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Effect of Three-Dimension Fertilizing Mode Using CO_2 and Liquid/Solid Fractions of the Digesate From Anaerobic Digester on Yield and Quality of Lettuce

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Abstract

Effect of three-dimension fertilizing mode (3-DFM) using CO_2 and liquid/solid fractions of the digesate from anaerobic digester was investigated in quality and yield of lettuce. CO_2, solid fraction of digestate (SFD), and liquid fraction of digestate (LFD) were used as fertilizer for gaseous fertilizer, the base fertilizer, and foliar fertilizer, respectively. The result showed that the 3-DFM was appropriate for lettuce. Lettuce's plant height, width and number of leaves growth amount were 29.25 cm, 22.38 cm, and 16.2 cm, which were 26.8%, 16.5%, and 18.4% higher than the control, respectively. The overall yield of lettuce was 30.78% more than that of the control. The soluble sugar content and the chlorophyll content in lettuce was the highest, which were 29.4% and 11.4% higher than the control. Under the CO_2 and SFD condition, the content of vitamin C in lettuce, the content of free amino acid, crude protein and crude fiber contents of lettuce were the highest. The experimental study can provide an effective way to improve yield and quality of lettuce and utilize biogas plants wastes.

Keywords: CO_2, solid fraction of digestate (SFD), liquid fraction of digestate (LFD), lettuce, three-dimension fertilizing mode (3-DFM)

1. Introduction

CO_2 is a greenhouse gas of tremendous hazards, and is also an indispensible important raw material during photosynthesis of green plants. A large amount of CO_2 will be absorbed in the photosynthesis of plants, for example, 1-6 tons of CO_2 will be consumed for synthesis of each ton of dry substances (Yu, 2008). Usually the content of CO_2 is 0.03% in the air. The content of CO_2 ordinary green plants need was 0.01%-0.1% (Zhu & Shi, 2008). However, due to the relatively closed environment in agricultural production in facilities with greenhouse sheds being the major construction type, the density of CO_2 in the greenhouse in winter, early spring and autumn of low temperature was far lower than that in the environmental atmosphere, and the crops were hungry for CO_2 with their growth and development being seriously restricted. CO_2 was used for over 90% of tomato, cucumber, sweet pepper and strawberry as fertilizer in Holland (Madsen, 1974).

The major sources of CO_2 fertilization at domestic and abroad mainly included liquid CO_2, fuel combustion, CO_2 generator, CO_2 particulate gas fertilizer and chemical reaction method. Liquid CO_2 (Zhang, 2007) was transported through pipelines during fertilization, in which CO_2 was injected through pressurization into the irrigating water flow, and the effect of yield increase was not desirable. Thus it could not be taken as the major way of CO_2 fertilization for vegetables. The CO_2 produced by fuel combustion could cause hazards of NO_x, and need to be purified (Zhang & Gao, 1993). CO_2 generator need natural gas, kerosene and propane as fuels, or specially burned coal ball, coke and charcoal etc., which had led to poor controllability, uneven gas thermal distribution, and misplacement of supply and demand for gas thermal energy, and hazards of polluting gases. Chemical reaction method was to produce CO_2 gases by making use of sulfuric acid and ammonium carbonate, and was inconvenient to operate with poor controllability and a relatively high cost. Gas hazards tend to occur due to improper operation sometimes (Deng, 1999). Thus it is urgent to develop sources of CO_2 fertilizer with stable supply and high effect which is free of environmental pollution.

CO_2 content is 35%-45% in biogas. China is reputed as "hometown of biogas in the world (Peng, 1997). By the end of 2011, there had been 80,558 biogas plants in China, with an annual output of biogas being 1,710 million cubic meters (Chen, 2013). There was about 700 million tons of CO_2 in biogas. And LFD was over 200 million tons (Li, 2011). The residues of such biogas plants could not be effectively utilized, especially those of large and extra-large scale biogas plants, waste of resources and secondary pollution will be inevitable. And such is a significant problem confronting people, which is also inevitable and must be solved.

