Earthworm as eco treatment tool for removing organic matter pollutant: A synergy between *L. rubellus* vermiculture farmer and traditional tofu maker in the effort for making zero waste in tofu production

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Abstract: Earthworms has been chosen as eco tool treatment since their ability to remove organic matter in waste has been reported everywhere. Due to high content of organic matter in tofu liquid waste and relative safe from pesticides and heavy metals contaminant and because of tofu makers release in water bodies without treating, therefore we are interested to study this liquid waste as an alternative food for feeding earthworm. *Lumbricus rubellus* is rich available in Indonesia and only view studies use this species for dealing with wastewater treatment. *L rubellus* culture is potential for feeding animal and curing some diseases of human. Every week tofu liquid waste was taken for testing its pH, turbidity, CODcr and BOD_5. After third week of studying there was increasing the population and bodies weight of 32% and 7.5% respectively. High efficiency removal of turbidity, CODcr and BOD_5 were obtained in reactor that inhabited by *L rubellus* rather than without having *L rubellus*. Study shown that *L rubellus* grows faster in tofu liquid waste rather than another slurry by breaking down high concentration of organic matter in its digestive pathway use their enzymes. *L rubellus* produces a large number of cocoons, juveniles and granules cast appeared on the top layer of bed reactor which contains a number of organic matter eater-organism and attached as well as growth on the surface of aggregate gravels. There is possibility created between *L rubellus* culture farmer and tofu makers for taking mutual benefit in term of economic point of view.

Key words: *L. rubellus, tofu liquid waste, eco-treatment tool, organic matter, microorganism.*

Introduction

Tofu is made by coagulating soy milk to create curds. The curds are then pressed and compacted into the gelatinous white blocks recognized as tofu. It is naturally gluten-free and low in calories. It contains no cholesterol and is an excellent source of iron and calcium.

It also contains isoflavones such as phytoestrogens, isoflavones may have both estrogen-agonist or estrogen-antagonist properties which is good for protecting some diseases. It is an important source of protein especially for vegans and vegetarians (Zhang et al. 2018; Megan Ware, RDN 2017; Alamu et al. 2017). Nonetheless if over-consumption of soybean products may also present some risks such as hormonal disturbances, carcinogenic and organotoxic of soy protein based on the clinical and experimental studies (Sukalingam K, Ganesan K, Das S 2015) and created worse memory of elderly in China and Indonesia had been reported (Hogervorst et al. 2008; Xu et al. 2015). Tofu used in numerous culinary dishes as protein bioavailability has been increasingly and easily obtain in almost all of traditional and modern market. However, the consequence of the tofu production causing a greenhouse gas emission (carbon foot print), grey water footprint which has been calculated throughout of cultivation, harvesting and production theoretically. For 1 kg of packed tofu, 16% CO_2e resulted from soybean production, 52% from tofu manufacturing, 23% from packaging and 9% from transportation referred to carbon footprint(Mejia et al. 2018) and for 1 kg of soybean curd product (Tofu) in Bandung-Indonesia requires 2.154 M^3 of fresh water referred to water footprint(Haidir and Sudrajat 2014). Tofu makers in Indonesia still use traditional production for maintaining the texture and sensory quality of tofu from one generation to another generation. However, this process is considered to be inefficient not in term of large fresh water uses only but also the negative impact of environment since tofu makers have discharged large amount of liquid waste into sewer system. Studies shown that concentration level of BOD_5 and CODcr was varies in the range of 5.981 – 6.525 mg/L for CODcr and 2.900 mg/L for BOD_5 (Oktariany and Kartohardjono 2018) and also 6.000 – 8.000 mg/L for BOD5 and 7.500 – 14.000 mg/L for CODcr has been reported(Faisal et al. 2015). Due to high concentration of organic matter in tofu liquid waste, researchers put a hope for studying about biogas, however tofu liquid waste could not be used directly as biogas substrate due to high nitrogen content which was not suitable to methanogen microorganism on biogas digester(Rahmat, Hartoyo, and Sunarya 2014). For controlling ratio carbon-nitrogen in tofu liquid waste is not easy for operator and also the investment for biogas process is considered not feasible for traditional tofu makers presently. Therefore, to reduce high level of BOD_5 and CODcr in tofu liquid waste, we use eco-treatment tool for removing organic matter in laboratory scale. In this study earthworm was chosen since their ability for removing organic matter has been reported everywhere(Nie et al. 2015; Kumar and Ghosh 2019; Sinha, Bharambe, and Chaudhari 2008) and tofu liquid waste used as alternative food for feeding earthworms instead of cow dung which usually used in vermiculture in Indonesia. By doing so, all process of tofu production will be relative zero waste (fig. 1) and the treated water can be reused for the process of production. Earthworm species *Lumbricus rubellus* has chosen due to rich available in Indonesia and only view of study use this species for dealing with wastewater treatment. The growth of organic matter eater-microorganisms on aggregate gravels in column reactor and removal efficiency of organic matter pollutant are studied as well. We would like to understand a suitable environmental condition relates to the healthy growth of *L rubellus* if use tofu liquid waste as food due to there is demand of healthy and uncontaminated earthworms which is used as protein sources and additive feed for aquaculture and poultry (Byambas et al. 2019; Edy Parwanto et al.

