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Penulis : Triastuti Sulistyaningsih, Nuni Widiarti, Widi Astuti, Dewanto Harjunowibowo

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Gmail

Triastuti Sulistyaningsih <triastuti.s@mail.unnes.ac.id>

Special Issue Submission-Journal of Chemical Technology and Metallurgy

2 messages

Triastuti Sulistyaningsih <triastuti.s@mail.unnes.ac.id> Wed, Nov 29, 2017 at 6:19 PM
To: Heri Septya Kusuma <heriseptyakusuma@gmail.com>

Dr. Heri Setya Kusuma

The Guest Editor

Journal of Chemical Technology and Metallurgy

November 29, 2017

Dear Dr. Heri Setya Kusuma:

I am pleased to submit an original research article entitled "The proliferation of effective microorganism (EM) in vinasse and its application in the fertilisers manufacture of livestock-wastes based" by Triastuti Sulistyaningsih, Nuni Widiarti, and Dewanto Harjunowibowo for consideration for publication in the ***Journal of Chemical Technology and Metallurgy***. We previously uncovered a simple method to recycling alcohol fermentation process by-product , and this manuscript builds on our study to determine role strategies to solve pollutant in society caused by vinasse in Indonesia.

In this manuscript, we show that liquid waste of alcohol industry could be successfully processed as an effective growing medium of microorganisms and can be used as starter bacteria organic fertiliser manufacture. The effective microorganism has many benefits to be used directly in the soil as a soil enhancer or used to accelerate the maturation of organic fertiliser. The EM inoculum is added to the prepared media and the breeding result is used for the manufacture of organic fertiliser based on goat farm waste. The EM culture analysis result showed an increase in microbial number, while organic fertiliser analysis showed that organic matter content was 43.42%, nitrogen 3.05%, phosphate 0.40% and potassium 1.23% which could result in proper condition for the crops.

We believe that this manuscript is appropriate for publication by the ***Journal of Chemical Technology and Metallurgy*** special issue because it well-matches with the scope of the journal. Our manuscript creates a paradigm for future studies of the built environment.

This manuscript has not been published and is not under consideration for publication elsewhere. We have no conflicts of interest to disclose.

Thank you for your consideration!

Sincerely,

On behalf of authors,

Triastuti Sulistyaningsih

Chemistry Departement
Universitas Negeri Semarang
Indonesia

Triastuti Sulistyaningsih <triastuti.s@mail.unnes.ac.id> Wed, Nov 29, 2017 at 6:21 PM
To: Heri Septya Kusuma <heriseptyakusuma@gmail.com>

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The proliferation of effective microorganism (EM)_Triastuti.docx
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The proliferation of effective microorganism (EM) in vinasse and its application in the fertilisers manufacture of livestock-wastes based

Triastuti Sulistyaningsih[1)], Nuni Widiarti[1)], Widi Astuti[2)], Dewanto Harjunowibowo[3)4)]

[1)]*Chemistry Departement, Faculty of Matematics and Natural Science, Universitas Negeri Semarang, Indonesia*
[2)]*Chemical Engineering, Faculty of Engineering, Universitas Negeri Semarang, Indonesia*
[3)]*Physics Education Department, University of Sebelas Maret, Surakarta, Indonesia*
[4)]*Department of Architecture and Built Environment, University of Nottingham, UK*
E-mail: triastuti.s@mail.unnes.ac.id

ABSTRACT

Liquid waste of alcohol industry has been successfully processed as an effective growing medium of microorganisms that can be used as starter bacteria organic fertiliser manufacture. The effective microorganism has many benefits to be used directly in the soil as a soil enhancer or used to accelerate the maturation of organic fertiliser. This research begins with the characterisation of chemical content in 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 result showed an increase in microbial number, while organic fertiliser analysis showed that organic matter content was 43.42%, nitrogen 3.05%, phosphate 0.40% and potassium 1.23%.

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

INTRODUCTION

The increasing population of the worldwide and the current industrialisation make energy as an essential requirement [1–3]. Petroleum and coal as sources of non-renewable energy become very limited and the primary cause of environmental damage. The United Nation issued The 2030 Agenda for Sustainable Development to increase the global energy production from renewable sources by 2030 to tackle the climate change and the impact for every country [4]. Therefore, biofuel such as biodiesel [5,6] and bioethanol becoming an idol to alter the fossil fuel [7,8].