In the LFD, research contents mainly includes analysis of increasing of yield and quality, physical and chemical properties of the soil, prevention and control of pest damage, and content of pollutants after application of LFD. And a wide range of crops have been studied, including field crops, vegetables, fruit trees, flowers and Pennisetum purpureum Schum etc.. SFD was used as base fertilizer by Yang (2013). The agronomic traits, fruit quality and tastes of tomato could be improved through spraying LFD of a density between 20% to 50%, and reduced the content of nitrate. And the best effect could be achieved through spraying LFD of a density of 50%. The use of LFD was greatly influenced by the growth period of the crops, and crop fertilization was non-successive, while the production of LFD was successive. The amount of fertilizer the crops need was unchanged, and excessive LFD would lead to leakage into underground and pollution of underground water. It had been discovered in research that the release amount of CO_2 in the land was be increased, and the greenhouse effect was also aggravated during agricultural irrigation with excessive LFD (Thomas et al., 2009). At present there was limited information on the fertilizer value of SFD from energy-cropping systems. Herrmann et al. (2013) thought that SFD application resulted in similar maize yielding performance to pig slurry and cattle slurry. Svensson et al. (2004) believed that application of SFD resulted in higher yield and grain quality than compost.

The objective of this study was to investigate effects of biogas plant byproducts (3-DFM) on yield and quality of lettuce. If the 3-DFM was effective, which has not only effectively solved the sources of the CO_2 for the greenhouse sheds, but also has made full use of SFD and LFD, thus saving energy, reducing emission and purifying environment.

2. Materials and Methods

2.1 Materials

The soil used in the experiment was the nutritious soil matrix purchased from Chinese Academy of Agricultural Science, and such soil matrix was obtained as follows: mix the turfy charcoal and marvel evenly according to the proportion of 1:2:1, and put them into the flower pot (Length 50 cm, width 30 cm, depth 35 cm). Then added enough water was added to the flower pot, and mix evenly to obtain sufficiently wet the nutritious soil. The soil water holding capacity was 60%. The lettuce seeds used were those of the No. 2 glass lettuce produced by Chinese Academy of Agricultural Science.

The SFD and LFD used in the experiment came from the outputs of anaerobic digestion tank of Changping experimental base of Beijing University of Chemical Technology with the straw being the raw material. Solid-liquid separation was carried out by the solid-liquid separator. Solid fraction of digestate(SFD) is biogas residue and liquid fraction of digestate (LFD) is biogas slurry. SFD was dried at 105 °C drying box for future use. The soil, LFD and SFD properties are shown in Table 1. The CO_2 gas came from the biogas of anaerobic digestion tank. Biogas was pured by pressurized washing way, CO_2 gas was separated and stored for future use.

Table 1. The characteristic of soil, SFD and LFD

Item	Soil	SFD	LFD
Nitrogen ($mg \cdot kg^{-1}$)	135	700	430
Phosphorus ($mg \cdot kg^{-1}$)	97	352	277
K^+ ($mg \cdot kg^{-1}$)	68	42	54
Mg^{2+} ($mg \cdot kg^{-1}$)	84	55	88
Ca^{2+} ($mg \cdot kg^{-1}$)	31	180	103
Organic matter ($mg \cdot kg^{-1}$)	16800	43000	14500
pH	6.5	6.9	7.4

2.2 Experiment Device

Artificial greenhouse system (Figure 1) was adopted in the experiment, which included two independent subsystems A and B, and various parameter conditions between the systems did not influence each other. The maximum error range of temperature control was ± 2 °C, while that of humidity control was ± 5%. The maximum error range for control of the quantity of CO_2 was ± 50 ppm. The intensity of illumination was regulated through controlling the switches of different numbers of lights.

