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Maintaining Favourable Fermentation Conditions in Sargassum Liquid Fertilizer Production using Siphon in A Two-Containers System Resulting in Acceptable Levels of Heavy Metals

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Abstract. A U-shapedsiphon was used to connect two 600 mL disposed of mineral water-plastic bottles called container **A** and **B**. Container **A** served as a decomposer drum for mixing*Sargassum* porridge, EM4 (effective microorganisms 4), organic nutrients, and water to allow an anaerobic fermentation to occur within 25 days.**B** was an aeration drum containing an acceptable volume of freshwater. Between these two containers, the siphon was set to bridge the air between containers above the suspension. There were 14 different configurations including a siphon with three different diameters (0.5, 1, 1.5 cm), four different lengths (16, 18, 20, 22 cm), and three different connections (connected to aeration drum, opened to air, closed). The last two configurations did not need the siphon. During the fermentation process, gas and heat were perceptibly released inone way-flowing from **A** to **B** and creating bubblesin **B**leading to a maintained condition of the fermentation. As the one of main challenging aspects in the production of sargassum liquid fertilizer (SFL) is the considerable number of heavy metals, therefore, we measured some transition metals levels extracted in the filtrate post-25 days to estimate the safety level of the product compared to those mentioned in the Regulation of the Indonesian Ministry of Agriculture (Permentan70/SR/140/10/2011). All data excluding sample S4 were below the limit, except Cd level, whereby two configurations gave the least values of potentially toxic elements, which might be linked to the role of the siphon in giving a favourable condition for preventing the release of the heavy metals trapped in the cell walls.

1. Introduction
In 2017, The Food and Agriculture Organization of The United Nations (FAO) identified the degraded quality of land and water as one of the serious threats in the security of agriculture and food

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1. Introduction

In 2017, The Food and Agriculture Organization of The United Nations (FAO) identified the degraded quality of land and water as one of the serious threats in the security of agriculture and food systems[1].Excessive usage of artificial fertilizer, nitrogen-rich fertilizer,for instance, has been linked to the direct and indirect detrimental effects to soil acidification and eutrophication in the aquatic environment because of the water-soluble nitric ions byproduct and aqueous nitrogen and phosphorous, respectively[2].Given the non-biodegradable nature of the synthetic fertilizer as the possible reason behind the trends, efforts in creating alternative biodegradable fertilizer have already been made to deal with not only those issues associatedwithfood and water security but also climate change as the majority of the inorganic fertilizer is most likely to rely on fossil fuel manufacture.The organic fraction of household wasteis one of the renewable raw materialsbesides other organic residues from post-harvest, livestock slaughtering, and food production [3]recognized as the environmentally friendly resource to tackle such long-term global circumstances. In contrast, according to Klinglmair and Thomsen [4], some drawbacks mainly related to the relatively high cost could potentially arise from the function of technology, handling processes, transportation, and maintenance that could hinder its application on small-scale household production. Indeed, addressing the above global issues should be started from the household level to induce awareness of future environmental sustainability.

With regards to the accessible resources, a micro-scale production of *Sargassum* liquid fertilizer (SFL) could be considered as a promising approach to meet the aforementioned purpose for those whose residential area is nearby a coastline like Banda Aceh and Aceh Besar.The coastal water is typically tropically characterized by temperature and pH of 25–30 ^{o}C and 5–10, respectively,that is suitable for the growth of*S. polycystum*CA Agardh, a brown macroalgae species with numerous nutrient contents.Previous screening of basic extract of the species revealed that this marine plant comprises a high level of essential transition metals such as Fe [5]. Some authorsreported that*S. polycystum*fertilizer obtained by either physical or biological methodsshowed beneficial effects onimproving the health, growth, and yield of organic plants linked to the remarkable level oforganic metabolites, trace elements, vitamins, minerals, growth plant hormones, and amino acids[6-8].Even though water-based extraction seems like the most efficient way to reveal bioactive, micro-, and macronutrients [9], microorganism-assisted SFL production *via* anaerobic fermentation has recently been confirmed to exert better advantages to stimulate plant germination and growth[10]. Simple installation and fewer resources, most importantly, make the fermentation method reasonably practicable.