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2016; Julendra, Zuprizal, and Supadmo 2010; Damayanti et al. 2008). It is also used as raw material of some kind of medicine(Septianda, Debora, and Rochmanti 2012; Reynolds, Wilma, and Reynolds 2017; Cooper, Hirabayashi, and Balamurugan 2012; Shen 2010; Mihara et al. 1991).

Materials and Methods

Materials

Tofu liquid waste

Tofu's liquid waste was collected from central of Tofu makers Cibuntu at Bandung City, West Java. Indonesia. Traditional tofu makers use the procedure for production of tofu as shown in figure 1.

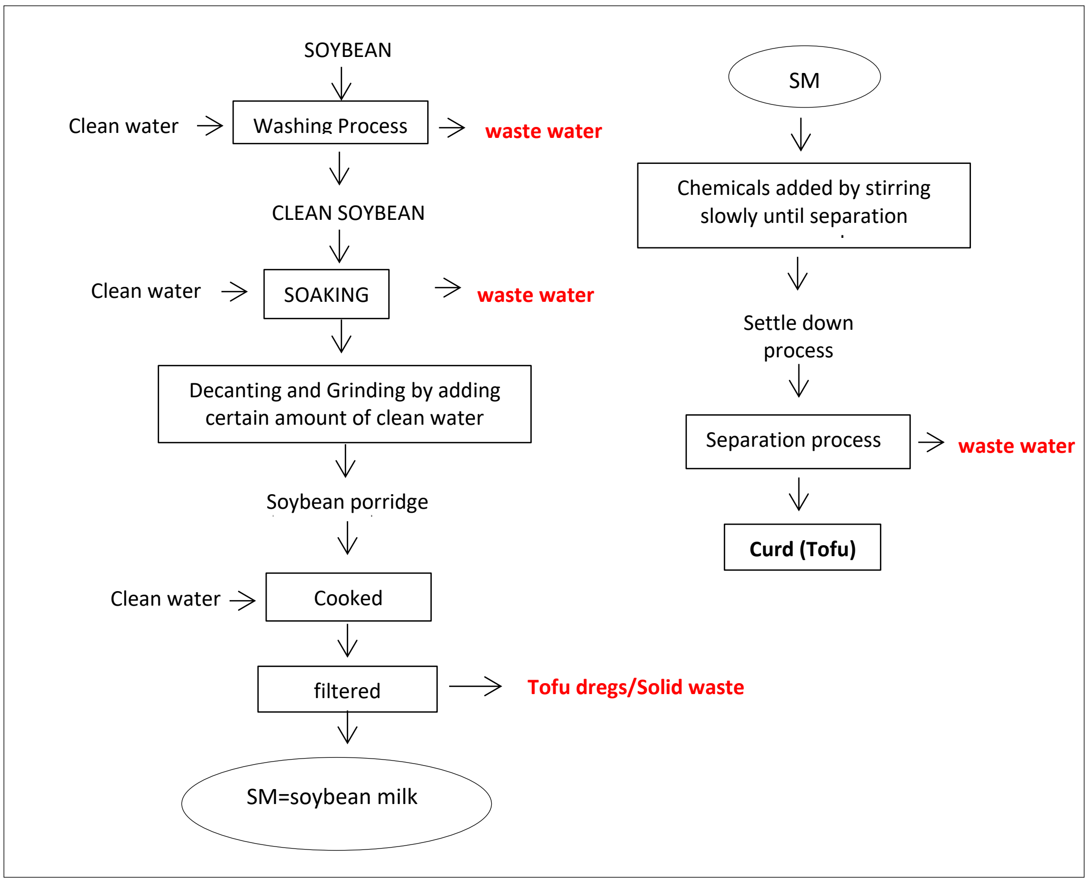

Figure.1. Flow chart of Tofu making

20 liters of tofu liquid waste randomly and well mixed was taken and put into the plastic drum and bring to Laboratory and then transferred to 7 litter of plastic bucket (Fig.3) which was equipped small capacity aerator to blow air into this tofu's liquid waste in order to avoid foul odor. Study was conducted at ambient temperature around 25-27 ^{0}C. Sampling was done twice a week for observing the fluctuation of effluent waste during three months and the characteristic of tofu liquid waste (table 1) does not change as has been

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done before in previous study(Persulessy A E, Rosmalina RT, Hartati E 2020). Triplicates batch experiment has been performed and the data was calculated as average and shown in this research.