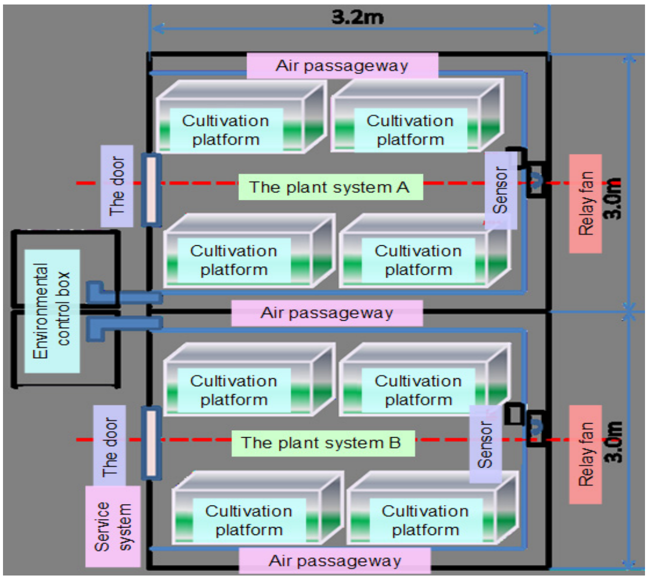

Figure 1. Artificial greenhouse system

2.3 Experiment Method

We present three-dimension fertilizing mode(3-DFM). CO_2, SFD, and LFD were applied on the lettuce in the self-built manmade greenhouses. CO_2 was used as gasous fertilizer, LFD and SFD were used as foliar fertilizer and base fertilizer, respectively, for three-dimension fertilizing(3-DF) of the lettuce (Figure 2).

The germination ratios of lettuce under different temperatures, humidity and amounts of CO_2 was measured in the initial period of the experiment in order to select the optimal planting conditions of lettuce. The external conditions finally selected were 18 °C (temperature), 50% (humidity) and 1000 ppm of CO_2. The two subsystems were set the same the temperatures, humidity and illumination intensity.

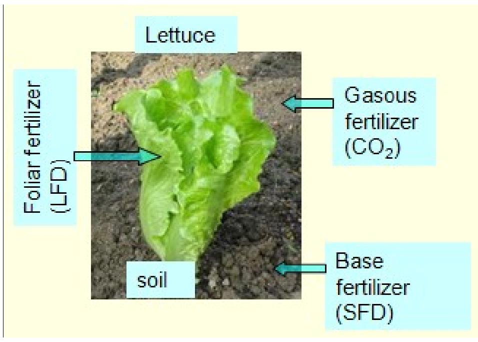

Figure 2. Scheme figure for 3-DFM

A pot planted 10 seedlings of lettuce, totally 10 pots for 5 treatments, with 20 seedlings per treatment; Taken the SFD, LFD and amount of CO_2 as the control factors. LFD was mixed in the water, added 0.05 L of LFD, and the water for each pot was 0.3 L. SFD was mixed in the soil, added 500 grams of SFD per kg of soil. The treatments are shown in Table 2.

Table 2. The different setting conditions of SFD, LFD and CO_2

System	Treatment	SFD (g· kg soil^{-1})	LFD (L·0.3L^{-1})	Amount of CO_2 (ppm)
System A	1 (control)	0	0	300*
System B	2	0	0	1000
	3	500	0	1000
	4	0	0.05	1000
	5	500	0.05	1000

*-The content of CO_2 is 300ppm in the air.

According to the need of experiment, removal of large and small seedlings, the uniform growth of seedlings were used to experiment since the 7th day of planting. Lettuce's the number of leaves, the plant height, and plant width were measured per weekly. The fresh weight and dry matters of the above ground part, and underground part, and the total yields for each treatment was recorded at the maturity period of the lettuce.

