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THE PROLIFERATION OF EFFECTIVE MICROORGANISM (EM) IN VINASSE AND ITS APPLICATION IN THE MANUFACTURE OF LIVESTOCK-WASTE BASED FERTILISERS

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ABSTRACT

Applying long term vinasse without pre-treatment to soil causes eutrophication to water and soil. The paper aims to process vinasse of the alcohol industry as an effective growing medium of environmental-friendly microorganisms that can be used in the organic fertiliser manufacture. The effective microorganism has some benefits such as becoming a soil enhancer when applied directly to the soil or an accelerator in the maturation of the organic fertiliser. This research starts with the chemical content characterisation of vinasse and the addition of microorganisms nutrients. After the media is ready, the EM inoculum is added and the breeding result is used for the manufacture of organic fertiliser based on goat farm waste. The EM culture analysis reveals an increase of the microbial number. Moreover, the organic fertiliser obtained has a content of an organic matter, nitrogen, phosphate, and potassium referring to 43.42 %, 3.05 %, 0.40 % and 1.23 %, respectively. It is in accordance with the Indonesian government regulation.

Keywords: effective microorganism, livestock waste, organic fertiliser, vinasse.

INTRODUCTION

The increasing number of the worldwide population and the current industrialisation require an enormous amount of energy [1-3]. Petroleum and coal as non-renewable energy sources become very limited and are suspected as the primary cause of the environmental damage. The United Nation issued the 2030 Agenda for Sustainable Development aiming to increase the global energy production generated from renewable sources by 2030 and hence to tackle the climate change and its impacts in every country [4]. Therefore, the biofuel as biodiesel [5, 6] and bioethanol become a favourable alternative to substitute the fossil fuel [7,8]. Around 80% of the carbon emissions are decreased because of bioethanol usage [9].

Until now, molasses are mostly used in ethanol production because of the high glucose content [10 - 12], the low price [13] and its ability to convert into bioethanol without any pre-treatment [14] using yeast such as *Saccharomyces cerevisiae*. Various kinds of microbes can be used in the fermentation process [15], such as *Zymomonas mobilis* [16], *Saccharomyces cerevisiae* [10, 11, 17, 18], and *Klueromyces marxianus* [19]. Whereas, *S. cerevisiae* is one of the most commonly used organisms in the fermentation process because of their high tolerance to ethanol [20, 21].

Indonesia produced 450 million litter bioethanol from sugarcane in 2015 but it has contributed only ca 1 % of total gasoline consumption. Therefore, the government policy supports the industries to reach 11.48 billion litre of production in 2025, which in turn will require 2.76 Mha land [22]. Meanwhile, the alcohol molasses-based refining industry is rated as one of the 17 most polluting industries [23]. In addition to the high organic content, the distillated water contains nutrients in the form of nitrogen (1,660 mg/L - 4,200 mg/L), phosphorus (225 mg/L - 3,038 mg/L) and potassium (9,600 mg/L - 17,475 mg/L) [24]. It has also a very high Chemical Oxygen Demand (COD) (80,000 mg/L - 100,000 mg/L)

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and Biochemical Oxygen Demand (BOD) (40,000 mg/L -50,000 mg/L) [25]. Furthermore, vinasse has a low pH value, a strong odor, it is corrosive and has a dark brown color [26]. It causes eutrophication of the water bodies. That is why the negative effects of the bioethanol industries' by-products on the environment are an urgent issue which have to be addressed and tackled.

Therefore, recycling these by-products using commercial effective microorganisms (EM) is reported in the present paper. The EM consists of yeasts, lactic acid, photosynthesising bacteria, fermenting fungi, and bacteria actinomycetes [27] which can be used to break down the organic content into a nutritious one. The EM culture which contains a useful natural organism can improve the soil fertility by enriching the diversity of the soil microbial ecosystems. In addition, the application of a soil fertiliser is also discussed.

EXPERIMENTAL

Material

Vinasse was taken from Bekonang alcohol industry of Indonesia with the initial ingredients described in Table 1. The commercial EM used contained fermentation bacteria of Lactobacillus sp, Actinomycetes photosyntetic bacteri (+), Phosphate solubilizing bacteria and yeast (Table 3). In addition, a shrimp paste, urea, a rice bran, herbs, molasses and distilled water were also used.