Tabel 1. Characteristic of Tofu liquid waste

C	Parameter	unit	Concentration range
1	pH	-	4 – 5
2	Turbidity	NTU	1.206 – 1.600
3	CODcr	Mg/L	5.000 – 8.000
4	BOD5	mg/L	5.400 – 7.700

Earthworms species *lumbricus rubellus*

Earthworms were collected from the local vermiculture farmer. A little amount of origin cow dung-soil mixture together with earthworms were transferred to laboratory as shown in fig 2. This study used 40 adult earthworms (approx. 20 grams) with length average 10 cm in 0.002 m^3 of cow dung-soil mixture bed exist in the reactor A. Cow dung-soil mixture with the same amount was transferred into reactor B without having earthworms. These earthworms were provided 5 days for acclimatization at new environment while trickling of tofu's liquid waste passed through entire of media layer slowly for acclimatization before the effluent was collected for analyzing.

Figure 2. *Lumbricus rubellus* earthworms in the West Java, Indonesia

Setting up a column reactor

Two identical column-type reactors were made of transparent acrylic and equipped with four drain valves (sampling ports) of each reactor, with rectangular shape of 10 x 10 x 100 cm were used. To protect worms from predators and to avoid a direct contact with sunlight, reactors were monitored every day. Material support in this column reactors consists of four layers with different size of aggregate gravels as shown in Fig.3.

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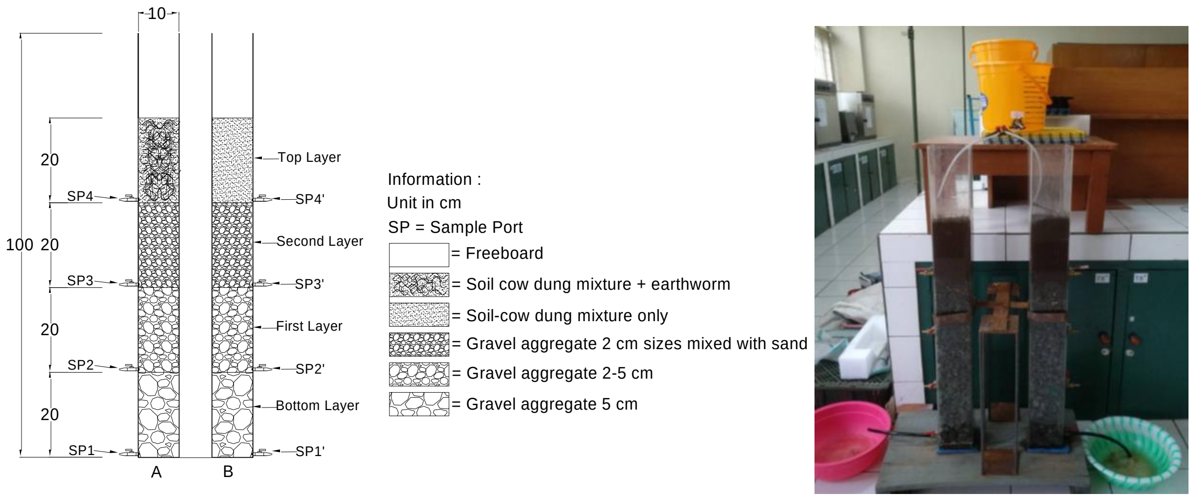

Fig 3. Two identical column-type reactors

Two sprinkler system used for trickling tofu liquid waste that allowed uniform distribution on bed material (top layer) of both reactors A and B as control. Tofu liquid waste from the drums above these reactors flowed through flexible plastic hose by gravity. Tofu liquid waste percolated down through aggregate gravels of two reactors A and B. All valves of sampling ports were closed when tofu liquid waste flows into both of reactor until this liquid waste goes up to second layer or below of top layer. Furthermore, the valve of bottom layer (sampling ports P1and P1') plays as the effluent control to keep the flow rate of influent and effluent on these two reactors without letting the beds become flood with tofu liquid waste. Of P1, P1' and P4, P4' treated tofu liquid waste was collected and analyzed for BOD, COD, pH, turbidity, and visual observation on support materials (aggregate gravels) relates to growth of organic matter eater-microorganism was performed.

HRT and HLR

HRT (hydraulic retention time) is an actual time has been spent by earthworms with tofu liquid waste to retrieve organic matter (turbidity, CODcr and BOD_5) from it as food. In this study tofu liquid waste was flowed into reactor A and B was 4 ml/minute of each with porosity of entire media was 18% and HRT for each reactor was 1 hour and 30 minutes and kept constant during study was taking place. Hydraulic loading rate (HLR) depends upon the number of live adult earthworms functioning per unit area in bed reactor. In this study, HLR was 0.024 m^3/m^2.hour as have done in previous study(Persulessy A E, Rosmalina RT, Hartati E 2020).

