Effective concentration of Water Hyacinth (*Eichhornia crassipes*) and Corncob (*Zea mays*) as a growth medium for the development of Sludge Worm (*Tubifex spp.*)

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**Abstract.** Feeds with high protein characteristics and relatively small size are needed to support the growth of fish seeds. One source of feed that meets these criteria is sludge worm. Water hyacinth and corncobs are waste in the community. The mixture of these two ingredients contains organic material that is able to provide nutrients for the growth of sludge worms. This study aims to optimize the development of sludge worm cultivation media based on water hyacinth and corncobs as raw material. Sludge worms are cultured with recirculation system with 3 design combination treatment variation of water hyacinth and fermented corncob. Those 3 design are 15% water hyacinth and 5% corncob (treatment I), 10% for both of water hyacinth and corncob (treatment II), and the last is 5% water hyacinth with 15% corncob (treatment III). The parameters measured in this study were water quality and dissolved oxygen. Treatment I and treatment II present good results as potential sludge worms cultivation media.

**Keywords:** Corncob, cultivation, fermentation, protein, sludge worm, water hyacinth

1. Introduction

High protein content and relatively small size are feeds characters that required to support the growth of fish seeds. Among living organisms that is used as feeds in fish seeds on fish farming is the organism from subclass of Oligochaeta [1]. Sludge worms (*Tubifex spp.*), a widely distributed fresh-water Oligochaetathat has been commonly used for various water pollution testing especially for the effect of some metal compounds. In addition, *Tubifex spp.* is also commonly used as a live food for fish cultivation [1, 2]. *Tubifex spp.* is used as live food for the juvenile phase of fish cultivation. Juvenile phase is one of the important phases that should be controlled because in this phase they are instinctively prefer to accept those live food items which are easily detected and captured while moving, swimming or having any type of motility in water [3].

Sludge worms can be easily found on silt which is rich of organic reservoirs, but it is also occurs on sandy and rocky soils even in small quantities. All this time, fulfilling the need of sludge worms in fish farming is just depend on their presence in nature which is quite risky with contaminated water and invasion with precursor of diseases. Besides that, the catch is still unstable because this method depends on environmental conditions so it is still not enough to fulfill of market demand. Nowadays, people are trying to find the solution to overcome this problem. Cultivating the sludge worms in a growth media
with optimum and controlled conditions to get the good quality sludge worms for fish cultivation is one of the solutions for this problem [2].

Sludge worms are aquatic invertebrates that have small sizes, usually 3-4 cm long. The front end of the body is constantly in soil of 5–10 cm [4]. Usually, they are found forming reddish big colonies in the mud and obtain food from the nutrients deposition resulting from the degradation of microorganism which will be selectively absorbed through the body wall [5]. Organic compounds are the natural feeds for sludge worm. Based on this fact, the artificial growth medium used to cultivate the sludge worm should contain nutritious organic compounds [2]. Therefore, the experiment to find potential organic material for the growth of sludge worms needs to be done. The organic material that is potential for the sludge worms growth media is media that consisted of fermented water hyacinth (*Eichhornia crassipes*) and corncobs (*Zea mays*).

The existence of water hyacinth in nature were frequently realized as weeds in waters, and corncobs are by-products that haven’t been optimally utilized. The nutrition content in both can be potentially used as a medium for sludge worm cultivation. Research about organic matter like water hyacinth has already been done in mustard cultivation but not in sludge worm cultivation [6]. So this research will compare the effective concentration of water hyacinth and corncobs for the development of sludge worm growth medium.

2. Materials and method

2.1. Preparation of culture unit

The experiment was conducted to find the best concentration of water hyacinth and corncobs that resulted suitable environment quality for the growth medium of sludge worm. The sludge worms were cultured for 20 days in plastic container (52 × 38 × 15 cm³) culvert system. The system was placed under a rooftop to protect the worms from excess light and high rain intensity. The system also provides good drainage by arranging the culvert above ground level. Then, to get the best circulation for worms, continuous water flow through the culvert should be always maintained. Twenty culverts were used to conduct 4 × 5 factorial design (1 control and 3 treatments, each with 5 replications). The media combination shown by table 1.

2.2. Collection the ingredients

Water hyacinth collected from Jalan Baru Lake at Margonda site in Depok, Indonesia. The water temperature when the water hyacinth collected was 28 °C. Before the water hyacinth was used for fermentation, it was acclimated first. Pre-treatment was conducted by soaking water hyacinth in water at the laboratory for 5 days. This step aims to reduce heavy metal content or other compounds [7]. After that, Water hyacinths are chopped using a grinding machine to produce smaller pieces (about 0.5–1 cm). Chopping aims to optimize the process of degradation of water hyacinth fibers due to increase contact area between microorganism and fermentation substrat [8]. The mud was obtained from soil at the location where the water hyacinth was collected. The corncobs were obtained from corn waste in Bogor. The corncobs were also chopped using a grinding machine into smaller pieces (about 0.5–1 cm) to optimize the process of degradation or fermentation [8].

