IMPROVEMENT OF YIELD AND QUALITY OF CHINESE CABBAGE (*BRASSICA RAPA PEKINENSIS* L.) BY AUGMENTING SOIL FERTILITY, NUTRIENT STATUS, AND MICROBIAL ACTIVITY WITH BIOGAS SLURRY APPLICATION

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Abstract. In order to provide experimental basis for the optimal application of biogas slurry in field conditions, this study was conducted to investigate the effect of different doses of biogas slurry (return to the field) on soil fertility and crop production (Chinese cabbage) from China. The experiment was conducted in 7 different groups (with 3 replicates each) using nitrogen equivalent chemical fertilizer (CF) and nitrogen equivalent biogas slurry (BGS) as the base fertilizer and four topdressing treatments including (1) NF (2) 100% CF (3) 50% BGS with 50% CF (4) 100% BGS (5) 200% BGS (6) 300% BGS (7) 400% BGS. The results showed that with increasing BGS dosage during all growth phases of Chinese cabbage, soil fertility increases linearly with significantly higher soil organic matter, soil enzymes (sucrose, alkaline phosphatase, and catalase) and nutrients (N, P, K, and ferrous ion) status. These impacts of BGS treated soils have been translated to the significant improvement in yield and quality of cabbage. After comprehensive analysis, it has been concluded that the soil amended with 200% BGS (treatment 5) was suitable to meet the nutrient requirements for optimal plant growth and development that leads to high quality and maximum yield of Chinese cabbage.
Keywords: *biogas slurry return, Chinese cabbage, soil physical and chemical properties, yield, quality*

Introduction

In recent years, the rapid urbanization, population explosion, agricultural transformation and regional development, scaled up and intensified the livestock and poultry farming to meet the challenge of ever-growing food demand and supply gap. The remarkable growth in livestock industry not only led to the improvement of regional economy but consequently, generated large amount of organic waste that contributes to environmental pollution. The efficient utilization of huge amount of organic waste as agricultural manure is considered as a challenging task to timely treat and utilize in eco-friendly manner for agricultural productivity is an integrated approach and became a key factor in determining the sustainable development of livestock and poultry farming. The environment friendly strategies demand the scientific basis and treatment process of livestock manure to meet the requirements of relevant standards before it can be returned to the field. At present, solid manure is mostly composted to

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achieve harmless treatment, while liquid livestock manure treatment and management has become a difficult task due to its large volume, low nutrient content and high biochemical treatment cost. Biogas technology is not only a green technology to produce large amount of methane gas to relieve energy shortage but also an effective way to eliminate liquid manure and turned into biogas slurry as a byproduct with potential to utilize as organic fertilizer for crop growth and productivity (Kadam et al., 2017).

Biogas slurry (BGS) is rich in Total Nitrogen (Total N), Total phosphorous (Total P), Total Potassium (Total K) and other nutrients, Calcium, Iron, Zinc, Copper and other micronutrients, as well as amino acids, vitamins, active enzymes, hormones and other microbial metabolites, which have good potential for improving crop yield, nitrogen utilization, soil mineralization rate and soil structure, crop yield and quality (Angelidaki et al., 2018; Oral, 2018). Therefore, biogas is often used in agricultural production as a liquid fertilizer. At present, most of the domestic and international studies on the use of BGS in agricultural production focus on the proportion of chemical fertilizer (CF) replaced by BGS and the effects of nutrients and organic matter in BGS on the conversion of nutrient elements in soil, soil texture, crop yield and quality under different replacement ratios. The study showed that under the application rate of 8000 kg/ha the yield and quality of Chinese cabbage (*Brassica rapa pekinensis* L.) were significantly higher and batter than that of CF treatment (Debebe et al., 2019). Another study found that the combination of biogas and CF significantly enhanced the nutrients in the soil tillage layer compared to the application of CF or biogas alone (Mdlambuzi et al., 2021). The results of numerous studies emphasize the positive contribution of BGS to agricultural production on agroecosystems, but some studies also show the negative effects of BGS use in agricultural production. It was found that when BGS completely replaced with CF, crop yields tended to decrease, and when the amount of biogas application was increased, there was a risk of groundwater contamination (Yasar et al., 2017; Tsachidou et al., 2019). In addition to the differences in soil texture and farm management practices, the differences in the composition of nutrients in BGS and CF, contributes in this contradiction in various finding. The total nutrients in CF are all inorganic nutrients, while the total nutrients in biogas are partly organic nutrients, which need to be converted to inorganic nutrients through the mineralization process before they can be absorbed by crops, which determine the delay in the supply of effective nutrients in BGS. In actual production, the amount of BGS is mostly converted based on the principle that the nitrogen nutrients of BGS are equal to those of CF, and the effect of BGS is delayed compared with that of CF after application into the soil. For this reason, in order to maximize the crop yield potential, the application of BGS, on farmland needs to be increased. The increased levels might enhance the potential risks of BGS application on the Plant-soil-environment continuum linked with plant growth and productivity. The analysis of the causes of the potential risks and measures to avoid them is also neglected and need to be addressed.