Methods

Tofu liquid waste which has been treated through column reactor was analyzed for pH, turbidity, chemical oxygen demand and biological oxygen demand by following the Indonesia Standard which were SNI 06-6989.11-2004 for pH, SNI 06-6989.25-2005 for turbidity, SNI 6989.73:2009 for CODcr and SNI 6989.72:2009 for BOD_5. After completion of experiment, support material of each layer was taken to be analyzed under the microscope for microorganism growth observation.

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Results and discussion

In situ observation

Daily observation has been done for reactors (A and B). Symptoms like foul odor, percolation of tofu liquid waste through the bed reactors (top layers), appearance of top layer, second layer, first layer and bottom layer were observed. Direct sunlight exposure to earthworm was not good and also if no sunlight at all caused earthworm dead within 7-10 days. Moderate sunlight emission will be good for this earthworm to maintain healthy life. No choked occurred and smooth percolation of liquid waste. After 5 days acclimation no cocoons appeared however at one week after acclimation and the beginning of second week, copulation of earthworm occurred by ejecting many of "cocoon" attached on the wall of reactor A and the color was whiteness. This was indicated that earthworm fertilize the eggs. At third week there was a bit fault odor detected in SP2, SP2' and SP3, SP3' (sampling ports) when we did release of this liquid waste of both reactors. The possibility came from very less dissolved oxygen in these layers or the anoxic condition had been taking place. At reactor A where earthworms were living, seems the agility and movement of earthworm quite good and after completion this experiment at third week, we found the number of earthworms become average 53 worms of three batch experiment so there were another 13 young worms with the length average 6 cm or showed 32% population increased and some juveniles appeared in top layer of reactor. We did not calculate a number of juveniles due to they did not show their significant role for removing organic matter. After separating these 13 young worms, we weigh of 40 adult worms to found out total body weight and surprisingly there was increase of body weigh from 20 grams becomes 23 grams or average 7.5% of each body weigh increased after 3 weeks staying in reactor A. Meanwhile feeding worms with swine manure shown there was 30% population increased during 4 weeks experiment by using *Eisenia Andrei* species as has been reported(Li et al. 2008). This was indicated that tofu liquid waste is good food for feeding earthworm because of high organic matter and some minerals in it and no interfere heavy metals during production of Tofu. Temperature, lighting and moisture also quite support for this study. No lighting exists during this experiment and only lighting and fresh air comes from windows in laboratory in day time.