| Table 1. Media combination for sludge worms growth medium |
|---------------------------------------------------------|
| **Medium composition**                               |
| Treatment | Mud (%) | Corncob (%) | Water Hyacinth (%) |
| Control   | 100     | 0           | 0                  |
| Treatment I | 80    | 15          | 5                  |
| Treatment II | 80   | 10          | 10                 |
| Treatment III | 80   | 5           | 15                 |
2.3. Fermentation

The first step is preparation for fermented water hyacinth and corncobs. This consists of molasses, water, and probiotic treatment with Effective Microorganism (EM4). By giving the EM4 15% to the composition, it can have an effect such as increased activity of microorganism in the degradation organic matter [9]. Fermentation was done in a HDPE water drum with 60 L capacity. Fermentation converts water hyacinth and corncobs to become easily ingested by sludge worms. Moreover, the fermentation has been proven to be effective in the increase of the nutrient of culture media [10].

2.4. Media preparation

Proximate composition of water hyacinth was determined following standard given by Zhang et al. [11] and Oyemi et al. [12] (table 2 to table 3). Proximate composition of corncobs was determined following standard given by Deutschmann et al. [13] and Menon et al. [14] (table 4 to table 5).

### Table 2. Composition of water hyacinth organs

| Organs      | Contents (%) |
|-------------|--------------|
|             | Cellulose    | Hemicellulose | Lignin | Others       |
| Leaf        | 15.42 ± 0.08 | 29.75 ± 0.15 | 9.79 ± 0.06 | 45.04 ± 0.29 |
| Stem        | 17.14 ± 0.12 | 21.82 ± 0.06 | 8.01 ± 0.07 | 53.03 ± 0.25 |
| Whole Plant | 18.07 ± 0.20 | 28.21 ± 0.11 | 7.03 ± 0.09 | 46.69 ± 0.40 |

### Table 3. Composition of water hyacinth leaf protein concentrate

| Nutrient    | Concentrate (%) |
|-------------|-----------------|
| Crude protein | 56.38 ± 2.15    |
| Fat         | 4.11 ± 0.55     |
| Ash         | 4.88 ± 0.24     |
| Crude fiber | 1.02 ± 0.05     |
| Carbohydrate| 56.38 ± 1.55    |

### Table 4. Lignocellulosic concentration of corncob

| Content (%) |
|-------------|
| Cellulose   | Hemicellulose | Lignin |
| 45 % to 55 %| 25 % to 35 %  | 20 % to 30 % |

### Table 5. Nutrient from 9 Mg ha⁻¹ corncob

| Nutrient        | Concentrate (%) |
|-----------------|-----------------|
| Dry matter, Mg ha⁻¹ | 1.8             |
| C, Mg ha⁻¹       | 0.8             |
| N, Mg ha⁻¹       | 7               |
| P, Mg ha⁻¹       | 0.5             |
| K, Mg ha⁻¹       | 21              |
2.5. **Collection of Tubifex spp.**
Wild sludge worms were collected from a fish farmer in Bumi Kepanduan Sentul (BKS), Bogor, West Java, Indonesia. The sludge worms were cleaned over 24 hours before inoculation into the culverts by using continuous water flow [15].

2.6. **Inoculation of sludge worms**
The sludge worms were inoculated at the rate 1.25 mg cm\(^{-2}\) using recirculation system. The water rate was observed every 10 days. The circulation system was supported by a water pump (DABAQUA Q 2007). The water flow rate was based on research from Ahmad et al. that is maintained by stop cork of pipes (PVC). Also, the periodic supply of culture media was started from the 10\(^{th}\) day with 10 days interval.

2.7. **Environmental data collection**
Sampling was completed after 30 days from inoculation. The worms are separated from its growth media. Water temperature of the culture was recorded with digital thermometer. Dissolved Oxygen (DO) and pH of water were determined using DO meters (Seven GoPro and MW 600 DO).

3. **Results and discussion**
The result showed that on the first day, the environmental parameters obtained from water hyacinth and corncobs fermented under 3 treatments and 1 control were as follows (table 6).

Table 6 showed that water temperature in the cultivation has a narrow range, 27.2–27.5 °C. Meanwhile in Situ Agathis where the sludge worm was also found in nature, the water temperature (measured at 2.30 pm) is about 28.7–29.8 °C (table 7). So the water temperature of cultivation and nature is quite different. For the air temperature, it is always controlled in the system with the help of the rooftop to protect the sludge worm cultivation from the over light and high intensivity of rain.

The pH medium for cultivation also has a narrow range, about 6.7–6.8. Meanwhile in Situ Agathis where the sludge worm found also in nature, the pH water in nature (measured at 2.30 pm) is about 6.94–8.50. So, the medium pH for cultivation is more acid than the water pH in nature. The value of dissolved oxygen (DO) in water for cultivation is much lower than the DO value in natural water where the sludge worm is naturally found. It is about 1.8–3.4 ppm whereas the DO value in nature can reach 9.6 ppm.