Procedure

Liquid waste alcohol characterisation. Before used, the vinasse was characterised by its composition (pH, organic C, Organic matter, N, C/N Ratio, P_2O_5, K_2O, Ca, Mg). The organic carbon and the organic matter were analysed using the Walkley and Black method in the Microbiology Laboratory of UNS. Basically, 1 N $K_2Cr_2O_7$ solution was used to oxidise the soil. It was assisted by the heat produced when one volume of dichromate was mixed with two volumes of H_2SO_4. The reaction product was titrated with ferrous sulphate solution using diphenylalamine as an indicator. The inverse titre represented the amount of C in the soil.

Table 1. A chemical composition of vinasse from Bekonang alcohol industry.

Parameters	Methods	Concentration values (%)
pH		4.55
Organic carbon	Walkley & Black	31.17
Organic matter	Walkley & Black	53.74
N	Kjeldhal	0.52
C/N Rasio		59.94
P_2O_5	HNO_3 & $HClO_4$ extraction	0.59
K_2O	HNO_3 & $HClO_4$ extraction	1.26
Ca		0.08
Mg		0.15

Breeding EM. A thousand millilitres of vinasse were sterilised by boiling and the mixture of 50g of a shrimp paste, 100 g of herbs, 50 g of urea, 100 g of a rice bran and 50 ml of molasses of sugar cane had been dissolved in 1000 ml of distilled water. The mixture was boiled again, then cooled at a temperature of 25°C and 5 mL of a microorganisms inoculant was added. Then the mixture was placed in a tightly sealed container. After a month, the breeding result of the microbe was assayed and analysed. The method used to estimate the concentration of the microorganisms in the sample referred to the Most Probable Number (MPN) method [28]. The procedure was carried out by replicating liquid broth growth in ten-fold dilutions.

Composting. One quintal of livestock waste was mixed with 100 ml of diluted EM and then added to 1000 ml of distilled water. After mixing evenly with 60 % of the water, the mixture was covered by a thick plastic to provide an anaerobic growth proceeding. The mixture reversal was done every five days to control the temperature and the moisture content of the material. The temperature measurements were carried out every day until the temperature equaled the room temperature. The constant value reached was treated as a sign of the compost maturation. The product then was tested and analysed in the laboratory.

The nitrogen content was analysed by the Kjeldahl Auto Distillation method. The method procedure involved three major steps, such as digestion, distillation, and titration. The digestion procedure was done to break down the nitrogen bonds and convert the organically bounded nitrogen into ammonium ions. After the process completion, the sample was diluted with distilled water and the concentration of the ammonium ions present was determined by a direct titration. Then the conversion of NH_4^+ to NH_3 was carried out.

RESULTS AND DISCUSSION

Molasses is a viscous liquid of a bit bitter taste and a dark colour. It is obtained as a byproduct in the course of sugar production [29] and contains many minerals [25].

Table 2. An average ntrients composition of a rice bran (w/w) [37].

Parameters	Content / %
Moisture	12.12±0.25
Protein	12.32±0.24
Fat	20.31±0.92
Ash	8.73±0.08
Digestible carbohydrates	17.92±0.26
Dietary fiber	28.60±0.32
pH	6.85±0.10

Usually, one tonne of fresh sugar cane can produce 4 % - 5 % of molasses [30]. The latter is primarily used worldwide as an animal food because it increases the growth of microbes in rumen animals and improves the digestion of fibres and non-protein nitrogen [31]. This indicates that vinasse as the most abundant effluent from a sugarcane biorefinery has a great potential of reuse.

However, as shown in Fig. 1(a), vinasse contains inorganic minerals and organic pollutants, it is corrosive, toxic and has a low biodegradability [32]. The direct vinasse application to the soil within 7 consecutive years causes cane maturation and reduction in sucrose content as a consequence of potassium excess [33]. Therefore, prior to its utilization as a breeding medium, the vinasse sample is tested to identify its original composition. The

Fig. 1. An appearance of the liquid waste of bioethanol production: (a) a good one; (b) a bad one.

liquid waste alcohol used in this study is black in colour, thick and has a sweet aroma, while the unprocessed one is brown and distributed between the sugar solution and the stench (Fig. 1(b)). In addition, the laboratory test of the alcohol waste sample shows (Table 1) a high content of an organic matter, carbon, and nitrogen. This provides its use as a medium for breeding of microorganisms counteracting the soil degradation. The most important nutrients of the microbes refer to carbon and nitrogen as nearly 50% of the dry weight of the cell consists of carbon. The autotroph prokaryotes use CO_2 as the only source of carbon, whereas heterotrophs use organic molecules as a source of carbon for growth [34].