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Microscopic Observation

Top layer 1st layer 2nd layer bottom layer

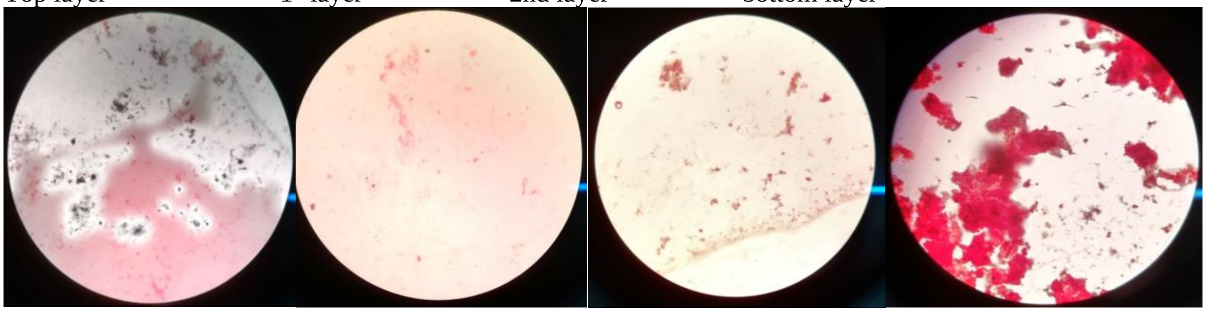

Fig. 4. Microscope photograph of each layer on reactor A

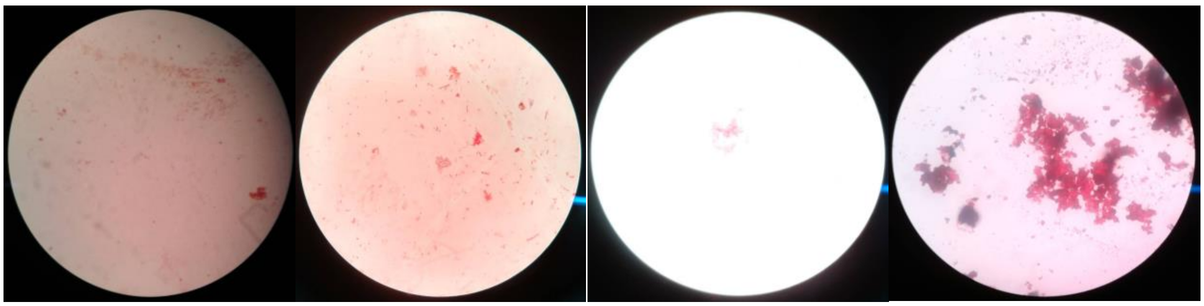

Fig. 5. Microscope photograph of each layer on reactor B

After treating tofu liquid waste completely, randomly aggregate gravel of each layer were taken for microscope observation with magnification 1000x. Results showed that all support materials were covered by basil and coccus microorganisms types in filament that consisted of *staphylo, strepto, diplo* and mono configurations on reactor A and B. Growth of microorganisms occurred more density on reactor A rather than reactor B. It showed that earthworms were release some microorganisms aerobic and anaerobic from casts that formed a colony of microorganisms on the surface of each aggregate gravels (Fig 4). According to Parle (1963) filamentous fungi and yeasts increased in number rapidly after the cast was produced(Parle 1963a). Microorganism in reactor B comes from tofu liquid waste as suspended phase which passed through aggregate gravels and also from cow dung-soil mixture taken from vermiculture farm as control (Fig 5). A number of studies were mainly focused in the earthworm guts and casts with relation to kind of diets and earthworm species(Parle 1963b; Gómez-Brandón et al. 2011; Gómez-Brandón, Lores, and Domínguez 2012; Aira et al. 2016; Knapp et al. 2009; Schönholzer, Hahn, and Zeyer 1999). Based on the DNA extracted was taken from substrates, the earthworms' gut contents and casts revealed that the gut and cast microbiota was strongly influenced by the food source ingested(Knapp et al. 2009). Another study reported that cocoons microbiome of the earthworms *Eisinia Andrei* and *E. fetida* were dominated by three vertically-transmitted symbionts, *Microbacteriaceae*, *Verminephrobacter* and *ca. Nephrothrix* and both ended showed high rate of sequence variation. This study was mentioned that during cocoons formation, cocoons are colonized with bacteria from its hosts and environment(Aira, Pérez-Losada, and Domínguez 2018). On the bottom layers (SP1 and SP1') of two reactors showed the excellent growth of microorganisms if compared to the top layers of two reactors (SP4 and SP4'). This was anaerobic microorganisms settled in the bottom of aggregate gravel and took

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action for eating organic matter. On the top layers (SP4 and SP4') of two reactors were aerobic microorganism which was dominant and on first layer and second layer (SP2, SP2', SP3 and SP3') were possible anoxic condition. Study showed that aerobic and anaerobic bacteria also presence in earthworm gut and casts(Alauzet et al. 2001) whilst has been mentioned also that microbes growth under both aerobic and anaerobic conditions more abundant in the earthworm intestine than in the beech forest soil from which the worms were obtained(Karsten and Drake 1995). During progress through digestive system in earthworm intestine there is a dramatic increase in numbers of micro-organisms of up to 1000 times have been reported(Edwards and Fletcher 1988). It shown that earthworms acted as biomagnification microorganisms in its intestine and then release in the casts form contains microorganism for helping maintain the existence of microorganisms in reactor. The length of intestine approximately 90% of whole *L rubellus* earthworm body that allows to absorb many organic and inorganic minerals in their diet and released through casts. Recent study regarding to C^{13} tracer showed that more than 85% mineral-associated organic matter (MOM) presence in casts(Vidal et al. 2019). The main diet for earthworm was organic and only a view microorganism like protozoans, Actinomycete, bacteria and fungi have been found to be part of earthworm diet(Byzov et al. 2007). Microbial presence in intestine are bacterial, fungal and actinomycetic populations were obtained in three species of earthworm; *Lumbricus terrestress*, *Allolobophora caliginosa* and *A. Longa*(Parle 1963b). Such genus *Bacillus*, *Aeromonas*, *Pseudomonas*, *Flavobacterium*, *Nocardia*, *Gordonia*, *Vibrio*, *Clostridium*, *Proteus*, *Serratia*, *Mycobacterium*, *Klebsiella*, *Azotobacterial*, and *Entorobacter* have been reported in the digestive tract of earthworm(Oligochaeta)(Hortensia and David 2009). Meanwhile in intestine of *L rubellus* earthworm was found *Bacillus* megatarium(Fischer et al. 1997), *Acidobacteria*, *Paenibacillus*, *Pseudomonas sp*., as well as *Actinobacteria* have been reported (Singleton et al. 2003). This study clearly showed that there was difference between aggregate gravels at reactor A and B in term of the density of microorganisms during observation. Source of microorganisms from earthworms' casts, cow dung-soil mixture and from suspended tofu liquid waste. *L rubellus* has casts size bigger than *Eisenia hortensis*(Canti and Piearce 2003) that means more microorganisms released into reactor. These microorganisms were attached on the surface of aggregate gravel and develop biofilms; some are growth and some detached on the surface of aggregate gravel. Physical, biologic and chemical processes determine biofilm formation. Organic matter which was transported to surface by molecular diffusion and forming a conditioning layer which will influence subsequence adsorption and attraction of microorganism to the surface of aggregate gravel. This process can be monitored clearly by using digital time lapse microscopy imaging (DTLM)(Lappin-Scott and Bass 2001).