As an edible leafy crop (Akter et al., 2020), Chinese cabbage is rich in calcium, vitamin C, flavonoids, polyphenols, alpha and beta carotenoids, thioglucosides and fiber (Šola et al., 2020), making it one of the main winter vegetable species. Due to the massive use of CF and pesticides in the agricultural production process of Chinese cabbage, it leads to the reduction of its taste and quality, while the BGS, as a potential organic fertilizer, can produce green and high quality vegetables to meet the demand for healthy, safe and high nutritional value vegetables. At present, there are few studies on

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the application of BGS on Chinese cabbage, mostly focused on the effect of a certain application rate on the yield and quality of Chinese cabbage. The information related to systematic studies on different application rates on the growth and development of Chinese cabbage, soil physicochemical properties and potential risk assessment is scanty. Therefore, this study was conducted to investigate the dynamic changes of soil physical and chemical properties and growth and development of Chinese cabbage under different application rates of BGS, to screen and establish the optimal amount of BGS, and to evaluate the potential risks, in order to provide theoretical reference for the application of BGS in agricultural production.

Materials and methods

Experiment materials

The trial started in mid-August 2021 and ended in late December 2021. The experiment was conducted at Yantai Institute of China Agricultural University, Yantai City, Shandong Province, China. The soil type was brown soil and the BGS was obtained from the biogas plant of Shandong Minhe Biological Technology Co., using chicken manure. Urea, superphosphate and potassium sulphate are applied as supplementary chemical fertilizers. The basic properties of soil and BGS are shown in *Table 1*. The Western White 65 variety of Chinese cabbage (*Brassica rapa pekinensis* L.) was used in this experiment. Chinese cabbage is a semi-hardy vegetable that prefers cooler climates and is suitable for sandy loam, loam or light clay loam soils with pH 6.5 to 8. The growing period of Chinese cabbage can be divided into seedling, rosette, heading and harvest stage, and the general yield of it ranges from 37500 to 75000 kg/ha. Chinese cabbage is susceptible to downy mildew, soft rot and other diseases at all stages of fertility and should be treated with methomyl and chlorothalonil before sowing or sprayed with agrobacterium streptomycin in the early stages of the disease.

Table 1. *Basic properties of testable soil and biogas slurry*

Test-taking materials	Organic matter (%)	Alkaline hydrolysis N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)	Ferrous iron (mg/kg）	Total N (%)	Total P (%)	Total K (%)	Ammonia N (%)
soil	1.03	97.48	129.13	30.73	22.35	/	/	/	/
biogas slurry	/	/	/	/	/	0.48	0.08	0.25	0.44

Experimental design

The experiment was conducted in a completely randomized group design with seven treatments and three replications. According to the long-term agricultural practice in the experimental area, 100% chemical fertilizer treatment can meet the nutrient requirements of Chinese cabbage in different growth stages. Because biogas slurry contains organic nutrients, there is a delayed supply of inorganic nutrients in the mineralization process. Therefore, treatment 3 in 50% biogas slurry + 50% chemical fertilizer treatment could protect the nutrient demand of Chinese cabbage in early stage and avoid the decrease of yield caused by delayed supply of biogas slurry. The other

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treatments are to discuss the reasonable application amount of biogas slurry in place of chemical fertilizer on the premise of guaranteeing the yield not to be reduced.

Treatment (1) no fertilizer application (NF), Treatment (2) 100% nitrogen equivalent fertilizer (CF), Treatment (3) 50% nitrogen equivalent biogas (BGS)+ 50% nitrogen equivalent fertilizer (CF), Treatment (4) 100% nitrogen equivalent biogas (BGS), Treatment (5) 200% nitrogen equivalent biogas (BGS), Treatment (6) 300% nitrogen equivalent biogas (BGS), Treatment (7) 400% nitrogen equivalent biogas (BGS). The experimental plot area was 12 m^2 (6 m × 2 m), and the Chinese cabbage plants were spaced at 0.35 m × 0.45 m, with a total of 76 plants. The target yield was 90,000 kg/ha, and the fertilizer application amounts of N, Phosphorus pentoxide (P_2O_5), and Potassium oxide (K_2O) were 135 kg/ha, 63 kg/ha, and 178 kg/ha, respectively, with 34% and 66% of the total N applied in basal and follow-up applications. The basal application was combined with soil tillage and applied at once. The follow-up nutrients were applied in four times, the first topdressing (rosette prophase, September 15, 2021), the second topdressing (rosette metaphase, September 20, 2021), the third topdressing (heading prophase, October 5, 2021) and the fourth topdressing (heading metaphase, October 17, 2021) of Chinese cabbage, with each nutrient application accounting for 25% of the total follow-up nutrients. To ensure the consistency of experiment conditions among treatments, each application was based on the amount of BGS applied in treatment 7, and the other treatments were treated with water to make up the difference in the amount applied. The basal and follow-up fertilizer and BGS application amounts for each treatment are shown in *Table 2*.

