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#### **ABSTRACT**

The Mekong Delta of Vietnam located in tropical monsoon area where produce such of agriculture plants and biomass sources. Due to temperatures are high all year round, water hyacinth is a highly biomass flora which was proof as a confidence source for biogas production. In the Mekong Delta, there were several studies on co-digestion water hyacinth and pig manure not only to produce more biogas but also to maintain the biogas plant in case no pig manure feeding. This investigation studied on applies the effluent from co-digester of pig manure and water hyacinth as organic fertilizer for Leaf mustard planting and as feeding for Tilapia fish growing. The results showed that the harvest yield of Leaf mustard fertilized by effluent from co-digester was 2.2 times higher in the treatment supplied with inorganic fertilizer. In addition to its contribution to higher yield of the plant, the effluent can remain more nutrients for soil layer, accelerate the flower formation that shortening cultivation time. For Tilapia fish culture, the experiments supplied with 50% bio-slurry + 50% commercial food produced the net production of fish of 43.81 and 51.92 kg.ha-1.day-1 in treatment of pig manure, of pig manure and water hyacinth, respectively. These growing rates were not significantly different to the treatment of fish culture with 100% commercial food. The results strongly confirm that the effluent from a co-digester of pig manure and water hyacinth is possible to use as organic fertilizers not only for vegetable planting but also for fish culture. By co-digestion, husbandry people could invest for a biogas system without any fearless on lack of pig manure feeding. This will bring a cobenefit on hygiene conditions to local communities due to less of husbandry waste freely discharge into open sources.

Keywords: anaerobic co-digestion, Leaf mustard plant, the Mekong Delta, Tilapia fish, VACB farming system, Water hyacinth

#### 1. Introduction

Knowing as a "rice bowl" in Vietnam - the Mekong Delta (MD) contributes more than 50% agriculture exports and about 60% of its combined fisheries and aquaculture output [Konishi, 2011]. To support this successful, farmer applied a cycle farming system VAC (Vuon - Garden, Ao - Finspond, Chuong - Pigsty) for long time in which the output from each component could be an input material to other components. Since '90 decade of last century, the biogas technology was introduce and combined to the VAC system that create a new VACB model, in which B is abbreviate of Biogas word [Nguyen, 2012]. The biogas technology is strongly confirmed not only a safe treatment for pig manure (PM) but also gas supply for household cooking and lighting, etc. There are some researches on apply bio-slurry from biogas plant into aqua-agriculture activities. Nguyen (1989) found that the use of biogas slurry as fertilizer resulted in a considerable increase in the crop yield of corn 26% and kohlrabi 31%. The research on the application of solid bio-slurry to potato crops undertaken by the Institute of Energy in 1990 confirmed that the yield of the potato crops applied the bio-slurry increased 64% compared to that of non-applied bio-slurry crops [Le, 2008]. Le (1998a) presented that the biomass yield and the protein content of cassava foliage were significantly increased when bio-slurry was used to fertilize the cassava in comparison with the same amount of nitrogen applied in the form of the raw manure. A similar finding was reported for the duckweed grown in ponds fertilized with the bio-slurry or the raw manure [Le, 1998b]. In 2003, the National Institute for Soil and Fertilizer carried out a research on applying liquid bio-slurry to cabbage cultivation, reporting that application helped increase the cabbage yield to 24% [Le, 2008]. Eije (2007) found that farmers saved money on chemical fertilizer and pesticides when applying bio-slurry to their tea crops in the Northern part of Vietnam.

In the South of Vietnam, due to the fact that farmers usually use inorganic fertilizers, the studies on bio-slurry utilization are limited. The research carried out by the Renewable Energy Center of Cantho University on using bio-slurry for soybean planting showed that the application of bio-slurry helps increase the yield more than 20%; the application of the bio-slurry to fish-pond helps increase fish yield up to 10% [Nguyen, 2012, cited from Le, 2010]. The primary study by Do et al. (1999) recorded that household earned an annual income of about 2,000,000 VND by application of bio-slurry from TG-BP plants to his/her orchards and fishery. Similarly, Duong et al. (2009) informed that bio-slurry provided the best effects on crop growth and yield on the alluvial soil, specifically with maize cultivation. In this regard, Duong et al. (2010a) reported that the application of bio-slurry to maize crops grown on old alluvial soil could increase plant weight 1.64 times compared to the direct application of pig manure. In case of the application of bio-slurry to maize crops grown on acid sulfate soil, the increase is 1.57 times [Pham et al. 2010]. In respect of aquaculture, Duong at el. (2010b) assessed the effect of supplementation of homemade feed to biogas effluent and pig waste on growth and yield of fish species of Tilapia, Snakeskin gourami,

Kissing gourami and Common carp.

