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Study on fish growing in hapa conditions using bio-slurry from co-digestion process

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Abstract

This study investigates the effects of bio-slurry taken from differently combined digesters and different feeding ratios on the growth of Tilapia fish (Oreochromis niloticus) in hapa conditions. It consists of seven treatments of different feeding formula, namely (i) 100% commercial food (CF); (ii) 100% bio-slurry (BS) from a digester fed by pig dung (PD); (iii) 50%CF+50%BS from a digester fed by 100%PD; (iv) 100%BS from a digester fed by 90%PM+10% spent mushroom compost (SMC); (v) 50%CF+50%BS from a digester fed by 90%PM+10%SMC; (vi) 100%BS from digester fed by 90%PM +10% water hyacinth (WH); (vii) 50%CF+ 50%BS from a digester fed by 90%PM+ 10%WH. The experiments were set up in hapa sizing 1 m × 1 m × 1 m installed in fishponds with triplicate for each treatment. The fish size of 5 - 7 g each was stocked at a density of 10 individual per hapa. The fish weight and length were measured every ten days in 50 continuous days. The results show that when feeding 50%BS+50%CF to the hapa, the fish biomass from the treatments PM, PM+SMC, and PM+WH got 43.8, 51.5 and 51.9 kg/ha*day^{-1}, respectively. The growth rates of fish were not significantly different ($p<0.05$) in the treatment of fish feeding by 100%CF. The results of the study indicate that the feeding by 50% commercial feed and 50% bio-slurry from the co-digestion biogas plant produces similar fish productivity to that of feeding by 100% commercial feed.

Keywords: *co-digestion, spent mushroom compost, the Mekong Delta, tilapia, water hyacinth*

1. Introduction

Although the bio-slurry laboratorial studies and bio-slurry application for crop performance have been implemented since the 1940s, the application for fish culture only processed since ’80 of the last century. For the aquaculture sector, Kaur *et al*. (1987) reported growth rates of the fish (in terms of weight) were 3.54 times higher in biogas slurry-treated tanks than in the controls, while growth rates of common carp in raw cowdung-treated tanks were only 1.18 to 1.24 times higher than in the control. Edwards *et al.* (1988) also found there might be potential for even greater yields from biogas slurry fed to fishponds because the relationship between net fish yield and slurry loading rate was linear. Balasubramanian & Bai (1994) confirmed the biogas effluent could be used as an organic fertilizer in fish culture. In another study, Sophin & Preston (2001) reported that all of the five fish species (Tilapia, Silver carp, Bighead carp, Silver barb and Mrigal) grew faster in ponds fertilized with biogas effluent than with manure.

In Vietnam, there is much research in applying bio-slurry to farming, but the study of applying bio-slurry to fish culture is limited. The study by Do *et al*. (1999) recorded that the farmer earned yearly income of about US$ 100 by applying bio-slurry from TG-BP biogas plants to their orchards and into their fishponds. In recent years, within the project “The biogas program for the animal husbandry sector of Vietnam”, some researchers conducted a study that applying bio-slurry into fishponds and to watering the vegetables cultivated at household scale. The participating farmers, who were interviewed about their opinions on the benefits of bio-slurry, seemed to be satisfied with this product (Le, 2008). Additionally, Duong *at el.* (2010) found the positive effect of supplementation of homemade feed to biogas effluent and pig waste on the growth and yield of fish species of Tilapia, Snakeskin gourami, Kissing gouramy and Common carp.

In an attempt to address the lack of input materials to feed into biogas plants in the Mekong Delta of Vietnam, some researchers conducted a study looking for potential additional input materials and found that water hyacinth (WH) and spent mushroom compost (SMC) are possibly used as such additional input materials (Nguyen *et al.* 2011; Vo & Fricke, 2012). However, the finding of their study poses a question about the potential of using the bio-slurry from this co-digestion of PM and WH or PM and SMC for fish cultivation. There is little research answering such a question. This study aimed to fill this gap.