In 2014, around 24 billion gallons bioethanol produced worldwide and increases up to 26.583 billion gallons in 2016 by 60% in US and 25% in Brazil, respectively [9], as seen in Figure 1. In addition to a source of cheap ethanol feedstock [6] and renewable such as sugarcane [10], corn [11] , grains [12], rice [13], starch cassava roots [14], and potato [15], around 80% carbon emissions reduction could be achieved because of bioethanol usage [16].

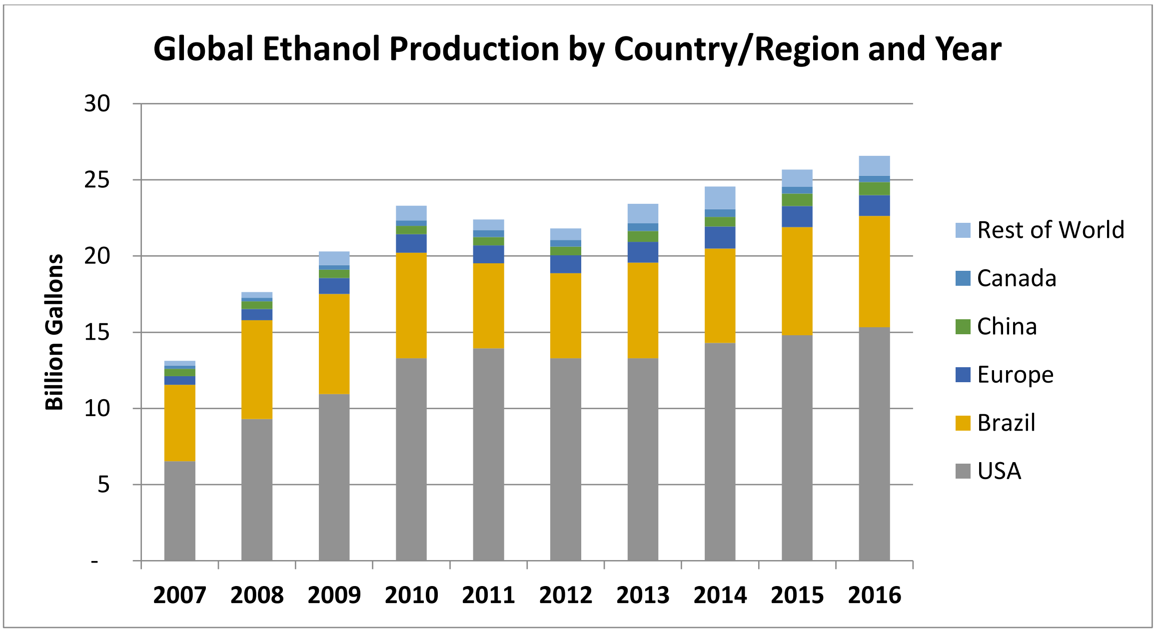

Figure 1. Worldwide Ethanol Production [9]

Nevertheless, an increase in bioethanol production with raw food becomes problematic when confronted with world food needs. Therefore, bioethanol manufacture feedstock based changed to food processing wastes based, which is much cheaper, easy to get and more environmentally friendly in the fermentation process [17]. They are whey [18], potato wastes [18,19], black liquor [20], molasses [10,21], and banana wastes [22], which is, potentially reduce the greenhouse gas emission [23]. Even now bioethanol could be produced from kitchen wastes with some pre-treatments and hydrolysis by physical, chemical and biological methods to enhance the productivity [24–26].

Table 1 reveals the summary of bioethanol production utilising food wastes.

Until now, molasses are mostly used in ethanol production because of the high glucose content [21,27,28], low price [29] and can be converted to bioethanol without any pre-treatment [25] by yeast such as *Saccharomyces cerevisiae*. Various kinds of microbes could be used in the process of fermentation [30], such as *Zymomonas mobilis* [31], *Saccharomyces cerevisiae* [14,19,21,27], and *Klueromyces marxianus* [18]. *S. cerevisiae* is one of the most commonly used organisms in the fermentation process because it has a high tolerance to ethanol so as capable of producing high concentration ethanol [32,33]. In addition, the yeast can produce ethanol of 9.8% (v/v) or its effectiveness reaches 88.1% of the maximum number theoretically [21].