2.4 Means of Analysis

The pH meter was used to measure pH value of the soil, SFD, and LFD. The content of organic substances was measured by solution titrimetric method. The total content of nitrogen was determined using a Kjeldahl apparatus. The total content of phosphorus and potassium were analyzed with atomic flame spectrometer. 2,6-Dichlorophenolin titrimetric method was used to measure the content of vitamin C, and anthrone colorimetry was used for measurement of soluble sugar. Protein was calculated according to coomassie brilliant G-250 method, and chlorophyll was determined by spectrophotometry. Moreover, ninhydrin colorimetric method was used to measure free amino acid, and phenol disulfonic acid was used to measure nitrate. Crude fiber and crude protein were obtained by acid digestion method and soxhlet extraction. The 10 parallel samples were measured for each parameter.

3. Results and Discussion

3.1 Evolution of Plant Height, Plant Width and Number of Leaves

Evolutions of the plant height, plant width and number of leaves for each treatment (or combination of fertilizers) are shown in Figure 3. The plant height of each treatment of lettuce was linear growth trend (Figure 3(a)). The 3-DFM for the lettuces was observed obviously effects. From the perspective of growth time, growth in the first 28 days was relatively slow. The plant height changed from 4.65 cm to 14.96 cm. And the difference range among each treatment was 1.45 cm. The greatest plant height was the 3-DF treatment. And the smallest plant height was the treatment to which CO_2 and SFD were applied. The plant height was increased from 17.28 cm to 29.25 cm in the later 28 days, accounted for 65.67%-74.93% of the total plant height. The greatest plant height of the 3-DF treatment was 29.25 cm greater than that of the control and other treatments by 26.8% and 8.4%-11.1%. The result was similar to the research results of Wang (2005).

The plant width and plant height of lettuce showed similar growth trends (Figure 3(b)). The plant width of the 3-DF was in a linear growth trend. As for the growth time, the growth in the first 28 days was slow. The plant width increased from 2.29 cm to 11.85 cm. And the range of differences among each treatment was 0.87 cm. The growth effect in the later 28 days was obvious, i.e. from 13.26 cm to 22.38 cm, accounted for 62.86%-69.43% of the whole plant width. The 3-DF treatment of the greatest plant width was 22.38 cm higher than the control and other treatments by 16.5% and 5.4%-10.1%.

The growth trend of the number of lettuce leaves was similar to that of plant height and plant width (Figure 3(c)). The number of leaves of 3-DF was basically taken on a linear growth trend. The growth in the first 28 days was relatively slow, and the number of leaves ranged from 2 to 8.5 with the range of difference among each treatment being 0.7 piece. The greatest number of leaves appeared the 3-DF treatment. The number of lettuce leaves was larger than that of the control by 8.97%. The growth effect of the later 28 days changed obvious from 9.5 pieces to 16.2 pieces, accounted for 64.93%-77.26% of the whole growth cycle. The number of leaves of the 3-DF was 16.2 pieces, which was more than that of the control and other treatments by 3.9 pieces and 0.8-1.4 pieces of leaves.

It can be seen from the trend data on the growth characteristics of lettuce that the lettuce of the 3-DF treatment showed more obvious growth effect than the lettuce that growth under other conditions. Duing to SFD and LFD was rich in nitrogen, phosphorus, potassium and other trace elements, which could be dissolved into the elements and directly absorbed by lettuces. In addition, CO_2 was in sufficient supply under the strong illumination conditions, thus the growth conditions of lettuces were superior to those of the lettuces of the same kind under other conditions.