pH of treated tofu liquid waste

According to Curry, earthworms are absent in very acid soils (pH<3.5) and are sparse in soils with pH below 4.5(Räty and Huhta 2003). However, in this study earthworms still alive in the extreme pH when acclimatization for first time experiment took place where pH of tofu liquid waste range between 4-5(table 1) passed through reactor A until third week of study. It was indicated that *L.rubbelus* be able

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to adapt in this condition in three consecutive study. Treated tofu liquid waste that passed through reactors was taken from bottom sampling port reactors and also from the top sampling port of A and B reactors every week after acclimatization for pH testing, turbidity, CODcr and BOD5.

Tabel 2. pH value of treated tofu liquid waste in both reactors

Week	**pHvalue before treating**	**pH value of treated tofu's liquid waste**			
		Reactor A		**Reactor B**	
		SP1	**SP4**	**SP1'**	**SP4'**
0	4,5	7.6	6.7	7.2	5.8
1st	4,5	7.1	5.6	5.7	6.6
2nd	4,7	7.1	6.2	5.6	6.6
3th	4,5	7.4	5.3	7.3	5.0

Note: SP1, SP4; SP1', SP4' are sampling ports of both reactors

In general, pH value of treated tofu liquid waste has changed from acidic became neutral in sampling port P1(Tabel.2) and interestingly pH on the top of reactor A become neutral followed by each layer where inhabits of earthworms. *L rubellus* earthworm most perhaps of release some granules contains calcium carbonate are known to be produced by the calciferous glands of earthworms through its casts(García-Montero et al. 2013; Gago-Duport et al. 2008; Canti and Piearce 2003; Crang, Holsen, and Hitt 1968; Versteegh, Black, and Hodson 2014) and also from excreta likes mucus and urine that plays important role for increasing and stabilizing of pH environment to be neutral and slightly basic. In calcic mull condition the density of earthworm higher than acidic mull(Salmon 2001) and has been proven that in this study the population and weight increased by 32% and 7.5% respectively. A large source of calcium intake by earthworm might come from tofu liquid waste where in production process was added one of calcium chloride or calcium sulfate or calcium lactate as coagulant in certain amount for determining the product texture, flavor and yield (Leiva, Rodríguez, and Muñoz 2011; Jayasena, Tah, and Nasar-Abbas 2014; Prabhakaran, Perera, and Valiyaveettil 2006; Shih, Hou, and Chang 1997; Zhang et al. 2018). Furthermore, this coagulant was absorbed by glands and release it in granules form as casts. Casts contains mineral nitrogen levels were greater than those of soil; the major part of the inorganic nitrogen occurred as ammonia which was rapidly converted to nitrate(Parle 1963a). Several studies also described another role of earthworms which works for nitrogen fixation and phosphorous solubilizing of composting process (Hoang et al. 2016; Aira, Monroy, and Domínguez 2007). Study has mentioned also that earthworm casts have been proven experimentally to have a higher acid–base buffering capacity in comparison with that of the initial soil(Kul'bachko et al. 2015; Didur et al. 2019). pH environment in this reactor might was regulated by *L rubellus*, since many reports have proven that during vermifiltration process pH environment changed become neutral, even though in these reports do not show the calcium

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compound content presence in their liquid waste. Other possibility since earthworm release urine which contains urea-ammonium compound which will increase pH value. This mechanism of pH changing still remain challenge for researchers. The role of buffer compound present in the reactor which has maintained to be stable in range 6-8 also another challenge to know its mechanism. Apparently at pH neutral as shown at SP1; earthworms can be a trigger for accelerating the microbe activities by enhancing the population of microorganisms which were spread out on the surface of aggregate gravels as shown in fig 4. This pH was good condition for organic matter eater-microorganism and the possibility was the neutralophiles microorganisms in range pH 5.5–9(Krulwich, Sachs, and Padan 2011) which growth onto the surface of aggregate gravels. However, in fig 5 does not shows excellent growth of microorganisms since earthworms absent in these aggregate gravels and only comes from remaining cow dung soil mixture also got from tofu liquid waste in suspended form.