Table 1. Bioethanol food processing wastes based [34]

Food waste source	Sugars or source of sugars fermented
Whey, whey powder	Lactose
Potatoes (whole)	Starch
Potato processing waste	Starch
Potato peel, mash	Starch, pectin, cellulose, and hemicellulose
Sweet potato	Starch
Fruit processing waste	Sucrose, glucose, and fructose
Pineapple peel	Sucrose and glucose
Raw sugar beet juices; sugar beet processing intermediates	Fermentable sugars
Molasses	Sucrose, glucose, and fructose
Corn processing wastes	Glucose and xylose
Coffee processing pulp	Xylose, arabinose, glucose, sucrose, fructose, maltose
Coffee bean husks	Cellulose and hemicellulose
Rice husks	Cellulose and hemicellulose

Ethanol or ethyl alcohol has molecular presentation CH_3CH_2OH with 52.15% carbon, 37.73% oxygen, and 13.12% hydrogen [35]. An ethanol production is a step series of pre-treatment, hydrolysis and fermentation. Moreover, the production during fermentation depends on many factors such as sugar concentration, agitation rate, pH, temperature, fermentation time, and inoculum's size [32]. After the fermentation process. The fermented juice is distilled to get ethanol and the by-products (vinasse). The fermentation process is carried by yeast and follows this reaction.

$$C_6H_{12}O_6 \longrightarrow 2CO_2 + 2CH_3\text{-}CH_2OH$$

(glucose) (carbon dioxide) (ethanol)

In Asia, India is the second largest ethanol producer with annual production projected about 2300 million litres in 2006-2007 [36]. Likewise, Indonesia in 2015, 450 million litter bioethanol has been produced from sugarcane but only gives contribution around 1% of total gasoline consumption. Therefore, the Indonesian government policy supports the industries to reached 11.48 billion Litre in 2025 which will require land by 2.76 Mha [37]. Whereas, the alcohol refining industry is rated as one of the 17 most polluting industries. Since in addition to high organic content, the distillate water also contains nutrients in the form of nitrogen (1,660-4,200 mg/L), phosphorus (225-3,038 mg/L) and potassium (9,600 -17,475 mg/L) [38] which may cause eutrophication of water bodies [39].

Hence, the negative effects of bioethanol's industry by-products on the environment are an urgent issue to address. Molasses-based refining is one of the most polluting industries because it produces a high volume of wastewater (Satyawali and Balakrishnan, 2008). In addition to corrosive, low pH, strong odor and dark brown color [40], this waste is characterized with very high Chemical Oxygen Demand (COD) (80,000-100,000 mg/L) and Biochemical Oxygen Demand (BOD) (40,000-50,000 mg/L) [41].

Thus, to tackle the problem, Corsano et al. offered a system to optimise the fermentation process as a non-linear programme (NLP) which concerns to the environmental safety. The model contributes to a more complete substrate consumption, water reuse and waste reduction [42]. Other than that, Rodriguez uses vinasse as a supplement to fertilise plant-cane and shows positive effect compared to plant-cane without vinasse [40]. Moreover, 2-3 years application in sugarcane fields resulted in soil improvement in physicochemical properties. In addition, soil hardening and soil acidification were not detected in the field [43]. However, it is still debatable since other researchers report that continuous vinasse application for 7 years caused cane maturation and reduction in sucrose content as a consequence of potassium excess [44]. In fact, vinasse contents like inorganic minerals, organic pollutants, suspended substantial, and corrosive which has high toxicity and low biodegradability [45] depend on sugarcane origin [46].

Furthermore, in the landfill, any wastes with high moisture will generate toxic leachate and require secondary wastewater treatment system [47]. The incineration of the liquid waste is obviously unrealistic because vinasse is a viscous liquid and there is a possibility of dioxin generation [26]. Accordingly, an alcohol industry waste treatment has been done to reduce levels of COD and remove the colour [39]. Moreover, a novel and economically strategy using *Pleurotus sajor-caju*, followed by electrochemical oxidation (EO) to sugarcane vinasse resulted in a colour reduction by 97%, COD 50.6%, turbidity 99%, and Total Organic Carbon (TOC) 57.3% [10]. Unfortunately, the method still needs to be socialised, and not simple. While in Indonesia, there are more than 5000 bioethanol home industries produce tonnes of vinasses. They often put it outside or just dumped into the land of rice fields and rivers which caused terrible smells, low soil acidity, and poisonous to plants and fishes without any pre-treatment.

Therefore, to tackle this problem, recycling the vinasse from molasses using commercial effective microorganisms (EM) is reported. The EM consist of yeasts, lactic acid, photosynthesising bacteria, fermenting fungi, and bacteriaactinomycetes [48]. The EM can improve soil fertility by enriching the diversity of soil microbial ecosystems because they contain useful natural organisms. In addition, the application of the products for soil fertiliser is discussed at once.