Table 2. *Basal and follow-up application dosage of biogas and CF*

Treatment	Base ertilizer (kg/ha)					Topdressing (kg/ha)				
	Biogas slurry	Water	Urea	Superphosphate	Potassium sulphate	Biogas slurry	Water	Urea	Superphosphate	Potassium sulphate
1	0	52520	0	0	0	0	26260	0	0	0
2	0	52520	73	0	188	0	26260	36	0	94
3	6505	46015	36	0	158	3282	22978	18	0	79
4	13130	39390	0	0	128	6565	19695	0	0	64
5	26260	26260	0	0	68	13130	13130	0	0	34
6	39390	13130	0	0	20	19695	6565	0	0	10
7	52520	0	0	0	0	26260	0	0	0	0

Sample collection and determination

The soil samples were collected on the second day after fertilizer application, respectively on the 2nd day of the Rosette prophase, the 6th day of the Rosette metaphase, the 2nd day of the Heading prophase, and the 14th day of the Heading metaphase and the samples were removed, air-dried, finely ground and stored in brown bottles for soil sample testing. Soil alkaline hydrolysis nitrogen (alkaline hydrolysis N), available phosphorus (available P) and available potassium (available K) were determined by alkali N-proliferation method (Zhejiang Topu Yunnong Technology Co., Ltd. KDN-08C Kjeldahl nitrogen analyzer), spectrophotometry (Shanghai Youke Instrument Co., Ltd. 722N Spectrophotometer) and flame photometry (Shanghai Precision Scientific Instrument Co., Ltd. FP6410 Flame spectrophotometer), respectively (Pereira et al.,

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2017), and organic matter was determined by dichromate oxidation-titration techniques (Fidêncio et al., 2002); The determination of ferrous ion content, sucrase, alkaline phosphatase and catalase activities in the soil was performed by the o-phenanthroline colorimetric method, 3,5-dinitrosalicylic acid colorimetric (Jeyasundar et al., 2021), sodium benzene phosphate colorimetric method and potassium permanganate titration method respectively (Bandara et al., 2017). Yield measurements were performed at the harvest period of Chinese cabbage, and Chinese cabbage leaves were also collected for quality and nutrient content measurements on the 10^{th} day of the Harvest period. Vitamin C (Vc) content, soluble sugar content and nitrate content in leaves were determined by 2,6-dichloroindophenol titration method, phenol method and colorimetric method with 5% salicylic acid, respectively (AOAC., 1984), and Total N, P and K contents were determined by automatic Kjeldahl distillation–titration unit (Königswinter, Germany). Phosphorus was determined spectrophotometrically by the phospho-vanadate colorimetric method (Hewlett Packard 8452A, Ontario, CA) and flame photometry (Carl Zeiss, Jena, Germany) (Milošević et al., 2013) (*Figures 1,2*).

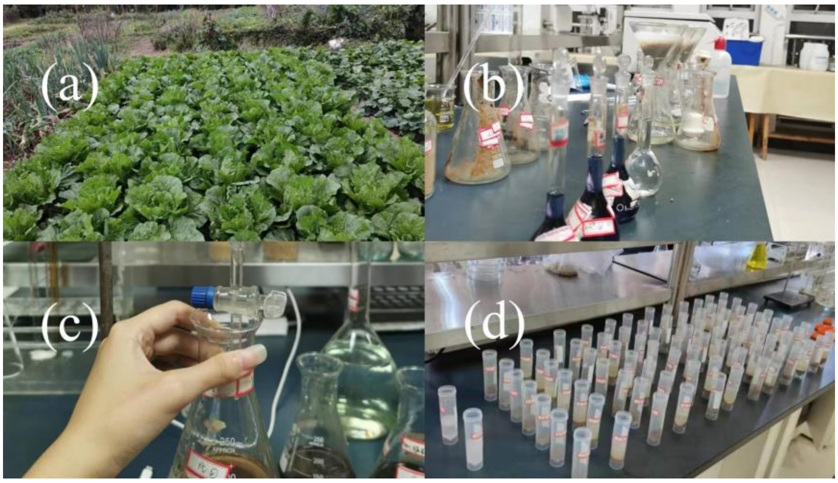

Figure 1. *(a) Chinese cabbage (b) available P (c) ferrous ion content (d) sucrase activities*

Figure 2. *(a) KDN-08C Kjeldahl nitrogen analyzer (b) 722N Spectrophotometer (c) FP6410 Flame spectrophotometer*

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Data processing

Statistical analysis of data was statistically analyzed using Microsoft Excel 2019, and the SPSS least significant difference (LSD) method was used to process the significant difference analysis.

Results and analysis

Effect of different application rates of BGS on soil organic matter content

In the rosette prophase to harvest period of Chinese cabbage, the soil organic matter content of treatment 1 and treatment 2 showed a decreasing trend and no significant difference between them from the rosette prophase to the harvest period of Chinese cabbage, while the soil organic matter content of BGS treatment gradually increased with the increase of BGS application (*Figure 3*). Compared with treatment 2, the soil organic matter content increased by 0.88%, 1.86%, 2.45%, 2.64% and 2.74% in treatments 3, 4, 5, 6 and 7, respectively, in the rosette prophase. The soil organic matter content of treatment 7 was consistently higher than the other treatments and was 1.025, 1.041, 1.045, 1.047, and 1.049 times higher than treatments 2, 3, 4, 5, and 6, respectively, up to the Chinese cabbage harvest period.