2. Materials and methods

2.1 Research location

The experiments were conducted at farm households located at acid sulfate soil areas in Hoa An Commune, Phung Hiep District, Hau Giang Province, Vietnam. At this area, with the fund supported by project VIE020-Water hyacinth, some of EQ2 biogas plants were built for co-digestion purpose.

1. Nguyen Tan Ho's household: EQ2 biogas plant with PM+WH feeding
2. Nguyen Van Lanh's household: EQ2 biogas plant with PM+SMC feeding
3. Cao Van Luc's household: TG-BP biogas plant with only PM feeding

Figure 1. EQ2 biogas plant with bio-slurry outlet pot (left) and WH feeding into inlet pot (right)

2.2 Experiment set-up

2.2.1 Design and treatments

In this study, Tilapia fish (*Oreochromis niloticus*) was selected due to their high tolerance in poor water conditions and high marketability with a stable price. Fish raising experiments were arranged in hapa of 1 m × 1 m × 1 m with triplicate for each treatment. The fish density was 10 units per hapa. The six-week-old fish were raised with the average weight of about 5 - 7 g each.

To make the supply of bio-slurries into fishponds easy, the hapa was installed onsite at the farmers' fishponds who owned the biogas plant for our experiment. The total four groups of treatments were arranged based on types of food supplied to fishponds.

- Applying bio-slurry from biogas plant fed by 100%PM into fishponds
  - PM0: without food supplied
  - PM1: 100% bio-slurry
  - PM2: 50% bio-slurry + 50% CF
  - PM3: 100% CF
- Applying bio-slurry from biogas plant fed by 90%PM+10%SMC into fishponds
  - SMC0: without food supplied
  - SMC1: 100% bio-slurry
  - SMC2: 50% bio-slurry + 50% CF
  - SMC3: 100% CF
- Applying bio-slurry from biogas plant fed by 90%PM+10%WH into fishponds
  - WH0: without food supplied

- WH1: 100% bio-slurry
- WH2: 50% bio-slurry + 50% CF
- WH3: 100% CF


Figure 2. Hapa for fish raising (left) and Tilapia fish used in the study (right)

Table 1. Treatment design

Treatment	100%PM			90%PM+10%SMC			90%PM+10%WH		
No food	PM0	PM0	PM0	SMC0	SMC0	SMC0	WH0	WH0	WH0
Only bio-slurry	PM1	PM1	PM1	SMC1	SMC1	SMC1	WH1	WH1	WH1
Bio-slurry + CF	PM2	PM2	PM2	SMC2	SMC2	SMC2	WH2	WH2	WH2
Only CF	PM3	PM3	PM3	SMC3	SMC3	SMC3	WH3	WH3	WH3

2.2.2 Feeding

The CF was applied into fishponds two times per day and the total amount of the CF per day was equivalent to 4 - 6% of the fish weight. We fed the food quantity equivalent to 6% of fish weight in the first month and 4% in the continuous months as suggested by Pham (2009). The bio-slurries were supplied into fishponds once or twice a day depending on the frequency of pigpen washing by the farmers. The bio-slurries volumes applied into fishpond based on COD values of bio-slurry (Veenstra & Polprasert, xxx), in which the offered COD from 50 to 150 kg COD/ha.day^{-1}. And to temporarily avoid bio-slurry exchanged between inside and outside of the experimental nets, a PVC film was used to close the outside of nets (except the treatments of PM0, SMC0, and WH0).

2.2.3 Measurements

After Tilapia fish were released into hapa, let the fish raising for one week to keep them in stable conditions before started measure them.

- Every ten days, three fish (random sample) were caught from each hapa and measured. Fish length and fish width were measured at the same time. After our measurement, the fish were released back to the hapa.
- In parallel, samples of bio-slurries were taken three times during the experiment period to analyze COD value that is used to adjust the bio-slurry volume applied into hapa.

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

3. Results and discussion

3.1 Fish pond characteristics

According to Hepher (1962), the primary production in fertilized fishponds was 4 to 5 times higher than unfertilized fishponds. In fact, by suppling organic fertilizers into fishponds, it contains more essential nutrients useful for fish productivities. Some of hapa characteristics were displayed in the below table.