In case lack of PM due to inconvenient market or pig diseases, some types of biomass could apply as additional feeding into biogas plant beside pig dung. In the MD, there are some studies on co-digestion PM and water hyacinth (WH - *Eichhornia crassipes*) to maintain the biogas production from biogas plant [Nguyen and Phan, 1989; Panning, 2003; Truong *et al.*, 2009, Nguyen *et al.*, 2011]. However, the effect of effluent from co-digestion PM and biomass to fish cultivation or plant fertilizing in this case not yet study.

The study aimed at testing the possibilities of application of bio-slurry from the co-digestion biogas plants into vegetable planting and fish cultivation. The growing rate of the Leaf mustard (*Brassica juncea H. F.*) and the Tilapia fish (*Oreochromis Niloticus*) would be evaluated when they are applied different kinds of fertilizers. Studies on applicability of bio-slurry from anaerobic co-digestion of PM and WH for aqua-agriculture could optimize the use of anaerobic digesters economically, and promote the VACB on broad scale. In parallel, it is offers more opportunities for husbandry people to invest the biogas digester which could improve the hygiene conditions for local communities.

#### 2. MATERIALS AND METHODOLOGY

#### 2.1 Research location

The experiments on fish culture were conducted onsite at farmer households in Hoa An commune, Phung Hiep district, Hau Giang province (40 km from Can Tho city). Time of fish raising in rainy season of the MD from April 2010 to September 2010.

The experiments on vegetable planting were conducted at the Experiment site of the College of Environment and Natural Resources, Cantho University, Vietnam. Time of fish raising in rainy season of the MD from April 2010 to September 2010.

For the effluent source, the mixing ratios of co-digestion of 90%PM+10%WH (based on organic dry matter [ODM] values of input materials) were finally selected. The physical - chemical analysis was processed according to APHA, AWWA and APCF (1995) and taken at the Environmental Engineering Laboratory of the College of Environment and Natural Resources, Cantho University.

## 2.2 Experiments set-up for Leaf mustard testing

#### 2.2.1 Design and measurements

Leaf mustard was grown from the seeds in lab-scale pots of  $30~\text{cm} \times 30~\text{cm} \times 25~\text{cm}$  (length  $\times$  width  $\times$  height) which were laid out in a randomized block design. The soil was prepared in homogenous form and then 10~kg of the soil was put into each of the pots.

Leaf mustard was planted at 2 - 3 cm depth and the space between the seeds was 15 cm. Each pot contained four units of Leaf mustard. The measurements were made on the 15th, 26th, 33rd and 43rd day from the seeding time. Each treatment

contained 12 replicates, so that three of the pots were used for one measurement.

Each of the measurements followed the following procedures:

- The plants in three of the 12 pots were used for one measurement. All of 12 plants were pulled out, weighed and measured for plant height in average (without their roots). Then, the biggest leaf was selected and measured for leaf height and leaf width.
- Right after the measurement was done, all of these 12 plants (excluding their roots) were used to analyze ODM value and Salmonella contaminate. The analysis methods were based on APHA, AWWA and APCF (1995).
- Soil samples were taken before and after the harvest for the analysis of humidity and primary nutrients of total Kjeldahl nitrogen (TKN), total phosphorus (TP) and potassium (K).

Statistical analysis: the data were analyzed by Duncan's Multiple Range Test using SPSS 13.0.

## 2.2.2 Fertilization

#### a) Treatment design

The planting work based on the guidelines on the amount and timing of fertilization applicable for Leaf mustard crop as suggested by Tran (2010). Accordingly, a Leaf mustard crop should be fertilized on the day 0, 10<sup>th</sup>, 15<sup>th</sup>, 20<sup>th</sup> and 25<sup>th</sup> of the growing period. The inorganic fertilizer (IF) treatment followed the amount and timing of fertilization in the guideline of Tran (2010). Meanwhile, in regard to the other treatments related to the bio-slurries, first the nutrients containing in the bio-slurries were determined in order to help make up a volume of bio-slurries equivalent to the required amount of fertilizer as suggested by the guideline. Then, the volumes of the bio-slurries equivalent to the required amount of fertilizer were daily applied to the treatments. The bio-slurries were directly watered into the foot of the plants that limit the spreading of the bacterium from the bio-slurry onto the plants.