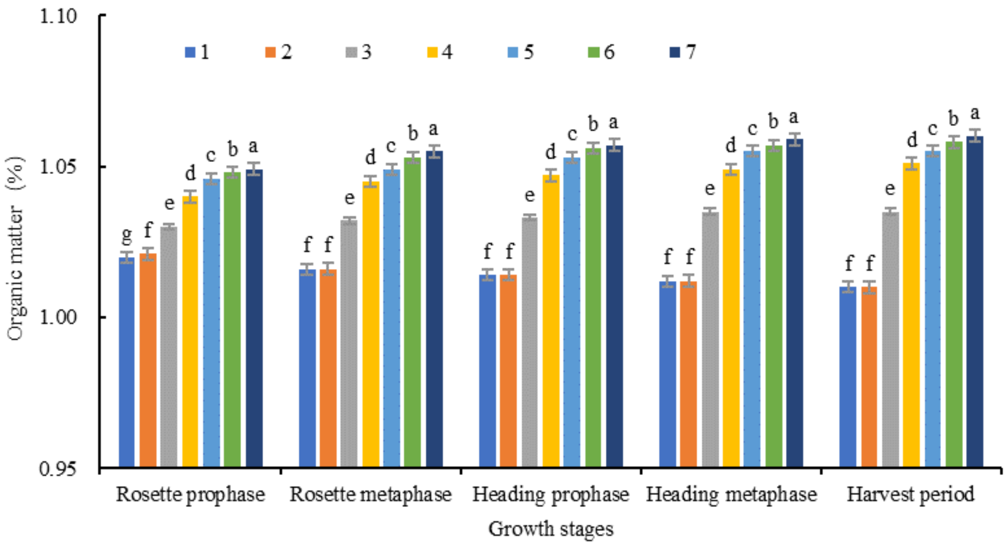

Figure 3. *Effect of different BGS application rates on soil organic matter content. All the values are the mean of triplications. Different letters above columns indicate significant differences between means by LSD at 5% level*

Effect of different application rates of BGS on the content of available nutrients in soil

As can be seen from *Table 3*, alkaline hydrolysis N, available P and available K contents of all BGS treatment soils increased with increasing BGS application. With the topdressing and the organic fertilizer BGS released through mineralization, soil nutrient contents are replenished during the vegetation period. The alkaline hydrolysis N, available P and available K contents of the soils in treatments 5, 6 and 7 were always higher than those in treatment 2 throughout the growth stages of Chinese cabbage.

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However, the soil in treatment 2 showed an increasing trend in the rosette prophase, rosette metaphase and heading prophase, and gradually decreased in the heading metaphase and harvest period. By harvest, the alkaline hydrolysis N, available P and available K contents of soil in treatment 7 were higher than those in treatments 1, 2, 3, 4, 5 and 6 by 215.98%, 110.77%, 63.20%, 50.97%, 20.80%, 5.62% and 595.62%, 141.29%, 48.22%, 15.29%, 7.15%, 1.49% and 549.13%, 206.57%, 165.42%, 154.13%, 44.85%, 11.46%.

Table 3. *Effect of different BGS application rates on soil available nutrient content*

Item	Growth stages	Treatment						
		1	2	3	4	5	6	7
Alkaline hydrolysis N (mg/kg)	Rosette prophase	41.42g	65.50d	59.05e	54.34f	117.04c	156.26b	182.24a
	Rosette metaphase	36.38g	77.50d	67.84e	62.34f	135.21c	180.24b	206.76a
	Heading prophase	34.42g	84.18d	72.88e	67.92f	154.25c	200.46b	223.43a
	Heading metaphase	32.23g	76.25f	87.74e	91.62d	162.46c	211.89b	232.34a
	Harvest period	49.80g	74.66f	96.42e	104.23d	130.26c	148.98b	157.36a
Available P (mg/kg)	Rosette prophase	37.65g	107.24d	102.52e	98.37f	133.97c	152.28b	162.74a
	Rosette metaphase	33.83f	110.50d	105.25e	101.26f	137.47c	155.66b	166.21a
	Heading prophase	27.71g	112.79d	107.33e	104.09f	140.70c	158.01b	168.97a
	Heading metaphase	16.32g	47.77f	60.25e	75.80d	80.64c	85.00b	87.06a
	Harvest period	14.86g	42.84f	69.74e	89.66d	96.47c	101.85b	103.37a
Available K (mg/kg)	Rosette prophase	13.68g	75.64d	51.26e	46.86f	109.96c	114.98b	117.25a
	Rosette metaphase	13.04g	79.00d	53.25e	49.06f	117.44c	120.35b	122.62a
	Heading prophase	41.42g	83.50d	59.05e	54.34f	117.04c	156.26b	182.24a
	Heading metaphase	36.38g	76.84f	77.50e	82.34d	135.21c	180.24b	206.76a
	Harvest period	34.42g	72.88f	84.18e	87.92d	154.25c	200.46b	223.43a

Different letters following the means indicate significant differences between means by LSD at 5% level

Effect of different application rates of BGS on soil ferrous ion content

From *Figure 4*, there was no significant difference in the soil ferrous iron content between treatment 1 and treatment 2 for Chinese cabbage from the rosette prophase to the harvest period, and the soil ferrous ion content in all BGS treatments increased significantly with the increase of BGS application. Compared with the rosette prophase, there was no significant difference in the soil ferrous ion content of treatment 1 and treatment 2 at rosette metaphase, heading prophase, heading metaphase, and harvest period, while with the extension of the growth stages, the soil ferrous ion content of treatment 3, 4, 5, 6, and 7 increased gradually by 16.63%, 15.79%, 15.93%, 21.73%, and 20.52% at harvest period.