Table 2. Hydro-biological characteristics of water body in hapa

Treatment		VSS (mg/L)	TS (mg/L)	BOD_5 (mg/L)	COD (mg/L)	N total (mg/L)	P total (mg/L)
100%PM	PM0	1.23	292	17.50	4000	17.45	1.43
	PM1	1.25	324	34.56	4800	25.73	1.87

	PM2	1.67	354	25.78	4000	19.34	1.53
90% PM+ 10% SMC	SMC0	1.23	292	17.50	4000	17.45	1.43
	SMC1	2.70	318	58.75	3600	30.40	1.79
	SMC2	2.45	376	56.25	8000	36.57	2.12
90% PM+ 10% WH	WH0	1.47	312	26.25	4800	23.57	1.83
	WH1	3.29	342	62.67	5600	29.65	2.10
	WH2	2.84	304	42.50	8000	23.73	1.77

Note: Both treatments 100%PM and 90%PM+10%SMC were arranged at the same fishpond and there was only one control treatment setup for these experiments. This means that the value of PM0 and SMC0 is similar.

At all experimental ponds, ponds factors remained at optimal values that are required for fish growth and survival. Most of the factors from the treatments SMC and WH higher than from the treatment PM showed that more nutrient remained in the experimental hapa which were applied with the bio-slurries taken from the co-digestion biogas plants. This finding is in line with the finding of other research that recorded higher fish yield in case of applying organic fertilizers into fishponds (Yadava & Garg, 1992). These nutrients can generate food for the algae and zooplankton in fishponds, which in turn can be eaten by the fish. Although in this study, there was no test for algae and zooplankton in the hapa, according to Balasubramanian & Bai (1994), these forms were observed to be increased considerably in the ponds supplied by biogas effluents. The authors also recorded that not only the bio-slurry affects fish growth directly but also the nutrient accumulation could remain several times after stopping applying the bio-slurry into the fishpond.

3.2 Fish growth

At the last record, all hapa were moved up and checked for the number of surviving fishes.

Table 3. Number of surviving fishes from the experiments

Food apply / Treatment	**No food (0)**	**Only bio-slurry (1)**	**Bio-slurry + CF (2)**	**Only CF (3)**	**Average**
100%PM	60%	90%	100%	90%	85%
90%PM+10%WH	80%	97%	80%	100%	89%
90%PM+10%SMC	60%	90%	87%	90%	82%
Average	67%	92%	89%	93%	

The number of fishes still alive after 52 cultivation days did not clearly show the differences among the treatments. As shown in the above table, in the treatments without food supply, the number of fishes alive is relatively high though it is lower than in the other treatments. One possible explanation for this is that the ponds of the former treatments were not closed by PVC firm as those of the latter ones so that the fishes could still enjoy the algae from water column due to the farmers still discharged the effluent from the biogas plant into their fishponds.

In the case of the treatments of food supplied, more fishes were alive in the treatments with the bio-slurry from digester with 100% pig manure input. However, there was a similar average value of surviving fish in the treatments with the bio-slurries from digester with 90%PM+10%WH and with 90%PM+10%SMC.

The results of the fish growth in the treatments fed by bio-slurry from the 100%PM digesters with or without supply commercial food were showed in the following table.

Table 4. Fish growth in the treatments with bio-slurry from the 100%PM digester

Treatment	**Fish weight (g)**	**Fish length (cm)**	**Fish width (cm)**
PM0	10.500 a	6.450 c	2.550 ef
PM1	9.295 a	6.192 c	2.465 e
PM2	17.740 ab	7.548 cd	3.047 fg
PM3	21.445 b	8.332 d	3.245 g

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

The fish growth factors showed that the highest values came from the treatment fed with 100% commercial food, following by the treatment fed with 50% bio-slurry + 50% commercial food, the control treatment, and the treatment fed with 100% bio-slurry. The result from Duncan testing displayed that there was not significantly different between the treatment fed with 100% commercial food and the treatment fed with 50% bio-slurry + 50% commercial food. In one study of the integrated pig - fish culture in the MD, Duong *et al.* (2010) recorded that fish yield from the treatment applied biogas effluent + supplemental food was higher than the treatment applied biogas effluent only.