Organic Matter removal

In general, efficiency removal of turbidity was not showing the same pattern between reactor A and B however both reactors showed the efficiency removal was increase versus time. At third week the efficiency removal was 99.3 % and 99.2% obtained on reactor A at sampling port (SP1) and sampling port (SP4) respectively. There was anomaly happened at (SP1') at reactor B which showed efficiency removal of turbidity was only 45.8% and in contrary showed at SP4' where efficiency removal of turbidity could be achieved 98%. High efficiency removal of turbidity most possibly comes from earthworms which digested this suspended particle and released a large number of bacteria through casts. Earthworms also improve the total specific surface area of aggerate gravel, by enhancing the ability to adsorb organic and inorganic substances from wastewater(Sinha et al. 2010).These microorganisms spread out not only on the top layer but penetrated until the bottom of reactor A. Created microorganism colony on the surface of aggregate gravels and attached on it. When tofu liquid waste passed through this media, suspended particles was slightly endured due to some physical force between tofu liquid waste (liquid phase) and aggregate gravel (solid phase) and influenced by HRT. It will provide the opportunities for organic matter eater-microorganisms to take action by eating this suspended particle as organic matter. HRT also quite important to determine efficiency removal of organic matter. If HRT is increased, the efficiency removal become increase(Sinha, Bharambe, and Chaudhari 2008; Singh, Bhunia, and Dash 2019).

Tabel 3. Type of waste and its removal efficiency of organic matter with different of HRT

Earthworm species	Type of waste	HRT (hours)	Initial parameter (mg/L) only pH unitless	pH	Turbidity removal (%)	BOD5 removal (%)	CODcr removal (%)	References
Earthworms	Dairy industry	6-10	pH:3.64-3.83 Turbidity:3.420-3.475 COD:>15.000 BOD:103.000-136.000	6.52-7.45	99.21-99.3	98.98-99.49	80-90	(Sinha and Bharambe 2006)
E. fetida, P. excavatus, and	Sewage	1-2		7-7.4			45	

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E. euginae			pH: 5-6		97-98			(Sinha,
			Turbidity:70-120					Bharambe, and
			COD:254-293			98		Chaudhari
Eisenia fetida	Sewage	2	BOD:260-328					2008)
				+/- 7			70	
			pH:6.45		98			
			Turbidity:336-386					
			COD:121-280			98		(Manyuchi,
L.rubellus	Gelatine	1	BOD:190-405					Kadzungura,
				7.02-				and Boka 2013)
			pH:6.22-6.30	7.12	-		89.9-90.3	
			Turbidity:			88.7-89.8		
			COD:1.818-1.845					(Ghatnekar et
Eisenia fetida	Domestic	-	BOD:433-457.5					al. 2010)
							55-66	
			pH:7.52-7.73	-		47-65		
			COD:59-78,3					
			BOD:29,6-38,4					(Meiyan,
Eudrilus	Urban	-					68-93	Xiaowei, and
Eugeniae	Grey					93-98		Jian 2010)
	water		COD:386-3584					
			BOD:350-3000					
	Tofu	1.5			99			(Ndiaye et al.
L.rubellus	liquid		pH:4-5	7.1-7.6		97	96	2019)
			Turbidity:1.206-					
			1.600					
			COD:5.000-8.000					
			BOD:5.400-7.700					This study

Table 3 shows the efficiency removal of organic matter related to several waste that has been treated by using some species of earthworms. At reactor B the efficiency removal of turbidity was inconsistency started from first week until third week of study. The effect of efficiency removal at reactor B, only come from cow dung-soil mixture microorganisms on top layer which was attached on the surface of aggregate gravels and some from microorganisms that inhabits tofu liquid waste which was suspended during passed through the reactor. The fluctuation of efficiency removal each week, might be possible due to complexity of interaction between microorganism and environment in reactors and relates to presence or absent of oxygen as described on microscope observation. Whereas CODcr and BOD_5 showed a similar pattern of removal efficiency in reactor A (SP1 and SP4) and also at (SP1' and SP4') in reactor B (Graph 1).