Effect of different application rates of BGS on soil enzyme activity

As can be seen from *Table 4*, the sucrase, alkaline phosphatase, and catalase activities of all BGS treatment soils showed an increasing trend at the rosette prophase, rosette metaphase, heading prophase, heading metaphase, and harvest period of Chinese cabbage, and were positively correlated with the amount of BGS applied. Unlike the BGS treatment, the enzyme activity of treatment 1 soil showed a gradual decreasing trend with the extension of the growth stages. The trend of sucrase and alkaline

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phosphatase activities in treatment 2 soil was similar to that of the BGS treatment, but the peroxidase activity showed a decreasing trend with the extension of the growth stages. Compared with treatment 2, the sucrase, alkaline phosphatase and catalase activities of soils in treatments 3, 4, 5, 6 and 7 of Chinese cabbage harvest period increased by 16.89%, 18.01%, 85.33%, 105.17%, 118.14% and 10.06%, 10.69%, 15.41%, 20.44%, 23.27%, and 38.85%, 48.80%, 54.82%, 60.24%, 65.66%, respectively.

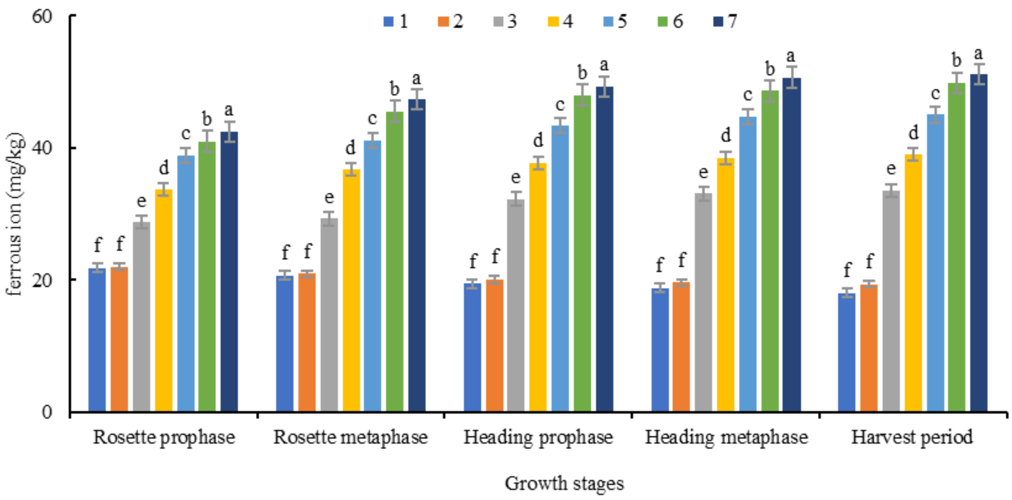

Figure 4. *Effect of different BGS application rates on soil ferrous ion content. All the values are the mean of triplications. Different letters above columns indicate significant differences between means by LSD at 5% level*

Table 4. *Effect of different BGS application rates on soil enzyme activity*

Item	Growth stages	Treatment						
		1	2	3	4	5	6	7
Sucrase /mg/(d×g)	Rosette prophase	3.94Ag	13.02Ef	14.37Ee	14.93Ed	19.28Ec	23.34Eb	25.66Ea
	Rosette metaphase	3.06Bg	13.91Df	15.67De	16.25Dd	22.16Dc	25.98Db	28.75Da
	Heading prophase	2.38Cg	14.62Cf	16.85Ce	17.07Cd	25.52Cc	28.84Cb	31.55Ca
	Heading metaphase	2.17Dg	15.21Bf	17.38Be	17.65Bd	27.48Bc	30.70Bb	32.82Ba
	Harvest period	2.12Eg	15.27Af	17.85Ae	18.02Ad	28.30Ac	31.33Ab	33.31Aa
Alkaline phosphatase /mg/g	Rosette prophase	0.195Ag	0.260Ef	0.286Ee	0.292Ed	0.307Ec	0.313Eb	0.318Ea
	Rosette metaphase	0.179Bg	0.283Df	0.314De	0.317Dd	0.337Dc	0.342Db	0.350Da
	Heading prophase	0.164Cg	0.302Cf	0.330Ce	0.334Cd	0.350Cc	0.369Cb	0.373Ca
	Heading metaphase	0.159Dg	0.311Bf	0.343Be	0.347Bd	0.362Bc	0.376Bb	0.385Ba
	Harvest period	0.158Eg	0.318Af	0.350Ae	0.352Ad	0.367Ac	0.383Ab	0.392Aa
Catalase /mg/(g×min)	Rosette prophase	0.366Af	0.368Af	0.432Ee	0.462Ed	0.483Ec	0.496Eb	0.502Ea
	Rosette metaphase	0.355Bf	0.359Bf	0.443De	0.473Dd	0.494Dc	0.513Db	0.519Da
	Heading prophase	0.343Cf	0.348Cf	0.452Ce	0.479Cd	0.502Cc	0.527Cb	0.532Ca
	Heading metaphase	0.332Df	0.336Df	0.457Be	0.488Bd	0.510Bc	0.531Bb	0.543Ba
	Harvest period	0.327Ef	0.332Ef	0.461Ae	0.494Ad	0.514Ac	0.532Ab	0.550Aa