The similar results were recorded by the groups of experiments supplied by bio-slurry from 90%PM+10%SMC and from 90%PM+10%WH digesters. The fish growth rate was the highest in the treatment fed with 100% commercial food, then the treatment fed with 50% bio-slurry + 50% commercial food, the treatment fed with 100% bio-slurry and the lowest was in the control treatment.

Table 5. Fish growth in treatments with bio-slurry from 90%PM+10%SMC digester

Treatment	Fish weight (g)	Fish length (cm)	Fish width (cm)
SMC0	10.500 a	6.450 c	2.550 e
SMC1	10.963 a	6.553 c	2.580 e
SMC2	20.147 ab	7.920 cd	3.200 f
SMC3	21.445 b	8.332 d	3.245 f

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

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

Treatment	Fish weight (g)	Fish length (cm)	Fish width (cm)
WH0	10.500 a	6.450 c	2.550 e
WH1	10.558 a	6.532 c	2.560 e
WH2	17.733 ab	7.558 cd	3.038 ef
WH3	21.445 b	8.332 d	3.245 f

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

Regarding the experiments supplied by bio-slurry from 100%PM digester, the fish growth values of the treatment fed with 100% bio-slurry were lower than in the control treatment. In contrast, the fish growth values of the treatment fed with 100% bio-slurry were higher than in the control treatment of other experiments fed with bio-slurry from 90%PM+10%SMC or 90%PM+ 10%WH digester. In fact, the treatment supplied with 100% bio-slurry should produce more nutrients than the treatment without any feeding. The different trend occurred as both groups of the experiments supplied with bio-slurry from 100%PM and from 90%PM+10%SMC digester were set up at the same pond. And this pond daily received free effluent from the 90%PM+ 10%SMC digester (daily about 150 L effluent discharged up to 60 m² surface water of fish pond), which supplied more nutrient to algae and zooplankton system outside the experiments nets. In respect with the group of experiments supplied with bio-slurry from the 90%PM+10%SMC digester, the fish growth of the treatment fed with 100% bio-slurry was higher than in the control treatment, while it is vice versa in the case of the experiments supplied with bio-slurry from the 100%PM digester.

In this study, the fishponds were loaded with bio-slurry based on the COD contents. Edwards *et al.* (1988) recorded that there was an increase in both the rate of growth of stocked fish and their mean size with an increase in organic loading of the bio-slurry. So in case farmers have more bio-slurry, they can supply more bio-slurry into fishponds but it should not exceed the value of 150 kg/ha.day^{-1} (Veenstra & Polprasert, xxx).

After 52 days of fish raising, the experiments supplied of 50% bio-slurry + 50% commercial food produced the net produce of fish by 2.28, 2.68 and 2.70 tons/ha in treatment PM, treatment PM+SMC, and treatment PM+WH, respectively. The experiments supplied with 100% bio-slurry produced the net yield of fish by 0.75, 0.77 and 0.98 tons/ha in treatment PM, treatment PM+SMC, and treatment PM+WH, respectively. Comparing the fish growth between the treatments of supplied effluent types, both of groups of experiments on supplied 100% bio-slurry and 50% bio-slurry + 50% commercial food showed the highest fish growth rate from treatment 90%PM+ 10%WH, treatment 90%PM+10%SMC and treatment 100%PM, respectively. It means that application of the bio-slurry from co-digestion PM+WH and/or PM+SMC into fishponds could help increase the fish yield more than in the treatment applying bio-slurry from the digester fed only by pig manure.