Four treatments were set up based on the three kinds of the fertilizers applied for the Leaf mustard. They included treatment fertilized by digestion effluent of 100% pig manure feeding (PM0), treatment fertilized by co-digestion effluent of pig dung and water hyacinth (WH0), treatment applied inorganic fertilizer (IF), and control treatment with only watered from tap water (C0).

Table 1 The Leaf mustard treatment design

Treatment	Applied bio-slurry		Others			
100%PM	PM0	PM0	PM0			$\overline{}$
90%PM+10%WH	WH0	WH0	WH0	><		><
Inorganic fertilizer				IF	IF	IF
Only water supplied				C0	C0	C0
(control treatment)						

## b) The bio-slurry volume used as plant fertilizers

Based on the nutrients content of each type of the bio-slurries, the volume of bio-slurry applied for the treatments was calculated to make an equivalent amount of nutrients to those contained in the IF applied. To do that, the bio-slurries were took and analyzed for their quality every week.

In this study, the bio-slurries were applied with the corresponding volume to the plants twice a day on a daily basis throughout the planting time. Meanwhile, the inorganic fertilizers were applied only 5 times for the whole planting time according to a fertilization schedule, and in this case water was supplied to the plants twice a day (in the morning and afternoon) on a daily basis so as to reach the required watering volume but there was no watering on rainy days.

Table 2 Bio-slurry volume in each treatment

Treatment	Day	Bio-slurry (L.m <sup>-2</sup> .day <sup>-1</sup> )	Water (L.m <sup>-2</sup> .day <sup>-1</sup> )
	0 - 10	0.98	14.02
	11 - 15	0.09	14.91
XX/110/XX/111	16 - 20	0.22	14.78
WH0/WH1	21 - 25	0.22	14.78
	26 - 30	0.44	14.56
	31 - 42	-	15.00
PM0/PM1	0 - 10	3.22	11.78
	11 - 15	0.28	14.72
	16 - 20	0.76	14.24
	21 - 25	0.76	14.24
	26 - 30	0.23	14.77
	31 - 42	-	15.00

Notes -: no nutrients supplemented during that period

# 2.3 Experiment set-up for Tilapia fish testing

## 2.3.1 Design and measurements

In this study, the fish was raised in the 1 m  $\times$  1 m  $\times$  1 m nets with triplicate for each treatment. The fish density was 10 units per net. The fish under the experiments started to be raised at the age of 6 weeks with the fish average weight of 5 - 7 g. To limit the exchanged water from the inside and outside of the experiments, the nets were enclosed by a transparent PVC layer.

The experimental fish were released into the nets in one week to get used to their living environment. After that, every ten days, three of the fish from each net were randomly picked up. The weight, length and width of the picked fish were measured by the scale (with maximum record of 500 g weight) and the ruler. After the measurement, the fish were released back to the experiment nets.

Statistical analysis: the data were analyzed by Duncan's Multiple Range Test using the SPSS software.

## 2.3.2 Feeding

### a) Treatment design

Based on the kinds of food supplied into the experiment nets, the fish were divided into three groups of fish raising treatments: no food supply (control treatment), bioslurry + commercial food (CF), and solely CF.

Treatment	100%PM			90%PM+10%WH		
No food supply	PM1	PM1	PM1	WH1	WH1	WH1
50% bio-slurry + 50%CF	PM2	PM2	PM2	WH2	WH2	WH2
Commercial food	PM3	PM3	PM3	WH3	WH3	WH3

Table 3 The Tilapia fish treatment design

The CF was fed into the fishponds two times a day. As suggested by NAEC (xxx), the amount of the CF for one time of feeding is estimated based on the weight of the fish. Accordingly, the amount of the CF should be equivalent to 4 - 6% of the fish weight. Based on such a suggestion, fish fed with the amount of CF equivalent to 6% of the fish weight for the first month and 4% for the later months. Like the CF, the bio-slurries was supplied into the experiment nets two times a day with the feeding volume, as suggested by [Veenstra & Polprasert, xxx], of 150 kg COD.ha-1.day-1. During the testing period, the COD values of the bio-slurries were analyzed three times in order to determine the volumes of bio-slurries to be supplied for each treatment.

