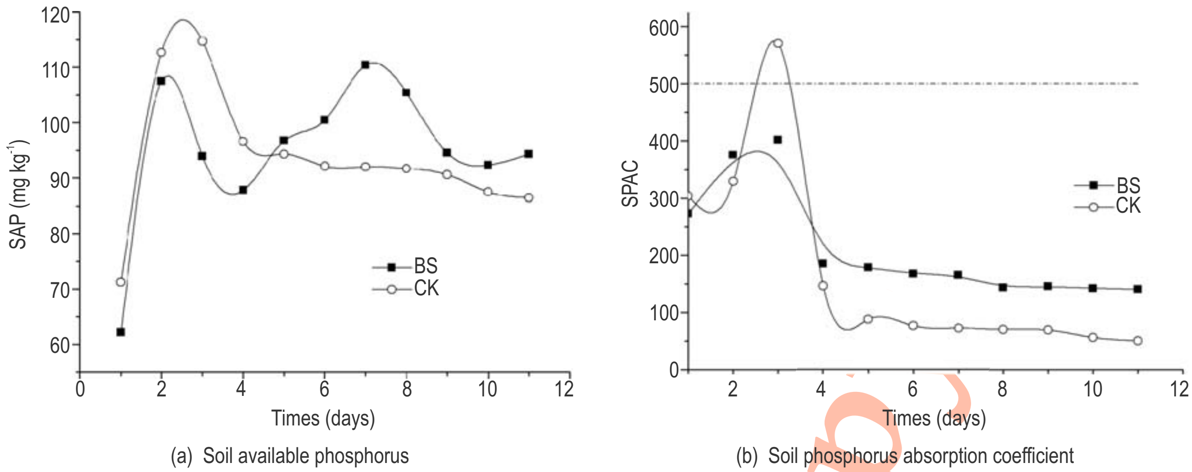


Fig. 2 : Soil available phosphorus and soil phosphorus absorption co-efficient under two different irrigation conditions