Capital letters after data in the same column in the figure indicate significant differences between treatments within different fertility periods (P<0.05), and lowercase letters after data in the same column indicate significant differences between treatments (P<0.05)

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Effect of different application rates of BGS on nutrient content, yield and quality of Chinese cabbage leaves

As shown in *Table 5*, the nutrient contents of Total N, Total P and Total K in Chinese cabbage leaves at harvest were significantly higher compared to treatment 2 and increased with the amount of BGS applied. There was no significant difference in leaves nutrient content between treatment 3 and treatment 4 and between treatment 6 and treatment 7. The yield of Chinese cabbage was highest in treatment 5, which was 1.76, 1.10, 1.05, 1.45, 1.16 and 1.14 times higher than treatments 1, 2, 3, 4, 6 and 7, respectively. The Vc, soluble sugar and nitrate contents of Chinese cabbage showed a good positive correlation with the amount of BGS applied, but the nitrate contents of treatment 3 and 4 were significantly lower than those of treatment 2. The Vc, soluble sugar and nitrate contents of treatment 5 were higher by 29.58%, 6.54% and 6.82% compared with treatment 2.

Table 5. *Effect of different amounts of BGS application on yield and quality of Chinese cabbage*

Treatment	**Leaves nutrient**			**Yield (kg/ha)**	**Quality**		
	Total N (%)	Total P (%)	Total K (%)		Vc (mg/kg)	Soluble sugar (%)	Nitrate (μg/kg）
1	1.820g	0.197g	0.943g	129000g	146.05g	1.03e	163.24g
2	2.410f	0.287e	1.080f	205500c	180.25f	1.07d	224.92d
3	2.506e	0.250f	1.152e	214500b	206.68e	1.11c	198.47f
4	2.579d	0.291d	1.195d	156000f	210.61d	1.13bc	216.44e
5	2.650c	0.315c	1.224c	226500a	233.56c	1.14b	240.25c
6	2.683b	0.337b	1.240b	195000e	252.63b	1.16a	252.16b
7	2.689a	0.343a	1.252a	198000d	261.72a	1.16a	259.63a

Different letters above columns indicate significant differences between means by LSD at 5% level

Discussion

The results of this study shows that the utilization of BGS (return to the field) increases the organic matter content in the soil and was positively correlated with the amount of BGS application (*Figure 3*), which is consistent with the results of Tang et al. (2020). This could be due to the availability of degradable organic substances (cellulose, hemicellulose, lignin etc.) in the BGS digestate. These substances are continuously degraded by microbial activity and increase the total organic content and fertility of soil (Iocoli et al., 2019).

The available soil nutrient content of treatments 3 (50% each CF + BGS) and treatment 4 (100% BGS) was lesser during the early phases, but higher in later phases, as compared to treatment 2 (100% CF). This is possibly due to the difference in nature of nutrient reserves existing in both sources (urea and BGS) (Vanotti et al., 1995). Urea rapidly release nutrients, while BGS contain many nutrients in the form of organic and humic substances, which needs to be mineralized in order to release in the soil. The time taken in mineralization process would be the reason of relatively delayed nutrients availability in treatment 3 and 4 soils. Although, the similar nutrient source (BGS) was applied in other treatments (5-7), but due to the large quantity of digestate (200% - 400% BGS), the amount of available nutrients was much higher than treatment 3 and 4.

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In later phases of cabbage growth, the higher contents of soil nutrients in all BGS treatments (3-7), indicating the gradual but continuous addition of organic and humic substances in soil's quick-acting nutrient pool, which also helped in activation and solubilization of insoluble nutrients in the soil. In addition, these substances can also promote the formation of larger agglomerates using smaller particles, which facilitates the effective adsorption and reduces leaching of nutrients (Hupfauf et al., 2016).

Iron is an important redox element, essential for plant growth and productivity. Changes in the ferrous ion content can help to characterize the redox state of soil substances (Bonneville et al., 2016; Watanabe et al., 2021). This study shows unchanged ferrous ion content in soils of treatment 1 and treatment 2. However, a linear increase in ferrous ion content with the application of increasing dosage of BGS (treatment 3-7) indicating a progressive decline in oxygen concentration with the soil depth, which create an anaerobic environment, partly resulting in the increase of ferrous ion content (Sposito et al., 2009).

In soil, enzymes are secreted by plant roots and their residues, animals and their remains, and microbial activity. It is an independent system that is sensitive to changes in the soil environment and generally affected by soil organic matter, fertility level, and redox potential (Gascó et al., 2016; Baddam et al., 2016). Our results have shown that BGS application was positively correlated with soil sucrase, alkaline phosphatase and catalase activities (*Table 4*). Increased organic matter, nutrients and regulated C/N of BGS soils stimulate microbial growth with balanced microbiota thus promoting activity of soil enzymes (Lagomarsino et al., 2015). It has been suggested that soil enzyme activity is directly linked with the effective availability of soil N, P, and K (Igalavithana et al., 2017). The decreased catalase activity of treatment 2 soil with increasing maturity of cabbage (Table 4) was possibly due to decrease in alkaline hydrolysis N, available P and available K contents, especially at the later stages of cabbage maturity (*Table 3*).