## b) The bio-slurry volume supplied into fishpond

The volume of bio-slurries loaded into fish nets was calculated based on COD

values of each bio-slurry type. Each fish net was supplied with the amount of bio-slurry equivalent to the value of 150 kg COD.ha-1.day-1 surface of the fish net. Bio-slurry was applied to fish nets twice a day in accordance to the pigpen cleaning daily routine. In parallel, CF was also applied twice per day at the same time as the bio-slurry was applied.

No. Treatment Volume of applied bio-slurries (L.m<sup>-2</sup>.day<sup>-1</sup>) Jun-27 to Jul-03 Jul-03 to Jul-31 Jul-31 to Aug-22 1 PM1 4 16 9.56 2.86 2 PM2 2.08 4.78 1.43 3 WH1 0.30 0.34 0.68 4 WH2 0.15 0.17 0.34

Table 4 Volume of bio-slurry fed into fish nets

#### 3. RESULTS AND DISCUSSION

## 3.1 Results of Leaf mustard planting

After two weeks of planting, most of the Leaf mustard plants in the control treatment died. Therefore, there is no discussion on the control treatment result in this study.

### 3.1.1 The changes in soil fertility

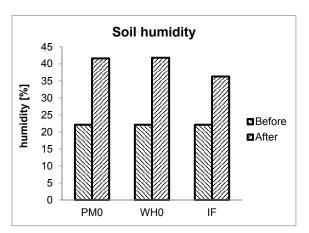
Before planting the Leaf mustard, there were no differences in the soil humidity and nutrient content among the fertilizer treatments due to the homogenous form of soil prepared at the initial phase. However, the soil humidity in all the fertilizer treatments became higher after growing the Leaf mustard, in which the treatment supplied with IF got the smallest change in the soil humidity. Similarly, there were variations on the soil humidity in the treatments with daily watering with various types of substrates. The substrates from the PM0 and WH0 treatments also equally brought in a positive prevention from water evaporation. By contrast, the IF treatment was applied no substrate but only daily watering that offered good conditions for water evaporation through soil surface.

The amount of nitrogen remaining in the soil after the Leaf mustard was harvested was larger than before the planting. Nitrogen contained in the harvest soil was larger than the initial nitrogen by 5.5, 6.2, and 7.2 times in the treatment of WH0, of PM0, and of IF respectively. Nitrogen was supplied for each treatment with the same amount but it remained the highest in the IF treatment. This could be due to the loss of nitrogen through soil surface by evaporation or nutrient from IF not easy to absorb by plant.

In addition, that nitrogen remained larger in soil after the harvest of Leaf mustard than before the planting signified that there could have been more nutrient supplied than the needed nutrient for the plants. In fact, the inorganic fertilizer scheme guidelines always suggest supplying a larger amount of nutrients than needed to plants so as to ensure the plant yield in case of loss of nutrients. Taking such a recommendation concerning fertilizer application, farmers will waste money on excessive fertilizers. The testing results showed that application of organic fertilizers for crops could help improve this "stork neck", thereby bringing in more income for growers.

Differences in phosphorus among the fertilizer treatments were not much, ranging from 0.11 - 0.18%, but lower than the initial phosphorus value. The results showed that Leaf mustard plant was good at absorbing phosphorus. In fact, phosphorus is an essential part of the process of photosynthesis and gets involved in the formation of sugars, starches, etc. Phosphorus will be strongly absorbed at the time of plant maturation or blooming. Besides, even though the phosphate was applied into the treatment IF as much as recommended, the phosphate value after the harvest was lower than before the planting. The explanation for this result is that there could be a significant loss of phosphorus contained in the inorganic fertilizer by discharging together with rainfall or by evaporation through soil surface.

The soil potassium was almost higher in all the fertilizer treatments after the planting but got the highest value in the IF treatment. This result indicated that the bio-slurries contained more potassium than the need of the plants. As such, in case of application of bio-slurries to plant growing, it is not necessary to fertilize the plants with extra potassium.