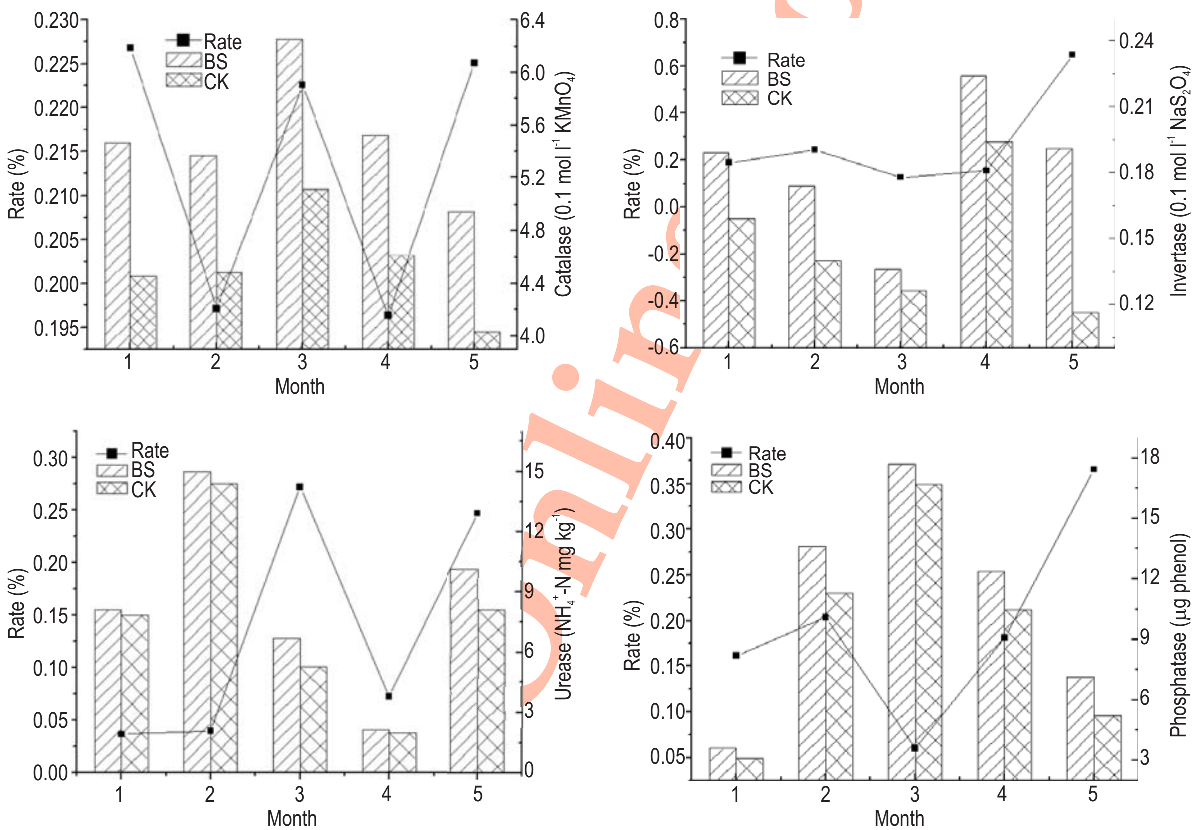
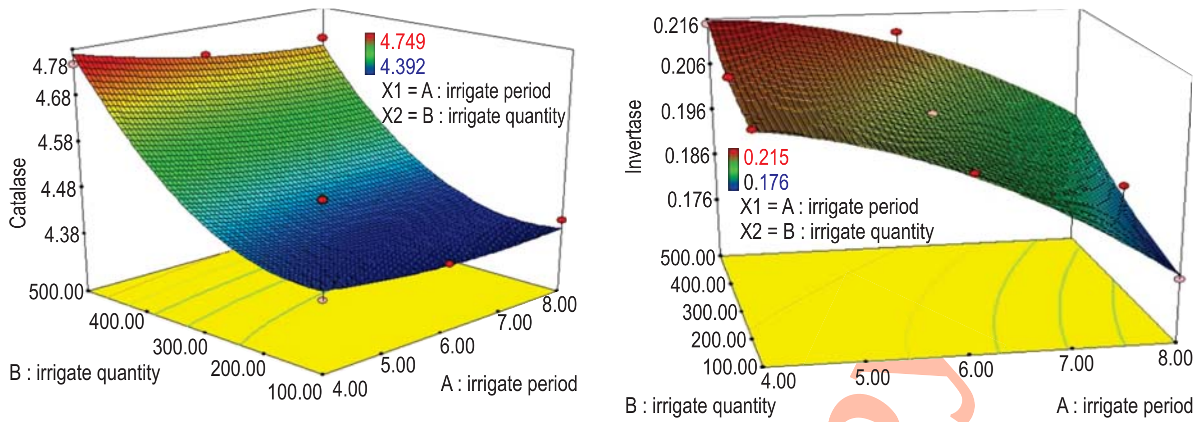


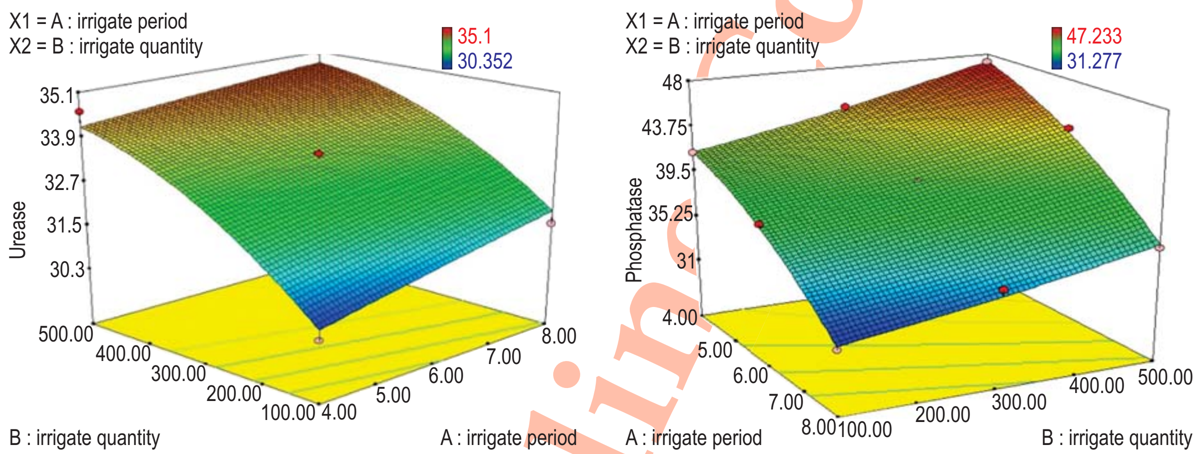
Fig. 3 : Comparison of soil enzymes between BS and CK irrigation