Nitrogen is needed in the proliferation of bacteria because it plays a vital role in cellular metabolism especially in cell division. Therefore, if the nitrogen content is less, the ability of bacteria self-division is smaller. As a result, bacterial growth is slow. In fact, the bacteria can assimilate organic and inorganic nitrogen compounds for their growth. Hence, the nitrogen compound will be decreased. Furthermore, it can be converted to ammonia [35].

Cheap and easy to obtain matewrials like vinasse, a rice bran, a shrimp paste, molasses and urea are used for microbes growth. The composition of the rice bran referring to the quality requirements is presented in Table 2. These materials contain nutrients which can be used by effective microorganisms for their growth and appropriate multiplication [36]. Besides, herbs are also added in the form of *Curcuma zanthorrhiza*, turmeric, *Kaempferia galanga*, and ginger to remove the stinging odour from the fermentation. The results of the microbial proliferation in a vinasse medium are presented in Table 3.

Table 3 shows that the presence of bacteria *Lactobacillales sp.*, yeast, as well as the total microbial content increase. *Lactobacillales* is responsible for the produc-

Table 3. Microbial analysis results referring to soil fertiliser starter products.

Microbial groups	Initial content (cfu/mL)	Final content (cfu/mL)
Phosphate solubilizing bacteria (PSB)	7.5×10^6	2.3×10^5
Lactobacillales sp.	8.7×10^5	4.9×10^7
Yeast	8.5×10^6	3.9×10^7
Microbial total	-	3.4×10^{10}

Table 4. Analysis results referring to the organic fertilisers (manure) and the planting media.

Sample	Percentage (%)			
	Organic matter	N Total	P Total	K Total
Manure produced	43.42	3.05	0.40	1.23
Planting media (manure:soil: charcoal husk = 2:1:1)	33.06	1.43	0.31	0.32
Pure Manure [44]	31.3	0.93	0.21	0.12
Organic fertilizer (SNI 19-7030-2004)	27- 58	Min 0.10	Min 0.20	Min 0.10

tion of lactic acid in the early stages of the composting and the anaerobic digestion process. At the beginning of the composting, other bacterial groups may be reduced due to the presence of *Lactobacillales* which produces hydrogen peroxide, bacteriocin and antibiotics [38]. Furthermore, the amount of indigenous microorganism inoculums which is less than 20 % can be insufficient for the composting [39]. Besides, the phosphate solubilising bacteria (PSB) cannot breed well and their number decreases. Whereas, PSB plays an essential role in the manufacture of fertiliser because it can increase the phosphate levels in the fertiliser [40, 41].

The liquids containing microbial cultivation are used to accelerate the process of organic fertiliser maturation. The essential ingredients of the organic fertiliser used in this research refer to a livestock waste (including that of goats) and forage feed residue. According to Arab et al. [42] the composting process can be enhanced by direct microbial inoculation. However, the quality of the produced compost is influenced by many factors such as the pre-processing, the particle size and the feedstock utilised, the C/N ratio, the bio-accelerator, the nutrients amendment, the pH value, the aeration, the moisture content, the temperature, and the maturation stage [43]. The analysis results referring to the total content of nitrogen, phosphorus, and potassium of the organic fertiliser used in this research are presented in Table 4.

Table 4 shows the total content of N, P, and K in the manure determined in accordance with SNI 19-7030-2004. In addition, the regulation of the Agriculture Ministry of Indonesia No. 70/Permentan/SR.140/10/2011 on organic fertilisers, biological fertilisers and soil enhancers states that the minimum technical requirement of solid organic fertiliser in respect to the organic matter is 15 %, while that for nutrient content ($N + P_2O_5 + K_2O$) is 4 % [45]. According to this research, the sum of the organic matter and the nutrient content ($N + P_2O_5 + K_2O$) is higher than that required by the regulations. The compost produced is better than the planting media which is defined as a mixture of manure, soil and charcoal husk. Moreover, the compost shows superior results when compared to those of pure manure.

CONCLUSIONS

The experimental study of recycling vinasse as a growth medium for natural beneficial organisms and its use to produce a fertiliser from a livestock waste by a simple method is successfully conducted. The discussion presented shows clearly shows that vinasse could be used as an alternative medium of effective microorganisms cultivation and as a microbial accelerator of organic fertiliser maturation. The organic fertiliser produced from goat livestock waste has high levels of organic materials, N, P and K and corresponds to the regulations of the Indonesian government.

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