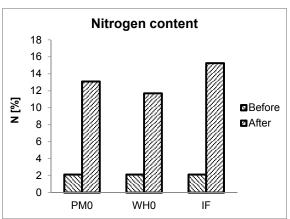
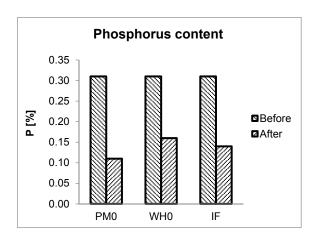


Fig 1 Soil humidity and nitrogen content in different treatments



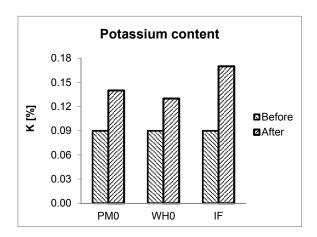


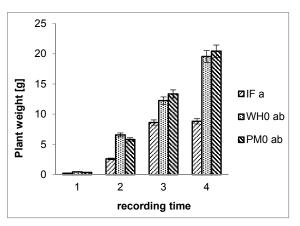
Fig 2 Phosphorus and potassium content in different treatments

### 3.1.2 Plant growing

The bio-slurries applied treatments could increase the plant weight more than the treatment only supplied with inorganic fertilizers. The Leaf mustard yield of 9.1, 8.7, and 3.9 tons fresh biomass per hectare was observed in the treatment of PMO, of WHO, and of IF respectively for a 43 day growing period. The increased yield pertaining to the Leaf mustard fed with bio-slurry could be attributed to the improvement of soil nutrients. In this connection, Garg *et al.* (2005) found that amendments of soil with biogas slurry could be attributed to the improvement of soil physical properties in terms of lower bulk density, higher hydraulic conductivity and greater moisture retention of soil.

In addition to the highest quantity of TKN and TP remaining from the PM0 treatment, the highest Leaf mustard yield was gained in this treatment. This result showed that nitrate nitrogen (NO<sub>3</sub>) from commercial fertilizer sources has less effect on plant yield compared to ammonia-N from the bio-slurry source, especially in case the applied bio-slurry taken from the biogas plant fed with PM+WH.

In respect with the results of plant height, the application of bio-slurry helped shorten the cultivation time of Leaf mustard. The WH treatment was the earliest flowering on the 35<sup>th</sup> day, the PM treatment started to flower on the 38<sup>th</sup> day, and the other treatments had flower formation from the 40<sup>th</sup> day onwards. The Leaf mustard plants must be harvested for sales before their flower formation. It means watering with bio-slurry could save from 5 to 10 days of Leaf mustard planting time. Actually, in this study, it was observed visually that the leaves of the plants began to dry and curl up since the flower formation occurred. This phenomenon strongly confirmed and signified that the plants were so mature and they should have been harvested earlier.



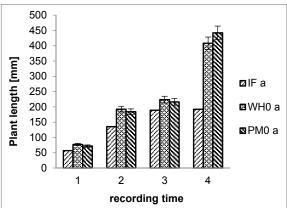
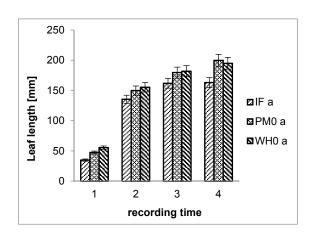


Fig 3 Plant weight (left) and plant height (right) at different fertilizer treatments



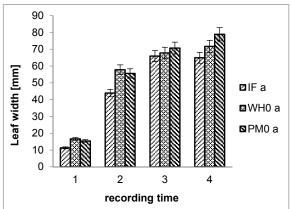
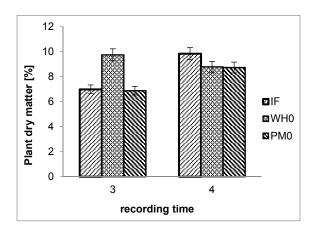


Fig 4 Leaf length (left) and leaf width (right) at different fertilizer treatments

Note: Treatments followed by a common letter are not significantly different at  $p \le 0.05$  based on Duncan's Multiple Range Test.



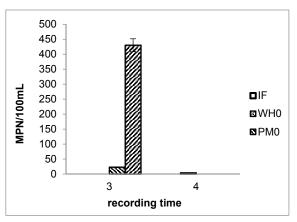


Fig 5 Plant dry matter content (left) and Salmonella contamination (right)

On average, the Leaf mustard plant dry matter content reached 8.4%, 9.3%, and 7.8% in the treatments of IF, of WH0, and of PM0 respectively. In the MD, assuming that irrigation is available throughout the year, in case of application of bio-slurries to Leaf mustard growing as prescribed in this study, the yield will be 7.3, and 6.4 tons of dry matter per hectare for the application of the bio-slurry from digesters fed with PM+WH, and with PM alone without an addition of any inorganic fertilizers, respectively.