EXPERIMENTAL

Material.

Vinasse is taken from Bekonang alcohol industry of Indonesia, commercial EM, shrimp paste, urea, rice bran, herbs, molasses and aquadest.

Procedure

Liquid waste alcohol characterisation. The vinasse is characterised by its composition in laboratory before used.

Breeding EM. A thousand millilitres of vinasse is sterilised by means of boiling. The mixture of 50g shrimp paste, 100g herbs, 50g urea, 100g rice bran and 50 ml molasses of sugar cane which has been diluted in 1000 ml of aquadest. The mixture is boiled again. The mixture was then cooled to 25 °C and added 5 mL of microorganisms inoculant. The mixture is placed in a tightly sealed container. After allowed 1 month, the amount of microbial result of breeding is assayed in laboratory and analysed.

Composting. One quintal livestock waste is mixed with 10 mL of diluted EM solution in 1000 mL of aquadest. After mixing evenly with water content ranges 60%, the mixture is covered in thick plastic to allow the anaerobic process. Reversal is done every 5 days to control the temperature and moisture content of the material. Temperature measurements are carried out every day until the temperature equals the room temperature and constant as a sign that the compost has matured. The product then is tested in laboratory and analysed.

RESULT AND DISCUSSION

Molasses is a viscous liquid, tastes a bit bitter and dark colour as evidently seen in Figure 2, resulted from the sugar factory [49] and contains many minerals [41]. Before utilised in breeding medium, the vinasse sample was tested in laboratory to investigate the original composition. Usually, one tonne of fresh sugar cane could produce 4-5% molasses [50]. Worldwide, molasses are primarily used as animal feed because it increases the growth of microbes in rumen animals that improve the digestion of fibre and non-protein nitrogen [51]. It indicates that vinasse very potential to be reused. The standard summary of vinasse composition is presented in Table 2.

To it, the liquid waste condition of the alcohol used in this study is the thick, black and sweet-smelling properties while the unprocessed is brown, separated between the sugar solution and water, and stench (Figure 2). In addition, the proximate laboratory test of alcohol wastes sample of molasses-based as shown in

Furthermore, nitrogen is needed in the proliferation of bacteria because nitrogen plays a vital role in cellular metabolism especially in cell division. Therefore, if the nitrogen content is less then the ability of bacteria to self-divide becomes slower. As a result, bacterial growth becomes low. In fact, bacteria can assimilate organic and inorganic nitrogen compounds for their growth. Hence, the nitrogen compound in such form will be reduced or catabolism by bacteria into ammonia [53].

Table **3** still contain lots of organic material.

Table **3** shows high organic matter, carbon, and nitrogen which contained in alcohol wastewater. Thereby, the nutritions can be used as a media for the breeding of soil microbial degradation. The most important nutrients for microbes are carbon and nitrogen since nearly 50% of the dry weight of the cell consists of carbon. The autotroph prokaryotes use CO as the only source of carbon, whereas heterotrophs use organic molecules as a source of carbon for growth [52].

Table 2. Vinasse component generation 1 and 2 [41]

Mineral analysis		Organic Analysis	
Component	**Value**	**Component**	**Value**
Cl^- (mg L^{-1})	59.4[a]	Organic matter(%)	3.96[a]
$SO4^{2-}$ (mg L^{-1})	1,680[a], 44–366[b]	C:N ratio	10[a], 49.2–124.9[b]
Na^+ (mg L^{-1})	8.6[a]	COD (mg L^{-1})	32,000–92,800[a], 75,800–109,700[b]
K^+ (mg L^{-1})	1,620[a]	BOD_5 (mgO_2 L^{-1})	13,514–36,847[a], 31,500–87,700[b]
Ca^{2+} (mg L^{-1})	3,160[a]	BOD_5/COD	0.18–0.34[a], 0.39–0.80[b]
Mg^{2+} (mg L^{-1})	162.4[a]	Phenols (mg L^{-1})	230–390[a], 0.4–12.4[b]
PO_4^{3-} (mg L^{-1})	560[a], 33.26[b]	NH_4^+ (mg L^{-1})	23.9[a]
NO_3^- (mg L^{-1})	823.7[a]	Protein (%)	2.92[a]
Fe (mg L^{-1})	44.9[a]	Fiber (%)	0.2[a]
Mn (mg L^{-1})	4.9[a]	Fat (%)	0.41[a]
Zn (mg L^{-1})	1.2[a]	Ash (%)	3.61[a]
BO_3^{3-} (mg L^{-1})	1.94[a]	Carbohydrate (%)	3.42[a]
Ba (mg L^{-1})	0.54[a]	Acetaldehyde (g L^{-1})	0.697[a]
Cd (mg L^{-1})	1.06[a]	Ethanol (g L^{-1})	3.83[a]
Cr (mg L^{-1})	0.15[a]	Propylene glycol (g L^{-1})	0.084[a]
Ni (mg L^{-1})	0.26[a]	2,3-butanediols (g L^{-1})	0.568[a]
Al (mg L^{-1})	72.5[a]	Glycerol (g L^{-1})	5.86[a]
MoO_4^{2-} (mg L^{-1})	0.17[a]	Erythritol (g L^{-1})	0.088[a]
Cu (mg L^{-1})	0.06[a]	Arabinitol (g L^{-1})	0.064[a]
Physicochemical analysis			