slurry itself contained certain number of soil organic matter, enzymes and culture medium. Liang *et al.* (2014) pointed out that manure increased enzyme activities in all particle-size fractions. Soil enzymes were mainly adsorbed on soil organic matter and mineral colloform. They existed in complex compounds. The capacity of enzyme adsorbed by soil organic matter was greater than minerals. Soil micro aggregate enzyme activities were higher than large aggregate activities (Lagomarsino *et al.,* 2012). Enzyme combined with soil organic matter affected the dynamic properties of enzyme. However, it



(a) Catalase activity (b) Invertase activity

Fig. 4 : Catalase and Invertase activities under different conditions by using the Design-expert model



(a) Urease activity (b) Phosphatase activity

Fig. 5 : Urease and phosphatase activities under different conditions by using Design-expert model

also enhanced soil stability.

Predicting the effects of biogas slurry irrigation on soil microbiological characteristics was a challenging task. As microbial communities were affected by multiple interrelated factors that operate at different spatial and temporal scales. Soil catalase activity was correlated with soil respiration intensity and microbial activities. Liu *et al.* (2014) reported that biological soil significantly increased soil enzyme activities. Soil catalase certainly reflected the strength of soil microbial processes. A response surface methodology (RSM) based on factorial design was performed to investigate and optimize the effects of biogas slurry irrigation methods (Francisco *et al.,* 2014). Fig.4(a), shows the result of catalase activity with different IP and IQ. When IP and IQ were 4 days and 500ml, catalase activity reached highest value of 4.76 (0.1mol.l^{-1} $KMnO_4$). According to software analysis, catalase activity was expressed in terms of IP and IQ conditions (Eq. 1) :

$$Catalase=4.61-0.0551\times A-0.0005167\times B-0.0000313\times A\times B+0.00424\times A^2+0.00000252\times B^2 \quad ..... (1)$$

A: IP (days); B: IQ (ml).

Therefore, low period and high quantity were key aspects for promoting catalase activity in the selected IP and IQ range (4 to 8 days and 100 to 500ml). The change in soil organic matter were due to organic input by biogas slurry irrigation. Catalase stimulated hydrogen peroxide to oxidize various kinds of compound. Thus catalase was one of the indicators of soil microorganism process intensity. Catalase belongs to oxidoreductase category and can accelerate hydrogen peroxide to O_2 and H_2O. Soil catalase activity depends on the activities of

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Table 1 : The results of soil enzymes under different conditions and precisions

Enzyme	Period (day)	Quantity (ml)	Activity (highest)	Activity (mean)	R^2 value	SD	Adjusted R^2
Catalase	4	500	4.76	4.51	0.9805	0.024	0.9666
Invertase	4	500	0.22	0.2	0.9840	0.0184	0.9725
Urease	4	500	34.7	33.11	0.9639	0.36	0.9382
Phophatase	4	250	47.2	39.73	0.9960	0.36	0.9932

soil organic matter and soil respiration. Invertase activity was positively correlated to soil organic matter, clay content and microorganism as well. When anthropogenic mellowing of soil increased, invertase activity was promoted. Thus, it was an indicator of soil fertility. Similarly, when IP and IQ values were 4 days and 500ml, the peak value of invertase activity was 0.22 (0.1 mol l^{-1} NaS_2O_4) as shown in Fig. 4(b). The possible reason was that typical purple soil itself had no anthropogenic cured or lacked of nutrients. As expected, higher IQ and lower IP motivated invertase activity. Using software analysis, invertase activity was expressed below in terms of IP and IQ conditions (Eq. 2) :

$$\text{Invertase} = 0.198+0.008362\times A-0.00003069\times B+0.000005\times A\times B-0.001405\times A^2+0.0000000469\times B^2 \quad \text{.......... (2)}$$

Invertase belongs to hydrolytic enzymes. It accelerates hydrolysis of sucrose glucose and fructose. Gu *et al.* (2009) suggested that urease and invertase activity in paddy soil may be positively influenced by allelopathic rice variety through release of allelochemicals. In the present study, biogas slurry acted as a catalyst which increased soil nutrients.