The fermentation takes place within three stages: (1) initial mesophilic, (2) thermophilic, and (3) final mesophilic phase [11] whereby the biocatalysts encounter the highest temperature during the second stage.To keep optimized, the inhibitory fermentation factors such as overheat condition should be controlled by removing excess heat and overproduced CO_2 through an aeration hose into an additional coolant reservoir[12].As a result, desired temperature and humidity of 60 ^{o}C and 66% could be met. According to Freegah et al. [13], the ratio of length (l) to diameter (d) of U siphon-pipes connecting condenser and evaporator in their thermo-siphon studies played a crucial role in the heat exchange. Besides, CO_2 gas has been believed to act as a working fluid because of its ability to get denser readily in a presence of heat[14]. Thus, modifying both variables could be beneficial forfermentation-based SFL production.

As marine organisms living in saline water environmentscan accumulate minerals, brown algae arenot surprising to contain a broad variety of metal ions, including transition metals and heavy metals such as Cd, Au, and Hg, up to 200–500 times higher than terrestrial plants [15]. The intense interaction between algal surfaces and the environment might lead to adsorption of the cations through the pore into the negative site due to electrostatic attraction [16]. Apart from this route, an internal process called mineral uptake could be taken into account since their metabolic processes need to capture cations for electron transfer in their entire cellular system[17]. Moreover, these ions are also involved in the catalytic activities of various enzymes and oxidation-reduction reactions. As a result, these substances are likely trapped in the cell walls or even in their cytoplasm that can be readily released when the fermentation has undergone. Concerning the harmful aspects of such toxic elements for human beings and their surroundings is a serious challenge in SFL production[18].A previous

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study [19]in anaerobic fermentation of Sargassum in a digestion bottle connected to double bottles with neglected connectors dimension failed to report heavy metals (As, Cd, Hg) content within the accepted limit corresponding to theRegulation of the Indonesian Ministry of Agriculture No 70[20]. Hence, this study aims to design a proper siphon-equipped decomposer to carry out a fermentation-based SFL production with minimum transition metal levels.

2. Materials and method

Materials were *S.polycystum*CA Agardh. from Lange coastal area (Aceh Besar), a commercial EM4starter purchased from a local distributor, post-harvest banana (*Musa paradisiaca*) trunk, and fish waste of *Euthynnusaffinis* collected from Lampulo local market. All chemicals including $HClO_4$, HNO_3, and H_2SO_4were supplied by Sigma-Aldrich (USA).

2.1. Preparation

The hand-harvested macroalgae were rinsed in running water in our laboratory, then grounded, and kept in a 4 °C fridge before further treatment. The preparation of siphon followed Nurhayati, Viridi (21)with varied diameters of0.5 cm (small), 1 cm (medium), and 1.5 cm (large) and length of the siphons immersed in the sludge of 16 cm (code 1), 18 cm (2), 20 cm (3), and 22 cm (4). A total of twelve bins of simple anaerobic decomposer were prepared from 600mL of used mineral water bottles equipped by the glass reverse U-shaped pipe connected to another half-filled water bottle. As the decomposition controls, non-siphon equipped with opened and closed bins were applied. The sample decomposition procedure was adapted from Sedayu, Erawan (22) with a slight modification. EM4 was diluted with water into a 2% solution and then sprayed onto the grounded macroalgae (± 20 mL for 1 kg of seaweed) followed by well-stirring. The growing media for bacteria used pieces of banana trees mixed with the fish waste in a ratio of 5:1 (w/w). The bottles were kept tightly closed at all decomposition times.

2.2. Analysis of metals content

Each sample was heated with acidic solvents (mixture of $HClO_4$, HNO_3, and H_2SO_4) and distilled water to boiling point. Filtration was then applied to afford the filtrate. All minerals content in the filtrate was estimated by using atomic absorption spectrophotometry (Shimadzu, Japan) following the instruction of the manufacturer.

3. Result and discussion

The process of anaerobic decomposition requires aerating hoses to allow fermentation gas materials from decomposition tube **A**to aerating tube**B**. In this research, the siphon was used as an aerating hose. During the compositing process, fermentation gas materials were flowing through the siphon-like pipe at a certain height to aerate the tube**A**. Here, it can be postulated that the deeperthe siphon pipe immersed in the suspension containing samples of fermentation in tube **A** and aerating liquid in tube **B**, the greater the pressure differenceof both ends of the pipe leading toincreased potential gravity energy, thus more transfer of heat and gaseous byproducts of the fermentation to the tube **B**.