The results of water hyacinth and corncob fermentation with EM4 starter in treatment-I, treatment-II, and treatment-III show a watery structure condition, with brownish color, and a slightly sour aroma typical of fermentation. Fermentation has been proven to be effective in the increase of the nutrient of culture media through the process of hydrolysis, acidogenesis, and amylolysis. Macromolecules in organic matter were transformed into micromolecules such as glucose, monosaccharides, and amino acids,. This method is useful to produce nutritious growth medium for sludge worms cultivation [10]. With fermentation technology, the utilization of cheap materials

| Table 6. Environmental parameters obtained from the fermented water hyacinth and corncobs. |
|---------------------------------|-----------------|-----------------|----|--------|
| Environmental variables in culture | Treatment | Air temperature (°C) | Water temperature (°C) | pH | DO (ppm) |
|---------------------------------|-----------|-----------------|-----------------|----|--------|
| Control                         | 30 ± 31  | 28.2            | 7.6             | 5.8|
| Treatment I                     | 30 ± 31  | 27.2            | 6.7             | 3.4|
| Treatment II                    | 30 ± 31  | 27.3            | 6.8             | 1.8|
| Treatment III                   | 30 ± 31  | 27.5            | 6.7             | 3.4|
Table 7. Environmental parameters in Situ Agathis UI.

| Coordinate          | Air temperature | Water temperature | pH Water | DO (ppm) |
|---------------------|-----------------|-------------------|---------|---------|
| 6°22'6" S          | 31 °C           | 29.1 °C           | 7.14    | 6.88    |
| 106°49'30" E       |                 |                   |         |         |
| 6°22'11" S         | 31 °C           | 29.8 °C           | 6.94    | 6.72    |
| 106°49'26" T       |                 |                   |         |         |
| 6°22.135' S        | 31 °C           | 28.7 °C           | 7.96    | 3.93    |
| 106°49.493 E       |                 |                   |         |         |
| 6°22'2" U          | 31 °C           | 29.5 °C           | 8.07    | 9.60    |
| 106°49'31" T       |                 |                   |         |         |
| -6.3709742,        | 31 °C           | 29 °C            | 8.50    | 9.26    |
| 106.8258381        |                 |                   |         |         |

like corn-cob and water hyacinth would go a long way in reversing the increasing feed costs and help in pollution and health hazards arising in the environment. Corn cob and water hyacinth containing cellulose which are not directly utilizable by sludge worms, could serve as renewable raw materials with the fermentation technology [16]. In addition, the use of water hyacinth as a feed ingredient has been investigated before. Liang et al. found that the addition of water hyacinth meal to vitamin-free channel can significantly improve fish growth and survival [17]. Similarly, Niamat et al reported that water hyacinth leaf meal (25 % CP) represented a possible source of cheap plant protein for silurid fish [18]. The WHLPC result from Adeyemi et al. showed water hyacinth contain essential amino acids. The data suggest water hyacinth can used as source of vegetable protein for supplement aquafeed [19].

Fermentation often produces slightly acidic product, so we can understand why the pH water in sludge worms cultivation is lower than the pH at nature conditions [10]. In this experiment, all of the treatment still have a good pH result. This is based on the facts that the pH range of the environment in which the optimum metabolism and respiration of sludge worm is at pH 6–8. But this condition is maximum only at pH 8, then decreased when the pH rises further [20]. So to optimize the growth of sludge worms and get the high quality sludge worms for fish cultivation, the pH for the sludge cultivation should be maintained at this range. It will be good if the sludge worm cultivation is maintained at neutral pH or pH values approaching the alkaline range were beneficial for Tubificidae.

Fermentation is also known as any process that involves the activity of microorganisms [21]. So, where fermentation occurs, there will also be O2 consumption for the metabolism of the microorganisms. This activity naturally results in low concentrations of O2 in the environment and substrate [10]. The low O2 concentration is showed by the low DO value in the water for sludge worms cultivation. The respiration rate of species belonging to Tubificidae increased when the DO concentration was about 1.5–6.5 ppm. The optimal DO value for the maximum respiration rate is when DO concentrate was at 4.5 ppm. Actually species belong to Tubificidae are adapted to live in habitats with very low oxygen content, some of them (e.g. Tubifex tubifex, Lumbriculus variegatus) are able to survive without oxygen for a long time (up to several weeks). The worms can survive in environment with low oxygen concentration by waving hemoglobin-rich tail ends to exploit all available oxygen and can exchange carbon dioxide and oxygen through their thin skins, similar like the frogs. It is important to ensure the system of sludge worms cultivation has optimum environmental conditions for growth, so the DO value in all treatment must be increased to the optimum range in the next experiments [18, 22].

Another environmental variable obtained from this experiment is the temperature of the sludge worm cultivation water. Temperature is an important factor for aquatic organisms such as Tubificidae as it affects their respiration, growth and reproduction. Lou et al. have stated in their experiment that sludge worm perform respiration very well at 22 °C and have tolerance temperature at 20–30 °C [20].
Therefore, if cultivation is carried out at higher than 30 °C, the cultivation process will not give optimal results [18].

4. Conclusion
The environmental variables of sludge worm cultivation have been investigated and compared to the environmental variables in their natural habitat. It was compared too with the literature about the relation between their common life function with their environment parametrical points. Treatment I and II have the environmental variables that match with the sludge worms natural environment. In the future, sludge worms biomass calculation will have to be conducted.

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