Urease is one of the amide enzymes which can promote peptide linkage in hydrolysis organic molecule. In addition, urease activities also present a positive correlation with soil organic matter, activities of microorganism and soil total nitrogen content. It catalyses hydrolysis of urease and has also been widely used in evaluation of soil quality. Urease activity increase due to organic fertilization and biogas slurry in soil. Therefore, urease was selected as indicator of nitrogen status (Pascual *et al.,* 1999; García-Gil, 2000; Kandeler and Gerber, 1988). Urease exhibited similar tendency as catalase and invertase with high activities in purple soil irrigated for 4 days and 500ml quantity (Fig. 5a). Urease responded to changes in N content in soil management faster than other soil variables. Therefore, it might be suitable as early indicators of biological changes. As results, trends of urease activities were in accordance with our hypothesis. Furthermore, according to software analysis, urease activity was expressed in terms of IP and IQ conditions (Eq. 3).

$$\text{Urease} = 40.26-5.21\times A+2.65\times B-0.31\times A\times B-1.38\times A^2+0.24\times B^2 \quad (3)$$

Urease is present in most bacteria, fungi and plants. Dick (1994) showed that amidase and urease activities were decreased by increasing the application of ammonia based N fertilizer. He hypothesized that addition of end product by enzymatic reaction suppressed enzyme synthesis. Soil microbial growth after addition of nitrogen source increased significantly with soil nitrate concentration (Katja *et al.,* 2014). According to Fig. 5(b), phosphatase activity reached highest value of 47.2 (µg phenol) when IP and IQ values were 4 days and 100ml respectively as compared with other enzymes, low biogas slurry quantity was used because phosphorus was stable during saturation period. By using software analysis, phophatase activity was expressed below in terms of IP and IQ conditions (Eq. 4).

$$\text{Phosphatase}=38.64+1.77\times A+0.0142\times B-0.000766\times A\times B-0.35\times A^2+0.00000602\times B^2 \quad \text{...... (4)}$$

Design-expert software was used to predict the reaction and formula. Phosphatase promotes hydrolysis of organic phosphorus compounds. Phosphatase activity is an indicator of soil fertility, especially phosphorus states. Comparing three enzymes studied above, phosphatase activity in biogas slurry irrigation soil was highest under low IQ condition, phosphorus was in saturated condition. If without the addition phosphorus source input , the content of phosphorus was basic constant in soil. Aaron *et al.* (2015) pointed out that high-phosphatase microsites contained higher C and N, but not P. The conclusion support our result that phosphorus was stable.

Biogas slurry used for irrigation not only recycled wastewater, but also improved the physico-chemical properties of soil. On the other hand soil microorganisms showed purifying effect on biogas slurry. Soil reduced the amount of biogas slurry as well as the concentration of contaminant. It suggests that biogas slurry enrich nutrient content by increasing N, P content and enzyme activities.

Comparing the test groups control check and biogas slurry of soil ammonium nitrogen, soil nitrate, the average biogas slurry content was higher than control check by 47.8% and 19%. The average content of soil available phosphorus and soil phosphorus absorb coefficient was slightly changed.

Relevant enzyme activity of N and P transformations were indicated by catalase, urease, invertase and phophatase. The results indicate that activities of enzymes, especially urease activity in purple soil irrigated by biogas slurry greatly increased. Biogas slurry increased the nutrient content by inputting the

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amount of both N and P. Using appropriate biogas slurry irrigation, period and quantity can stimulate soil enzyme activity and promote soil nutrient effectiveness.

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