Density (g mL^{-1})	1[a]	Chiro-inositol (g L^{-1})	0.114[a]
Ph	4.84[a], 4.0–4.9[b]	Sucrose (g L^{-1})	0.222[a]
DO (mg L^{-1})	4.3[a]	Acetic acid (g L^{-1})	1.56[a]
Moisture (%)	89.64[a]	Formic acid (g L^{-1})	0.582[a]
Eh (Mv)	260[a]	Lactic acid (g L^{-1})	7.74[a]
Conductivity(mS cm^{-1})	8.52[a]	Quinic acid (g L^{-1})	0.508[a]

[a]First generation, [b]Second generation

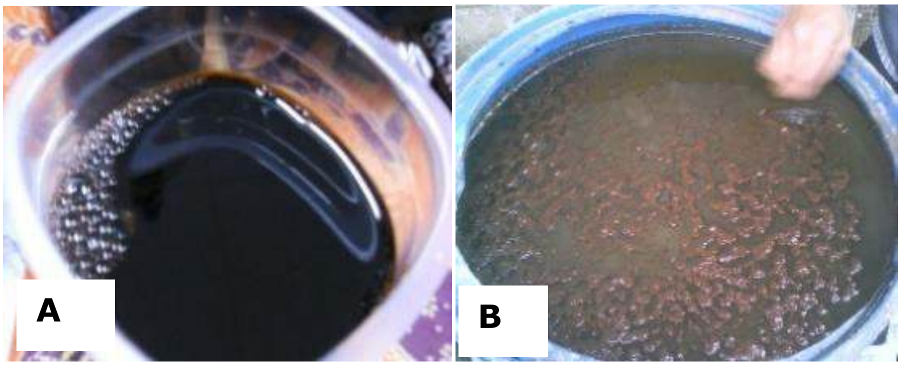

Figure 2. Waste of bioethanol appearance (a) good, and (b) bad condition

Furthermore, nitrogen is needed in the proliferation of bacteria because nitrogen plays a vital role in cellular metabolism especially in cell division. Therefore, if the nitrogen content is less then the ability of bacteria to self-divide becomes slower. As a result, bacterial growth becomes low. In fact, bacteria can assimilate organic and inorganic nitrogen compounds for their growth. Hence, the nitrogen compound in such form will be reduced or catabolism by bacteria into ammonia [53].

Table 3. Chemical composition of vinasse from Bekonang alcohol industry

Parameter	Method	concentration (%)
pH		4.55
C organic	Walkley & Black	31.17
Organic matter	Walkley & Black	53.74
N	Kjeldhal	0.52
C/N Rasio		59.94
P_2O_5	HNO3 & HClO4 extraction	0.59
K_2O	HNO3 & HClO4 extraction	1.26
Ca		0.08

Mg	0.15

In growing bacteria, an alternative media that is media used to grow microbes by using materials that are cheap and easy to obtain such as rice bran, brown sugar, monosodium glutamate shrimp paste, and molasses can be used. Hence, in addition to vinasse, in the breeding media for EM added rice bran, shrimp paste, molasses and urea. The average composition of rice bran according to the quality requirements is presented in Table 4. These materials contain nutrients can use to grow and multiply appropriately by the effective microorganism [54]. Besides, herbs are also added in the form of *Curcuma zanthorrhiza*, turmeric, *Kaempferia galanga*, and ginger to remove the stinging odour from vinasse. As the result, the microbial proliferation with the vinasse media is presented in

Table 5.