The BGS application showed a variable response in terms of the yield of Chinese cabbage (*Table 5*). Treatment 3 (50% each CF + BGS) and 5 (200% BGS) significantly increased the cabbage yield for about 9000 kg/ha (4.4%) and 21000 kg/ha (10.2%) respectively, while other treatments did not show yield promotion, as compared to CF treatment (2). The highest yield in treatment 5 may be due to the availability of 2x more N equivalent BGS that was continuously replenishing the large amount of essential nutrients (N, P, K), as well as by the multi-nutrient supply characteristics by the joint action of trace elements (B, Mn, etc.). In addition, BGS is also rich in bioactive substances such as vitamins and growth hormones required for better plant growth and yield production (Sogn et al., 2018).

All BGS treatments (3-7) significantly increased the quality of Chinese cabbage, by increasing their nutrient contents, while yield promotion was obtained only in treatment 3 and 5 (Koszel et al., 2016). When the amount of BGS application continues to increase, it causes greedy green growth of Chinese cabbage at the harvest stage, which is not conducive for yield production (Cinar et al., 2020; Kumar et al., 2022). BGS is a huge source of N, which can be used for synthesizing soluble sugar, amino acid rich molecules, vitamins and various factors for plant growth, hence increased the Vc and soluble sugar contents in Chinese cabbage. Therefore, N content of BGS treated plants was higher than the CF treated plants (Zhao et al., 2022), but it did not exceed the threshold limit of ≤3000 mg/kg recommended by the national standard (nitrate limit in vegetables GB19338-2003).

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Conclusion

It can be concluded that the soil supplemented with 200% BGS was suitable for optimal plant growth and development that lead to high quality and maximum yield of Chinese cabbage. Using BGS, is a natural, eco-friendly, and feasible approach to obtain maximum outcomes and better economic gains from agricultural soils.

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REFERENCES

[1] Akter, A., Miyazaki, J., Shea, D. J., Nishida, N., Fujimoto, R. (2020): Gene expression analysis in response to vernalization in chinese cabbage (*Brassica rapa* L.). – The Horticulture Journal 89(3): 268-277.

[2] Angelidaki, I., Treu, L., Tsapekos, P., Luo, G., Campanaro, S., Wenzel, H., Kougias, P. G. (2018): Biogas upgrading and utilization: Current status and perspectives. – Biotechnology Advances 36(2): 452-466.

[3] AOAC. (1984): Vitamin C (Ascorbic Acid) in Vitamin Preparations and Juices: 2, 6-Dichloroindophenol Titrimetric Method. – Association of Official Analytical Chemists, Washington, DC, pp. 844-845.

[4] Baddam, R., Reddy, G. B., Raczkowski, C., Cyrus, J. S. (2016): Activity of soil enzymes in constructed wetlands treated with swine wastewater. – Ecological Engineering 91: 24-30.

[5] Bandara, T., Herath, I., Kumarathilaka, P., Seneviratne, M., Seneviratne, G., Rajakaruna, N., Vithanage, M., Ok, Y. S. (2017): Role of woody biochar and fungal-bacterial co-inoculation on enzyme activity and metal immobilization in serpentine soil. – Journal of Soils & Sediments 17: 665-673.

[6] Bogunovic, I., Pereira, P., Brevik, E. C. (2017): Spatial distribution of soil chemical properties in an organic farm in Croatia. – Science of the Total Environment 584-585: 535-545.

[7] Bonneville, S., Bray, A. W., Benning, L. G. (2016): Structural Fe (II) Oxidation in Biotite by an Ectomycorrhizal Fungi Drives Mechanical Forcing. – Environmental Science & Technology 50(11): 5589-5596.

[8] Cinar, S., Ozkurt, M., Cetin, R. (2020): Effects of nitrogen fertilization rates on forage yield and quality of annual ryegrass (*Lolium multiflorum* l.) in central black sea climatic zone in turkey. – Applied Ecology and Environmental Research 18(1): 417-432.

[9] Debebe, Y., Itana, F. (2016): Comparative Study on the Effect of Applying Biogas Slurry and Inorganic Fertilizer on Soil Properties, Growth and Yield of White Chinese cabbage (Brassica oleracea var. capitata f. alba). – Journal of Biology, Agriculture and Healthcare 6: 19-26.

[10] Fidêncio, P. H., Poppi, R. J., de Andrade, J. C., Cantarella, H. (2002): Determination of organic matter in soil using near-infrared spectroscopy and partial least squares regression. – Communications in Soil Science & Plant Analysis 33(9-10): 1607-1615.

[11] Gascó, G., Paz-Ferreiro, J., Cely, P., Plaza, C., Méndez, A. (2016): Influence of pig manure and its biochar on soil CO_2 emissions and soil enzymes. – Ecological Engineering 95: 19-24.

[12] Hupfauf, S., Bachmann, S., Fernández-Delgado Juárez, M., Insam, H., Eichler-Löbermann, B. (2016): Biogas digestates affect crop P uptake and soil microbial community composition. – Science of the Total Environment 542: 1144-1154.