Table 7. Fish growth in treatments supplied with 100% bio-slurry

Treatment	Fish width (cm)	Fish length (cm)	Fish weight (g)
PM1	2.465 a	6.192 b	9.295 c
WH1	2.560 a	6.532 b	10.558 c
SMC1	2.580 a	6.553 b	10.963 c

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

Table 8. Fish growth in treatments supplied 50% bio-slurry + 50% commercial food

Treatment	Fish width (cm)	Fish length (cm)	Fish weight (g)
PM2	3.047 a	7.548 b	17.733 c
WH2	3.038 a	7.558 b	17.740 c
SMC2	3.200 a	7.920 b	20.147 c

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

In both group of experiments supplied 100% bio-slurry and that of 50% bio-slurry + 50% commercial food, the fish growth were not significantly different ($p \leq 0.05$) on Duncan test. It clearly confirms that farmers could raise fish by supplying merely 50% commercial food, the rest 50% of feeding can come from the bio-slurry taken from a biogas plant. In other words, farmers can save at least 50% of the expense for fish food when raising fish in a pig - fish integrated cultivation system.

Some research found higher growth rates of fish in ponds fertilized with biogas effluent than with manure (Kaur *et al*., 1987; Sophin & Preston, 2001). However, Duong *et al*. (2010) recorded fish yield from the treatment applied biogas effluent lower than from the treatment applied pig waste only. Indeed the authors did not arrange treatments that applied pig manure directly into fish ponds due to hygienic conditions of this kind of feeding.

In this study, there was no opportunity to make a profound study in fish yield between treatments supplied with bio-slurry from the 90%PM+10%SMC digester and from the 90%PM+10%WH digester. It was unclear why the fish growth in the treatment supplied with bio-slurry from the PM+SMC digester was recorded higher than in the PM+WH. According to previous study of Sunila *et al.* (2007), rice straw can be used to increase fish productivity through the development of bacterial biofilm and periphyton. In addition, by applying rice straw into fishpond, 87 genera of phytoplankton were identified belonging to the following groups: *Bacillariophyceae, Chlorophyceae, Cyanophyceae* and *Euglenophyceae*. Based on the findings of the research mentioned, it is assume that applying the bio-slurry from the PM+SMC digester into the fish pond could create some of the mentioned phytoplankton and zooplankton, which benefit the fish cultivated in this experimental nets.

4. Conclusions

The findings of applying bio-slurry from co-digester PM+WH and PM+SMC for fish farming in the Mekong Delta can be summarized as follows:

- Regarding Tilapia fish culture, the experiments supplied with 50% bio-slurry + 50% commercial food produced the net fish yield by 43.81, 51.50 and 51.92 kg/ ha.day^{-1} in treatment PM, treatment PM+ SMC and treatment PM+WH, respectively. The experiments supplied with 100% bio-slurry produced the net fish yield by 14.33, 14.75 and 18.81 kg/ha.day^{-1} in treatment PM, treatment PM+SMC, and treatment PM+WH, respectively. These values strongly confirm that it is possible to raise fish with bio-slurry feeding but without giving any nutrients supplementary or chemical fertilizers.

- In addition, the application of bio-slurry into a fish pond could keep the pH range at an optimal for fish growth, avoiding algae bloom as apply fresh manure that maintain DO value in water body. As observed by the farmers, the culture time for fish raising could be saved in the case of applying bio-slurry from a biogas plant inputted with pig manure alone. However, since our experiments lasted only in 52 days, such observation of time saving can hardly be strong evidence.

- In case of raising fish during the dry season in acid sulphate soil area, it should take into account the pH value from fishpond's water body. Actually, this study did arrange some experiments raising fish in the dry season, but most of the fish died due to more acidity water body in the study area, which is strongly affected by acid-sulfate soil. Even in the rainy season, the pH value from water body in the fishpond was only about 6.31 - 6.62.

The study's results offer good opportunity to farmers on apply bio-slurry from co-digester to fish culture. The benefits of co-digesters not only collecting biogas for energy purpose but also taking bio-slurry as fertilizer for fish raising. This giving a chance to promote the biogas plant and the VACB farming system in the Mekong Delta.

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This section can be kept at the end of the manuscript before reference section and should not be more than 50 words. This section can be used to acknowledge the help of those who do not qualify for authorship or to acknowledge funding, donated resources or significant contribution to the research.

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