The Salmonella contaminated in the Leaf mustard which was watered with different bio-slurries was tested. There was no Salmonella found contaminated in the treatments of IF and of WH0 in the first test, while in the second test Salmonella was found only in the treatment of WH0. The presence of Salmonella in some of the treatments may pose a risk to human health, particularly in case of supply of bio-slurry to such plants as vegetables which are normally eaten fresh.

# 3.3 Results of Tilapia fish raising

## 3.3.1 Fish growing

During the experimental period, the number of fish alive was frequently recorded based on the number of fish died floating visually on the water surface. And at the last record, all the experimental nets were hauled in and the number of living fish was checked. The number of fishes still living after 52 culture-days was not significantly different between the treatments. Obviously, this result gives to farmers a chance to use the biogas plant with co-digestion pig manure and biomass.

		=		
Foo	d apply No food (1)	50% bio-slurry +	Only CF (3)	Average
Treatment		50%CF(2)		
100%PM	60%	100%	90%	85%
90%PM+10%WH	80%	80%	100%	89%
Average	67%	89%	93%	

Table 5 Number of living fish in the experiments

The fish growth factors showed that the highest values came from the treatment fed with 100%CF, followed by the treatment fed with 50% bio-slurry + 50%CF, and the control treatment. After 52 days of fish culture, the experiments supplied with 50% bio-slurry + 50%CF produced the net production of fish of 2.3, and 2.7 tons.ha<sup>-1</sup> in the treatment of PM, and of PM+WH respectively.

Table 6 Fish growth of the supplied bio-slurry from 90%PM+10%WH digester

Treatment	Fish weight (g)	Fish length (cm)	Fish width (cm)
WH1: no food supply	10.500 a	6.450 c	2.550 e
WH2: 50% bio-slurry +50%CF	17.733 ab	7.558 cd	3.038 ef
WH3: 100%CF	21.445 b	8.332 d	3.245 f

Note: The means followed by a common letter are not significantly different at  $p \le 0.05$  based on Duncan's Multiple Range Test.

The fish growth rate reached highest in the treatment fed with 100%CF, followed by the treatment fed with 50% bio-slurry + 50%CF, and the last was the control treatment. However the result from Duncan testing displayed that there was no significant difference between the treatment fed with 100%CF and the treatment fed with 50% bio-slurry + 50%CF. It clearly confirmed that the fish could be raised by only feeding 50%CF and 50% bio-slurries but the fish yield was not significantly different from feeding the fish with 100% commercial food. In other words, farmers can save at least 50% expense cost on fish food when raising fish in an integrated pig - fish culture system.

### 4. CONCLUSIONS

Bio-slurry from a co-digestion of pig manure and water hyacinth is possible to use as organic fertilizers not only for vegetable planting but also for fish cultivation. The results of Leaf mustard planting as analyzed in this study showed that application of bio-slurry of the co-digestion to planting gave a better output compared to that of inorganic fertilizers application. Actually, the harvest yields of Leaf mustard were 2.3 and 2.2 times higher in the treatment supplied with inorganic fertilizers from co-digester of PM and of PM+WH respectively.

For Tilapia fish culture, the experiments supplied with 50% bio-slurry + 50%CF produced the net production of fish of 43.81, and 51.92 kg.ha-1.day-1 in treatment of PM, and of PM+WH respectively. These growing rates were not significantly different in the treatment of fish raising with 100%CF. This means farmers can raise fish by only feeding 50%CF + 50% bio-slurry from the codigestion biogas plant and get a similar output to that of feeding fish with 100% commercial food.

Farmers in the MD conventionally discharge fresh pig manure into their fishponds or for gardening, which causes negative impacts on water sources. Application of the biogas plant for pig manure treatment and using the bio-slurry as organic fertilizer for fish raising or for gardening activities could create the sustainable aqua-agriculture cultivations. As a result, the local environment could improve so much due to preventing the practice of either discharge fresh pig manure or bio-slurry into open sources.

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