Table 4. The Average Nutrients Composition of the Rice Bran (w/w) [55]

Parameters	%
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

Table 5. Results of microbial analysis in soil fertiliser starter products

Microbial group	Initial (cfu/mL)	Product (cfu/mL)
Phosphate solubilizing bacteria (PSB)	$7{,}5 \times 10^6$	$2{,}3 \times 10^5$
Lactobacillales sp.	$8{,}7 \times 10^5$	$4{,}9 \times 10^7$
Yeast	$8{,}5 \times 10^6$	$3{,}9 \times 10^7$
Microbial total	-	$3{,}4 \times 10^{10}$

Based on

Table 5, it maps out that the number of phosphate solubilising bacteria (PSB) cannot breed well and decrease the number of bacteria. Whereas, PSB plays an essential role in the manufacture of fertiliser because it can increase the phosphate levels in the fertiliser [56,57]. However, bacteria *Lactobacillales sp*., yeast, and total microbial increased. *Lactobacillales* are responsible for the production of lactic acid in the early stages of composting and anaerobic digestion process. At the beginning of composting, other bacterial groups may be reduced due to the presence of *Lactobacillales* which produces hydrogen peroxide, bacteriocin and antibiotics [58]. Furthermore, the low amounts of indigenous microorganism inoculums less than 20% can cause insufficient sources for the composting [59].

Liquids containing microbial cultivation results are then used to accelerate the process of organic fertiliser maturation. The essential ingredients of organic fertiliser in this research are livestock waste which includes goat livestock, forage feed residue so-called manure. According to Arab et al., the composting process can be enhanced by direct microbial inoculation [60]. However, the quality of produced compost is influenced by many factors such as the pre-processing, particle size and feedstock utilised, the C/N ratio, bio-accelerator, nutrients amendment, pH, aeration, moisture content, temperature, and the maturation stage [61]. The analysis results of nitrogen, phosphorus, and potassium total of organic fertilisers in this research are presented in Table 6.

Table 6. The result of organic fertilisers (manure) and planting media analysis

Sample	Percentage (%)			
	Organic	N Total	P Total	K Total

	matter			
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 [62]	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

Table 6 shows the organic material content, N, P, and K total in the manure yielded is in accordance with SNI 19-7030-2004. In addition, The regulation of the Agriculture Minister 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 for organic matter is at least 15%, and for nutrient content ($N + P_2O_5 + K_2O$) at least 4% [63]. In this research, the sum of nutrient content ($N + P_2O_5 + K_2O$) is 4.68%, agree with the regulation. The compost produced is better than planting media which is defined as a mixture of manure: soil and charcoal husk. Moreover, compared to pure manure, the compost resulted shows the superiority.

CONCLUSION

The paper aims to recycle vinasse into medium growth for natural beneficial organisms using EM and uses it to produce fertiliser from livestock waste in a simple method. Based on the discussion, it clearly shows that Vinasse could be used as an alternative media of effective microorganisms cultivation and then used as microbial accelerator of organic fertiliser maturation. Organic fertiliser produced from goat livestock waste has high levels of organic materials, N, P and K and in accordance with those set out in Indonesian government regulations.

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UNIVERSITAS NEGERI SEMARANG

TRIASTUTI SULISTYANINGSIH <triastuti.s@mail.unnes.ac.id>

Final revision of paper_18_105

1 message

Dewanto Harjunowibowo <Dewanto.H@nottingham.ac.uk> Thu, Oct 18, 2018 at 4:30 PM
To: Heri Septya Kusuma <heriseptyakusuma@gmail.com>
Cc: "triastuti.s@gmail.com" <triastuti.s@gmail.com>

Dear Dr. Heri S.K.

I am sorry for the very late response to submit the final revision of our paper ID 18-105. We have revised it and proofread carefully, please find the attached files above.

If you have other requirement for me to do, please don’t be hesitate to ask.

Thank you in advance.

Warm Regards,

Dewanto Harjunowibowo
Sustainable Energy Technology
Architecture and Built Environment Department
The University of Nottingham, Nottingham NG7 2RD

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2 attachments

18-105_Revised-Response to reviewers_Triastuti.docx
13K

18-105_Manuscript with corrections (triastuti).docx
191K

Dear Editor

Many thanks for your useful comments and suggestions about our manuscript (ID 18_105). We have modified the manuscript accordingly reviewers' comments and answered accurately. Some parts of the manuscript have been changed. All exerted changes can be seen in highlight manner.

Sincerely,

Triastuti S.