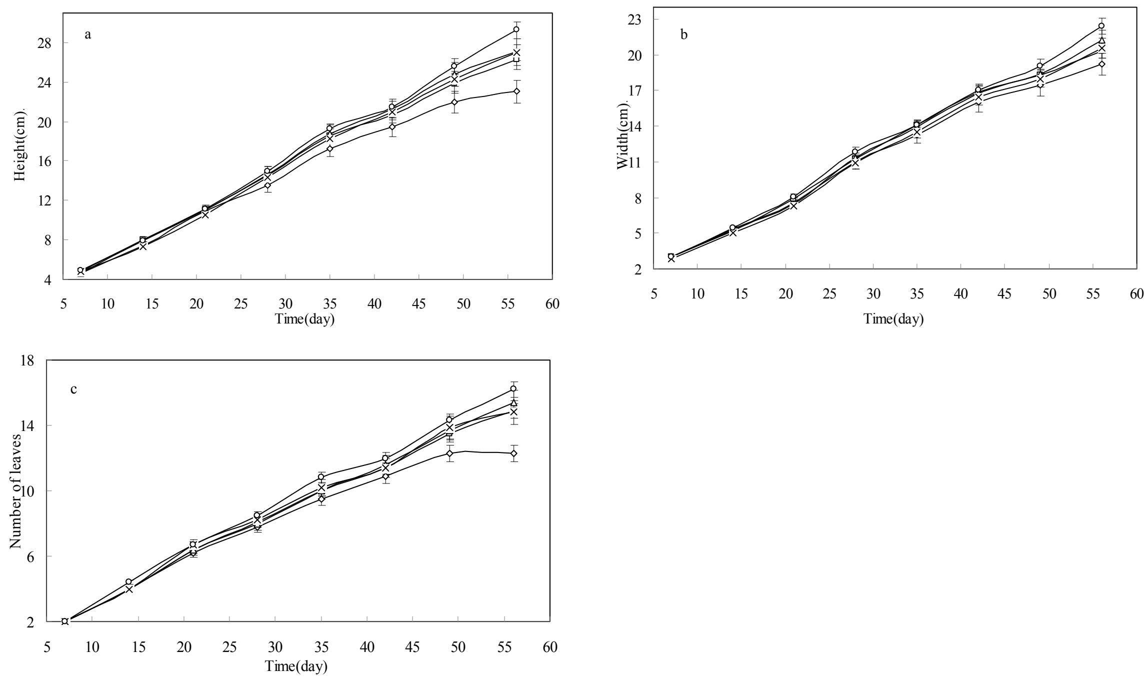

Figure 3. The changes of (a)the plant height, (b)plant width and (c) number of leaves with different phase of lettuce: ◇, Control; □,CO_2; △, CO_2 and LFD; ×, CO_2 and SFD; ○, CO_2, LFD and SFD

3.2 Yield of Lettuce

The fresh weight, content of dry weight, and the total yield were calculated of the ground part and underground part of each treatment of lettuce during maturity period (Table 3). The fresh weight with the 3-DF was more obvious than that of the control. The fresh weights of the ground part and underground part of the lettuce with the 3-DF were 748.01 g and 70.79 g respectively, higher than those of control and other treatments by 32.70%, 8.77%-22.06% and 13.45%, 1.13%-10.78%, respectively. The total fresh weight was 626.10-818.80g, and the range of yield increasing was 8.08%-30.78%. The total fresh weight of the lettuce with the 3-DF was higher than that of the control and other treatments by 30.78% and 8.08%-20.70%. The total scopes of the contents of dry weight and dry substances were 59.30-63.70g and 8.71%-9.47%. The ranges of variation were 8.88% and 12.33%. The yield of lettuce of the 3-DF could obviously increase by 30.78%, while the changes in the dry weight and content of dry substances were not obvious. However, the content of dry substances of the 3-DF was higher than that of the control, which showed that the yield of nutritious substances was lager than that of the control during growth of lettuce, and the growth conditions of lettuce with the 3-DF were superior to those of the lettuce of the same kind growing under other conditions.