Modifications were also made to the diameter of the siphon pipe. With a wider pipe's diameter, the volume of flowing gas could be assumed to get higher as described by Cai et al. [23]. However, when surpassing its critical point, the velocity of flowing energy and materials has been believed to be gradually decreasedwhich might be followed by lowering transfer of the byproducts.One major gas product of the fermentation is CO_2 that is dissolved in the aqueous suspension forming a possible anionic species, HCO_3^-. Laboratory models of desorption of heavy metals with such as a naturally occurred anion have shown the effective process of inexpensive and environmental-friendly approach of toxic cations removal [24].Carbonate-chelated complexes have widely been accepted to play a role in this idea that help reduce toxic minerals in waste-water treatment.

The length and the diameter of the pipe also influence maintaining the temperature and moisture of the decomposition process. As the length and diameter become larger, the temperature will not reach beyond 70°C, to keep bacteria active. When the temperature is well-controlled, the humidity will also be well-maintained, because the temperature and humidity are inversely proportional.Moreover, desorption of heavy metals from organic tissues into an aqueous medium can be thought of as a temperature-dependent process since the solubility of minerals tends to correspond to increasing temperature. This seems due to the increased kinetic energy of water molecules that allow higher molecular vibration to break up the cellular wall and help chelate the cationic metals by the dissolved carbonate anions. Therefore, evaluation of siphon-assisted removal of caloric energy and CO_2 is of great interest to confirm a promising application of siphon-applied SFL decomposer.

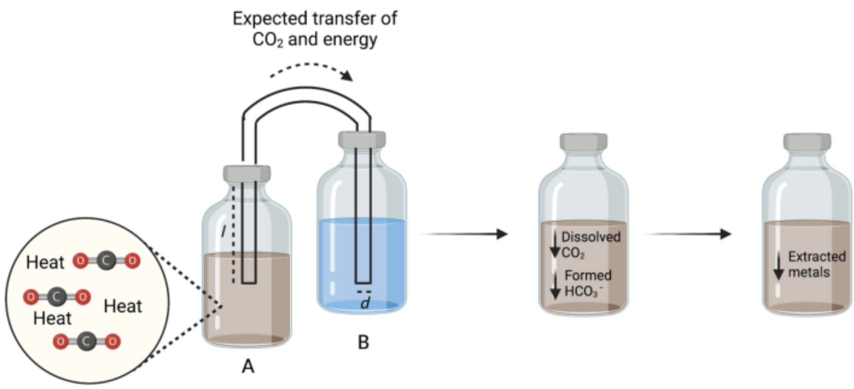

Fig 1. A representative illustration of modified siphon-like hoses in the production SFL in this study and hypothesized effect in the detected trace and heavy metals. The image was created with BioRender.com.

Table 1. Trace elements content in SFL was composted with siphon-modified decomposers.

No	Samples or reference	Concentration (ppm)					Concentration (ppb)	
		Pb*	Cd*	Co	Cr	Ni	As*	Hg*
1	S1	3.13	1.62	1.02	0.89	0.82	65.77	78.83
2	S2	3.40	1.72	1.75	1.16	0.83	< LoD	< LoD
3	S3	6.85	2.55	1.03	0.83	0.94	38.17	< LoD
4	S4	13.70	1.65	1.71	1.34	1.05	52.17	< LoD
5	M1	4.55	2.01	1.92	1.96	1.03	46.57	46.67
6	M2	10.03	2.29	1.87	1.77	1.06	< LoD	85.74
7	M3	4.74	2.21	0.85	1.58	1.11	< LoD	74.72
8	M4	4.11	2.60	0.72	1.35	0.80	29.78	56.29
9	L1	5.22	1.91	2.12	1.78	0.97	33.97	88.25
10	L2	3.15	2.34	1.60	2.02	1.08	< LoD	62.6
11	L3	3.29	2.43	0.89	1.50	0.85	< LoD	< LoD
12	L4	1.76	1.74	1.97	1.75	1.04	35.37	86.75
13	CO	1.85	1.72	0.69	1.32	0.92	49.37	43.17
14	CC	11.16	2.44	1.20	1.86	0.82	42.37	< LoD
15	Permentan 70	< 12.5	< 0.5	< 20	nd	nd	< 2500	< 250

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No	Samples or reference	Concentration (ppm)					Concentration (ppb)	
		Pb*	Cd*	Co	Cr	Ni	As *	Hg*
	/SR/140/10/ 2011							

Note:
* = heavy metal
LoD = *limit of detection* (20 ppb for As, 40 ppb for Hg)
nd = not defined