Comments from the editors and reviewers:

1. Part Introduction: It is very long and most of this part does not concern the problem (the utilization of vinasse wasted from the fermentation of molasses to bioethanol). In addition, the chemical equation that describes the fermentation of glucose to ethanol is not suitable for the illustration of bioethanol production from molasses as it mainly contains saccharose. Also, widely used methods, including such that are used in industry for utilization of vinasse, are not discussed (e.g. biomethanisation).

- fixed by revising the structure and made it much shorter. I deleted some information which is not so important in this part. For vinasse utilisation we only focus on fertiliser.

2. Part Experimental: This part is very short and incomplete. The content of the microbiological trade product (effective microorganisms EM) has to be described, as well as all methods used in the experimental work (e.g. Results of the analyses are shown in Table 4 without the description of the method applied).

- fixed by adding information regarding the laboratory test procedure in principle. Since the methods used to analyse are very common/well known.

3. Part Results and Discussion: Table 1 (Vinasse component generation 1 and 2) has been copied from the introduction of source [41] and has not to be shown in its full volume in this part. Some values can be used only for comparison of the results.
The text on page 12 repeats a text from page 11.
It is discussed the role in the process of composting only of the microbial groups with minor content in the start product (Table 4).

- fixed by deleting the previous Table 1, and restructured the content become much understandable. While for repetition and we couldn't find it since page 11 and 12 are references. The paper investigated the most important aspect of microbial species for fermentation especially who have a big contribution for it.

4. English text is very careless. It needs serious correction

- Fixed by proofreader

The proliferation of effective microorganism (EM) in vinasse and its application in the manufacture of livestock-waste based fertilisers

Triastuti Sulistyaningsih[1)], Nuni Widiarti[1)], Widi Astuti[2)], Dewanto Harjunowibowo[3)4)]

[1)]Chemistry Departement, Faculty of Matematics and Natural Science, Universitas Negeri Semarang, Indonesia

[2)]Chemical Engineering, Faculty of Engineering, Universitas Negeri Semarang, Indonesia

[3)]Physics Education Department, University of Sebelas Maret, Surakarta, Indonesia

[4)]Department of Architecture and Built Environment, University of Nottingham, UK

E-mail: triastuti.s@mail.unnes.ac.id, dewanto_h@fkip.uns.ac.id

ABSTRACT

Applying long term vinasse without pre-treatment to soil causes eutrophication to water and soil. The paper aims to process vinasse of alcohol industry as an effective growing medium of microorganisms that environmental-friendly and can be used as starter bacteria organic fertiliser manufacture. The effective microorganism has some benefits such as becoming a soil enhancer when applied directly on the soil or an accelerator in the maturation of organic fertiliser. This research began with the chemical content characterisation of vinasse and the addition of microorganisms nutrients. After the media was ready, the EM inoculum was added and the breeding result was used for the manufacture of organic fertiliser based on goat farm waste. The EM culture analysis revealed an increase in microbial number. Moreover, for organic fertiliser, revealed that the content of organic matter, nitrogen,

phosphate, and potassium were 43.42%, 3.05%, 0.40% and 1.23%, respectively. It is in accordance with 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 sources of non-renewable energy become very limited and is suspected as the primary cause of environmental damage. The United Nation issued The 2030 Agenda for Sustainable Development to increase the global energy production generated from renewable sources by 2030 to tackle the climate change and its impacts on every country [4]. Therefore, biofuel such as biodiesel [5,6] and bioethanol becomes a favourable alternative to substitute the fossil fuel [7,8]. As a result, around 80% carbon emissions was reduced because of bioethanol usage [9].

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

In 2015, Indonesia produced 450 million litter bioethanol from sugarcane but it only contributed around 1% of total gasoline consumption. Therefore, the government policy supports the industries to reach 11.48 billion litre of production in 2025 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 high organic content, the distillated water contains nutrients in the form of nitrogen (1,660-4,200 mg/L), phosphorus (225-3,038 mg/L) and potassium (9,600 -17,475 mg/L) [24], and it is also very high Chemical Oxygen Demand (COD) (80,000-100,000 mg/L) and Biochemical Oxygen Demand (BOD) (40,000-50,000 mg/L) [25]. Furthermore, vinasse has low pH, strong odor, corrosive and dark brown color [26] that cause eutrophication of water bodies. Hence, the negative effects of bioethanol industries' by-products on the environment are an urgent issue to address and to tackle.

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 nutritious. The EM culture which contains a useful natural organism can improve soil fertility by enriching the diversity of soil microbial ecosystems. In addition, the application of soil fertiliser is also discussed.