Table 3. The yield and dry weight of lettuce

Treatments	Upside fresh weight (g)	Root fresh weight (g)	Fresh weight (g)	Dry weight (g)	Dry matter content (%)	Increase (%)
Control	563.70 ±4.32	62.40±1.10	626.10±5.42	59.30±0.84	9.47±0.95	--
CO_2	612.80 ±7.64	63.90±0.94	676.70±8.58	61.20±0.93	9.04±1.20	8.08±2.06
CO_2, LFD	629.00 ±5.28	70.20±2.33	699.20±7.61	60.90±0.87	8.71±2.36	11.68±6.84
CO_2, SFD	687.70 ±4.27	68.00±1.75	755.70±6.02	58.50±0.91	8.43±1.42	20.70±7.83
CO_2, LFD, SFD	748.01 ±6.96	70.79±1.57	818.80±8.53	63.70±0.98	8.89±0.70	30.78±1.05

3.3 Nutritious Properties of Lettuce

3.3.1 Contents of Vitamin C and Free Amino Acid

Vitamin C is a water-soluble vitamin, and vegetables are an important source of vitamin C. The content of free amino acid is an important quality index (Lu, 1998) to describe the freshness level and tastes of the vegetable leaves. The content of vitamin C and free amino acid in lettuce are showed Figure 4.

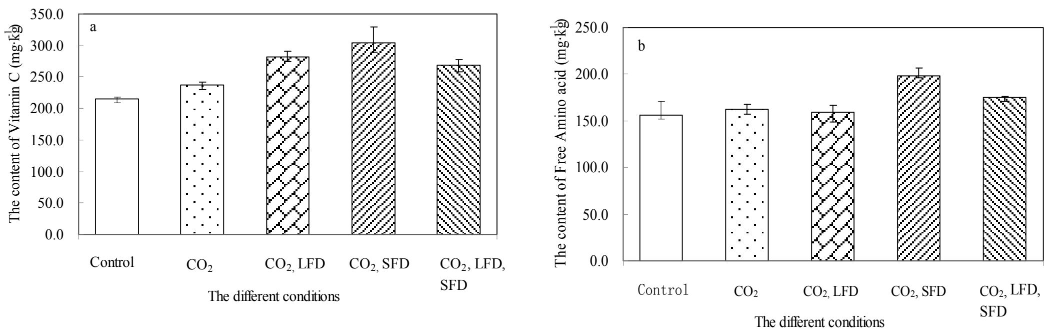

Figure 4. The content of vitamin C and free amino acid in different conditions

The contents of vitamin C (Figure 4(a)) and free amino acid (Figure 4(b)) of the control were the lowest with 214.6 mg·kg^{-1} and 156.3 mg·kg^{-1}, while the content of vitamin C and free amino acid with CO_2 and SFD were the highest, with the maximum value being 303.6 mg·kg^{-1} and 198.5 mg·kg^{-1}, rising by 41.47% and 27.00% respectively over the control. The content of vitamin C and free amino acid of the 3-DF was lower than that of the CO_2 and SFD treatment, which may be due to the reason that the LFD was sprayed at the back of the lettuce leaves, and the solid substances contained were attached to the leaf surface and was somehow adhesive. Thus the absorption of CO_2 was greatly influenced, and photosynthesis was also influenced. The synthesis of vitamin C and free amino acid in the leaves was restricted (Xu, 2009).

3.3.2 Content of Soluble Sugar, Crude Protein and Crude Fiber

The contents of soluble sugar, crude protein and crude fiber in the nutritious substances of lettuce are high, and are all one of the six nutritious elements recommended by scientists.

The contents of soluble sugar, crude protein and crude fiber are all the lowest in the control (Figure 5), with the minimum values being 10.27 g·kg-1, 11.26 g·kg-1, and 6.37 g·kg-1, respectively, while the content of soluble sugar of the 3-DF was the highest, which the maximum value was 13.29 g·kg-1, rising by 29.41%. The contents of the crude protein and crude fiber of with CO2 and SFD were the highest, which the maximum values were 13.08 g·kg-1 and 8.31 g·kg-1, respectively, rising by 16.16% and 30.45%, respectively. In comparison, the contents of crude protein and crude fiber of the 3-DF varied slightly. Thus contents of soluble sugar, crude protein and crude fiber of the lettuce under the 3-DF during lettuce planting were the highest. The results were similar to the research results of Ai Tian (Ai et al., 2006).