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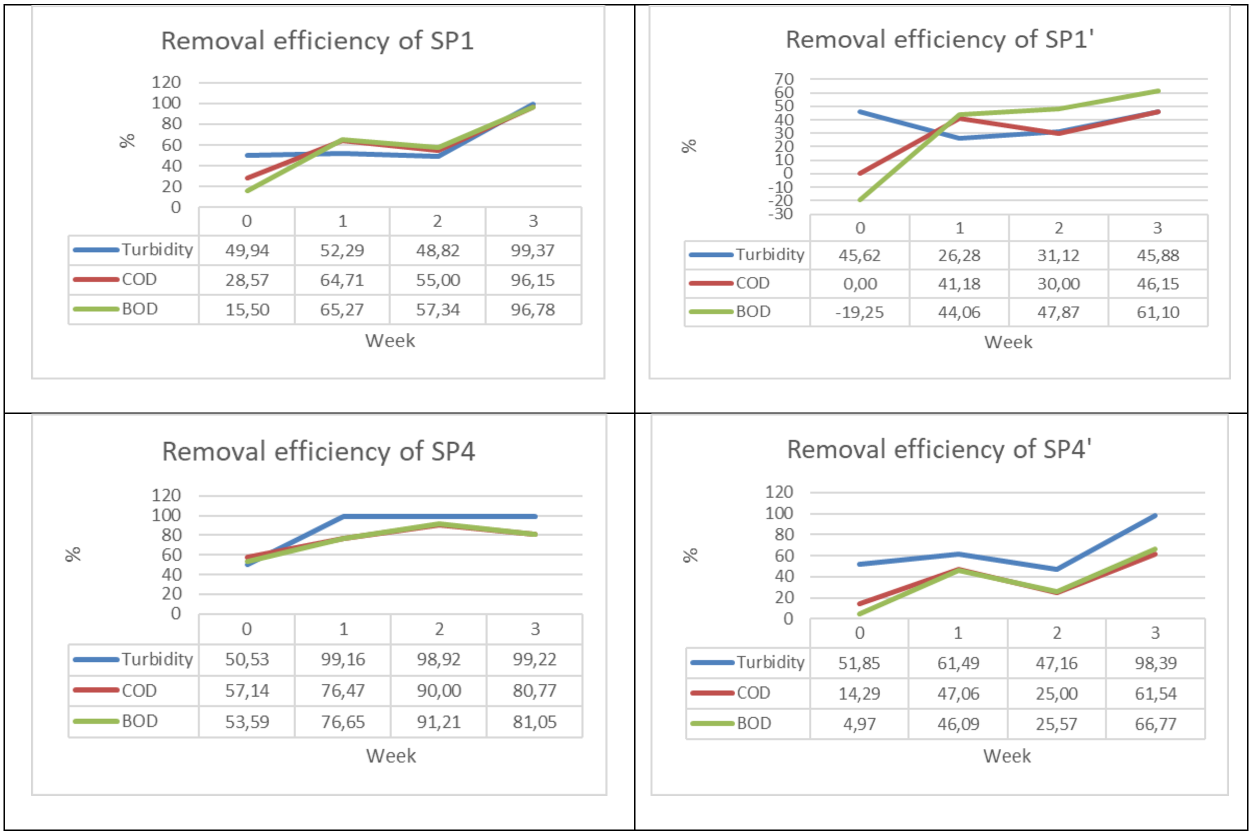

Graph 1. Organic Matter Removal of treated tofu liquid waste

Graphs of efficiency removal seems overlap between CODcr and BOD_5 and showed that there was a consistency of organic matter removal versus time. CODcr and BOD_5 at SP1 was 96% and 97% respectively and at SP4 was around 81% at third week of study whereas at SP1' for CODcr and BOD_5 46% and 61% respectively. CODcr and BOD_5 at SP4' were 61% and 64% more less the same with those in SP1'. Fluctuation of efficiency removal every week might be caused also by the age of casts and the bacteria formed resting stages for first and second week of study in reactor A. In the end of second week and beginning of third week there were young earthworms participated for digesting organic matter resulting the efficiency removal was high. High percentage of organic matter removal (turbidity, BOD_5 and CODcr) mainly mediated by microorganisms in the intestine of earthworms. The process of intake organic matter through its body wall(Sinha, Bharambe, and Chaudhari 2008) and break down through intestine which contains a large number of enzymes in its digest tract(Aira, Monroy, and Domínguez 2007; Prabha et al. 2007). Study has demonstrated that abundance and enzymatic activities of earthworm had significant correlation with treatment efficiency in reactor(Meiyan, Xiaowei, and Jian 2010). It is certainly proven that the growth of earthworm dependent on kind of food, environment condition and also type of species as has mentioned that *L. rubellus* has high protein and fat in its body rather than *L. terestris* and *P. excavates* and potential to be used as protein source for feeding animal (Damayanti, Sofyan, and Julendra 2008). It also indicated that *L rubbelus* might be absorbed and digested organic matter bigger that another species since has proven that its casts bigger than another species as described before.

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Conclusion

Study has indicated that *L rubellus* grows faster when feeding with tofu liquid in pH range 4-5. Body weight and population of *L rubellus* increased in room temperature. *L rubellus* can regulate pH become neutral in reactor to support the growth of organic matter eater-organisms. *L rubellus* also magnification microorganism in its intestine for removing high concentration of organic matter and then releases casts contains microorganism for removing organic matter in reactor. High removal efficiency of organic matter in tofu liquid waste ensure whole tofu production process become valuable especially for its liquid waste. Tofu liquid waste can be introduced as alternative food for feeding *L rubellus* in vermiculture. Several of supports material in reactor become our concern for future study, relates to growth of organic matter eater-organisms.

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