[13] Igalavithana, A., Lee, S., Niazi, N., Lee, Y.-H., Kim, K., Park, J.-H., Moon, D. H., Ok, Y. S. (2017): Assessment of soil health in urban agriculture: Soil Enzymes and Microbial Properties. – Sustainability 9(2): 310.

[14] Iocoli, G. A., Zabaloy, M. C., Pasdevicelli, G., Gómez, M. A. (2019): Use of biogas digestates obtained by anaerobic digestion and co-digestion as fertilizers: Characterization, soil biological activity and growth dynamic of *Lactuca sativa* L. – Science of The Total Environment 647 (PT.1-1664): 11-19.

[15] Jeyasundar, P. G. S. A., Ali, A., Azeem, M., Li, Y., Guo, D., Sikdar, A., Abdelrahman, H., Kwon, E., Antoniadis, V., Mani, V. M., Shaheen, S. M., Rinklebe, J., Zhang, Z. (2021): Green remediation of toxic metals contaminated mining soil using bacterial consortium and Brassica juncea. – Environmental Pollution 277: 116789.

[16] Kadam, R., Panwar, N. L. (2017): Recent advancement in biogas enrichment and its applications. – Renewable and Sustainable Energy Reviews 73: 892-903.

[17] Koszel, K., Kocira, A., Lorencowicz, E. (2016): The evaluation of the use of biogas plant digestate as a fertilizer in aflafla and spring wheat cultivation. – Fresen Environ Bull 25(8): 3258-3264.

[18] Kumar, A. M., Verma, L. M., Sharma, S., Singh, N. (2022): Overview on agricultural potentials of biogas slurry (BGS): applications, challenges, and solutions. – Biomass Conversion and Biorefinery. https://doi.org/10.1007/s13399-021-02215-0.

[19] Lagomarsino, A., Grego, S., Kandeler, E. (2012): Soil organic carbon distribution drives microbial activity and functional diversity in particle and aggregate-size fractions. – Pedobiologia - International Journal of Soil Biology 55(2): 101-110.

[20] Mdlambuzi, T., Muchaonyerwa, P., Tsubo, M., Moshia, M. E. (2021): Nitrogen fertiliser value of biogas slurry and cattle manure for maize (*Zea mays* L.) production. – Heliyon 7(5): e07077.

[21] Milošević, T., Milošević, N., Glišić, I. (2013): Tree growth, yield, fruit quality attributes and leaf nutrient content of 'Roxana' apricot as influenced by natural zeolite, organic and inorganic fertilisers. – Scientia Horticulturae 156: 131-139.

[22] Oral, E. (2018): Effect of nitrogen fertilization levels on grain yield and yield components in triticale based on AMMI and GGE biplot analysis. – Applied Ecology and Environmental Research 16(4): 4865-4878.

[23] Rakshit, S., Uchimiya, M., Sposito, G. (2009): Iron (III) Bioreduction in Soil in the Presence of Added Humic Substances. – Soil Science Society of America Journal 73(1): 65-71.

[24] Sogn, T. A., Dragicevic, I., Linjordet, R., Krogstad, T., Eijsink, V. G. H., Eich-Greatorex, S. (2018): Recycling of biogas digestates in plant production: NPK fertilizer value and risk of leaching. – International Journal of Recycling of Organic Waste in Agriculture 7(1): 49-58.

[25] Šola, I., Vujčić Bok, V., Dujmović, M., Rusak, G. (2019): Developmentally-related changes in phenolic and *L*-ascorbic acid content and antioxidant capacity of Chinese cabbage sprouts. – J Food Sci Technol 57(2): 702-712.

[26] Tang, Y., Luo, L., Carswell, A., Misselbrook, T., Shen, J., Han, J. (2021): Changes in soil organic carbon status and microbial community structure following biogas slurry application in a wheat-rice rotation. – Science of The Total Environment 757: 143789.

[27] Tsachidou, B., Scheuren, M., Gennen, J., Debbaut, V., Toussaint, B., Hissler, C., George, I., Delfosse, P. (2019): Biogas residues in substitution for chemical fertilizers: A comparative study on a grassland in the Walloon Region. – Science of The Total Environment 666: 212-225.

[28] Vanotti, M. B., Leclerc, S. A., Bundy, L. G. (1995): Short-term effects of nitrogen fertilization on soil organic nitrogen availability. – Soil Science Society of America Journal 59(5): 1350-1359.

[29] Watanabe, T., Katayanagi, N., Agbisit, R., Llorca, L., Hosen, Y., Asakawa, S. (2021): Influence of alternate wetting and drying water-saving irrigation practice on the dynamics

of Gallionella-related iron-oxidizing bacterial community in paddy field soil. – Soil Biology and Biochemistry 152: 108064.

[30] Yasar, A., Rasheed, R., Tabinda, A. B., Tahir, A., Sarwar, F. (2017): Life cycle assessment of a medium commercial scale biogas plant and nutritional assessment of effluent slurry. – Renew Sust Energ Rev 67: 364-371.

[31] Zhao, J., Pullens, J., Srensen, P., Blicher-Mathiesen, G., Olesen, J., Brgesen, C. D. (2022): Agronomic and environmental factors influencing the marginal increase in nitrate leaching by adding extra mineral nitrogen fertilizer. – Agriculture, Ecosystems & Environment 327: 107808.

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