3.4 Content of Chlorophyll

The content of chlorophyll not only reflects the freshness of lettuce, but also the strengthening photosynthesis, speed of photosynthesis, and the accumulation of photosynthetic products during growth of lettuce, thus it is also one of the indexes to be measured.

According to Figure 6, the content of chlorophyll of the 3-DF was 472.3 mg·m^{-2} much higher, which the maximum margin of increase was by 11.42%. The chlorophyll content of other treatment was 437.5-451.2 mg·m^{-2}, which was not obvious change in different conditions. The content of chlorophyll was not the nutrition index of vegetable, but it could reflect the strength of vegetable photosynthesis. Thus it was of great significances for reflecting the growth conditions of plant and accumulation level of nutritious substances. It showed that the photosynthesis and accumulation of nutritious substances had been greatly improved under the 3-DF, which may be due to the synergistic effect through the 3-DF to lettuces (Rustad et al., 1994).

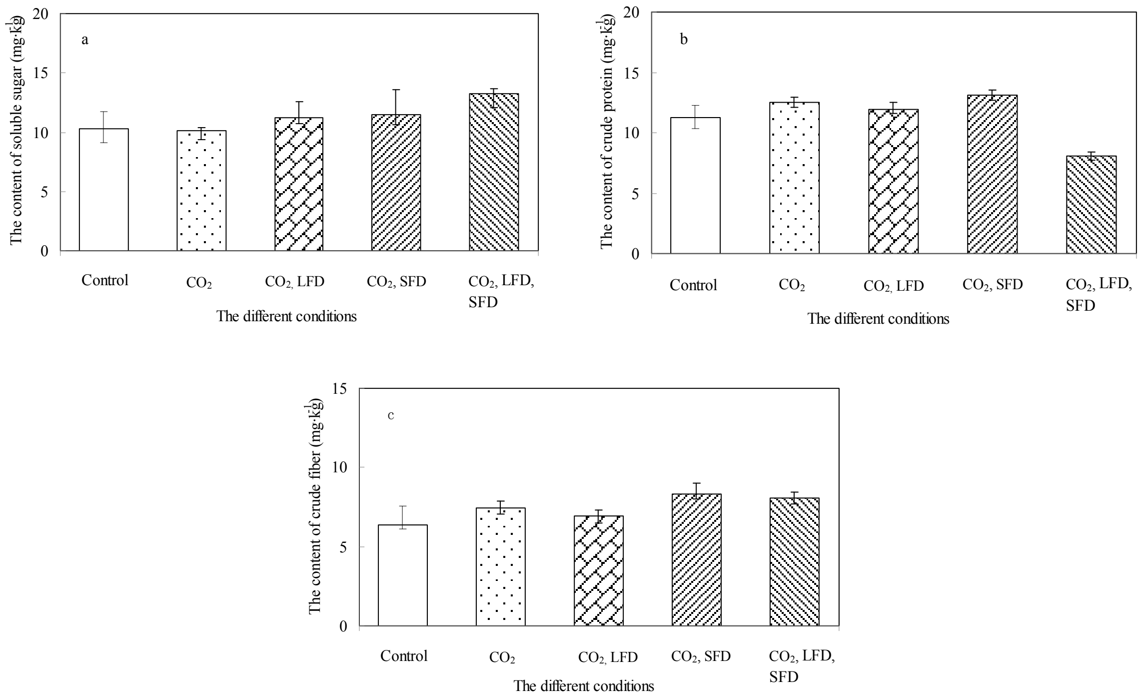

Figure 5. The contents of soluble sugar, crude fiber and protein in different conditions