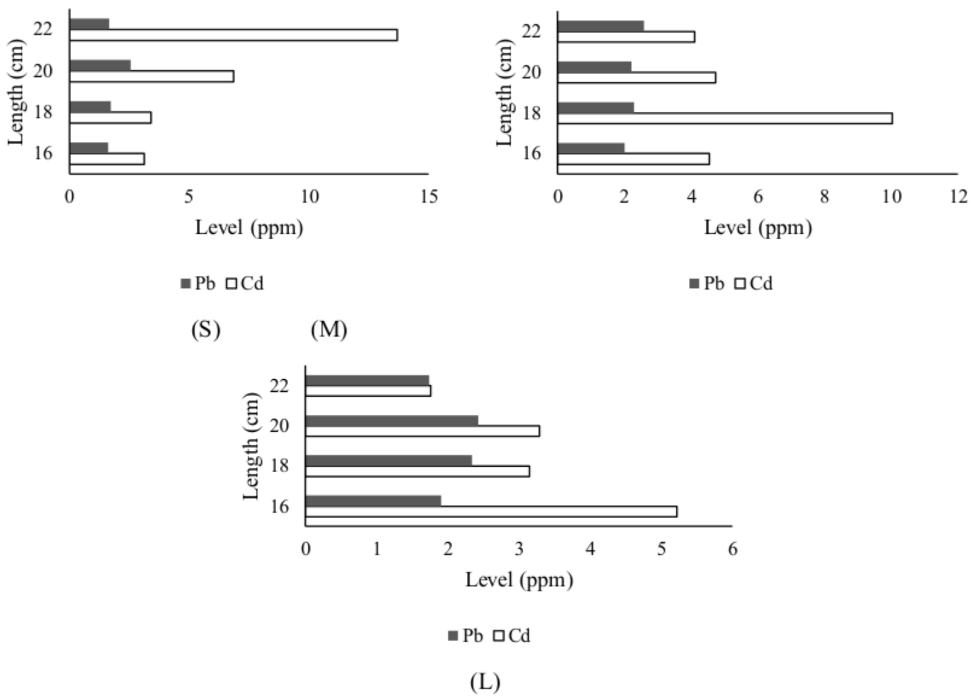

Fig 2. A comparison of level-representing curves of concerned heavy metals (Pb and Cd) among small (S), medium (M), and large (L) diameter of the siphon with four variations in the length.

In term of heavy metals level, all products could be excluded from the national standard of Cd level which should be below 0.5 ppm. However, by selective consideration, the concentration in S2 was observed as the closest value against the standard. For lead level, in particular, sample S4 was observed to have higher concentration as well as depicted in **Fig 2** followed by M2.Long-term exposure to these two metals in organisms has been believed to cause health problems such as gene mutations and poisoning.

Since the level of As in drySargassum is commonly reported up to 20–21 ppmt,a deep consideration regarding the utility of the seaweed for food and agriculture should be made[25]. The high levels of heavy metals in fresh brown algae are associated with the ability of polysaccharide molecules in the cell walls to absorb positively charged ions from Cd, Pb, and Au minerals [26, 27]. However, based on observed data which were below 2.5 and 0.25 ppm for As and Hg, respectively, the leachate seems to bearsenic and mercury-safe for the environment compared to the Indonesian regulation [20]. Moreover, all sample seems to contain the permissible concentration of trace elements such as cobalt mineral.

The trace elements could be released to the leachate when the microorganisms digested the biomass. The main idea of the fermentation is to break down macromolecules of the seaweed to a simpler form of organic nutrients mainly C, N, O, P as well as minerals.One factor that may contribute to the release could be the nature of the bio-activators that canform mineral coating surrounding their cell walls[28]. This allows the transition metals to be chelated into the networks. Some minerals have been observed to have agonist effects on the biocatalyst activities. For instance, at a certain point, Cu and Fe demonstrate a synergic effect on thedigestive activity of bacteria.

4. Conclusion

Except for sample S4, all SLFs obtained from fermentation with siphon-equipped decomposers are considered fairly safe for the environment due to a much lower concentration of heavy metals compared to Permentan 70/SR/140/10/2011 with Cd level as the exception.Especially, samples S2 and L3 are the most attracted SLF with the lowest concentration of As and Hg. Though the extracted cadmium level was somewhat beyond the maximum standard, S2 with diameter and length of 0.5 and 18 cm, respectively, could still be considered as the promising model for the application of siphon in SFL production. The further investigation remains needed to confirm the exact parameters involved in the process such as temperature, concentration of CO_2 and air pressure.

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