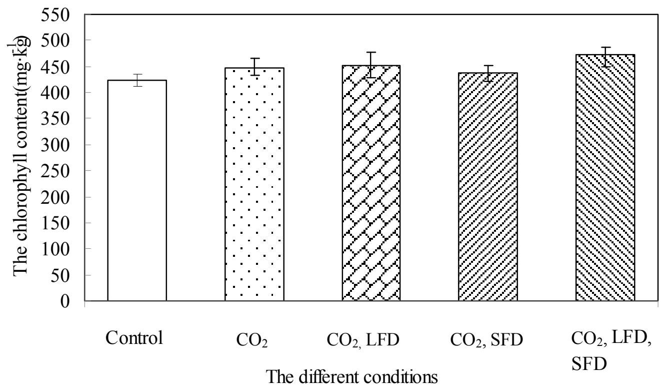

Figure 6. The content of chlorophyll in different conditions

3.5 Content of Nitrate

Nitrate can be converted into nitrite under certain conditions, while the latter is of great hazards to human body. Thus the content of nitrate in vegetable is one of the important indexes to appraise the vegetable quality.

According to Figure 7, the content of nitrate of the control was the lowest, which the minimum value was 534.82 $mg \cdot kg^{-1}$, that of other treatments higher than the control by 4.26%, 7.04%, 7.15%, 2.40%, respectively. According to the national standard "GB 19338-2003", the limit for the amount of nitrate in leaves vegetable cannot more than 3000 $mg \cdot kg^{-1}$. The different conditions had no obviously changes on the increase of the content of nitrate, which may be due to two reasons as follows: On the one hand, part of the nitrate in the SFD and LFD was decomposed through anaerobic digestion, but a small amount of substances was absorbed by lettuce during application (Ding et al., 2010). On the other hand, a certain amount of nitrate was produced by metabolism during which various nutritious substances of lettuce came into being.

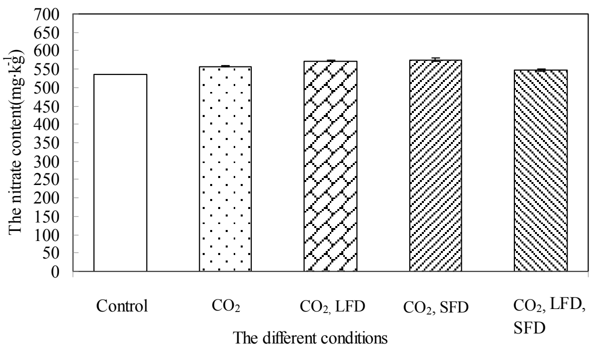

Figure 7. The content of nitrate

4. Conclusions

The 3-DFM is a very effective fertilization method for lettuce in ecological agriculture. The lettuce's plant height, plant width, and number of leaves increased the largest with the 3-DF, which the maximum values were 29.25 cm, 22.38 cm, and 16.2 pieces, higher than those of lettuce by 26.8%, 16.5%, and 18.4%, respectively. And the total yield increased by 30.78% in comparison. The content of soluble sugar and chlorophyll of the lettuce were 13.29 $g{\cdot}kg^{-1}$ and 472.3 $mg{\cdot}m^{-3}$ higer than those of fertilization 29.41% and 11.42% with the 3-DF. The contents of vitamin C, free amino acid, crude protein and crude fiber of the vegetable were 303.6 $mg{\cdot}kg^{-1}$, 198.5 $mg{\cdot}kg^{-1}$, 13.08 $g{\cdot}kg^{-1}$, and 8.31 $g{\cdot}kg^{-1}$, respectively, rising by 41.47%, 27.00%, 16.16%, and 30.45% in comparison the control. The 3-DFM could not only provide a new method to reutilize digestate from anaerobic digester and mitigate potential pollution with it, but also develop a new fertilizing mode for high quality vegetable production for eco-agriculture.

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