EXPERIMENTAL

Material

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

Procedure

Liquid waste alcohol characterisation. Before used, the vinasse was characterised by its composition (pH, C organic, Organic matter, N, C/N Rasio, P_2O_5, K_2O, Ca, Mg) in laboratory. Organic carbon and organic matter were analysed using Walkley and Black method in Microbiology Laboratory of UNS. Basically, 1 N $K_2Cr_2O_7$ solution was used to oxidise the soil, assisted by heat. The heat was 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. Inversely titre represents the amount of C in the soil.

Breeding EM. A thousand millilitres of vinasse was sterilised by boiling and the mixture of 50g shrimp paste, 100g herbs, 50g urea, 100g rice bran and 50 ml molasses of sugar cane had been diluted in 1000 ml of aquadest. The mixture was boiled again, then cooled in a temperature of 25 °C and 5 mL of microorganisms inoculant was added. The mixture was placed in a tightly sealed container. After a month, the breeding result of the microbe was assayed in laboratory and analysed.

The method used to estimate the concentration of microorganisms in the sample was Most Probable Number (MPN) method [28]. The procedure was done by means of replicating liquid broth growth in ten-fold dilutions.

Composting. One quintal of livestock waste was mixed with 100 ml of diluted EM, resulting 1000 ml of aquadest solution. After mixing evenly with 60% of water, the mixture was covered in a thick plastic to allow the anaerobic process. Mixture reversal was done every five days to control the temperature and moisture content of the material. Temperature measurements were carried out every day until the temperature equals the room temperature,

Commented [BSS1]: Plastic reversal?

and constant was the sign that the compost has matured. The product then was tested and analysed in laboratory.

Nitrogen was analysed using Kjeldahl Auto Distillation method. The method procedure involves 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 completed, the sample was diluted with water and distilled to convert the NH_4^+ into NH_3 by adding alkaline. The ammonium ions yielded was captured by absorbing solution and the concentration of the captured ammonium ions was determined using direct titration.

RESULT AND DISCUSSION

Molasses is a viscous liquid, tastes a bit bitter and dark colour, similar with the sugar factory production [29] and contains many minerals [25]. Usually, one tonne of fresh sugar cane can produce 4-5% molasses [30]. Molasses are primarily used worldwide as animal food because it increases the growth of microbes in rumen animals that improve the digestion of fibre and non-protein nitrogen [31]. It indicates that vinasse is very potential to be reused.

However, as can be seen in Figure 1a, vinasse contents like inorganic minerals, organic pollutants, suspended substantial, and corrosiveness has high toxicity and low biodegradability [32]. Continuous direct vinasse application to soil for 7 years causes cane maturation and reduction in sucrose content as a consequence of potassium excess [33]. Therefore, before being utilised in breeding medium, the vinasse sample was tested in laboratory to investigate the original composition. For this purpose, the liquid waste condition of the alcohol used in this study was the thick, black and had a sweet aroma while the unprocessed one was brown and separated between the sugar solution and water, and stench

(Figure 1b). In addition, the proximate laboratory test of alcohol wastes sample of molasses-based as shown in Table 1 still contain lots of organic material.

Table **1** shows high organic matter, carbon, and nitrogen which contained in alcohol wastewater. Thereby, the nutrition can be used as a media for the breeding of soil microbial degradation. The most important nutrients for microbes are carbon and nitrogen since nearly 50% of the dry weight of the cell consists of carbon. The autotroph prokaryotes use CO as the only source of carbon, whereas heterotrophs use organic molecules as a source of carbon for growth [34].

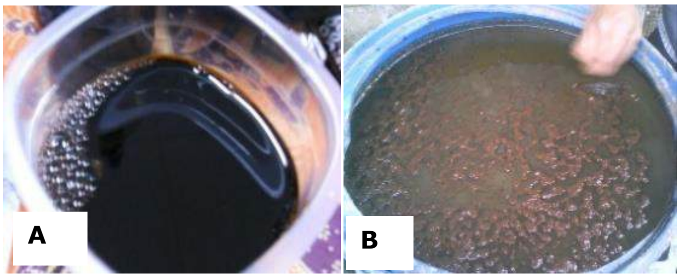

Figure 1. The appearance of liquid-waste industry of bioethanol (a) good, and (b) bad

Furthermore, nitrogen is needed in the proliferation of bacteria because nitrogen plays a vital role in cellular metabolism especially in cell division. Therefore, if the nitrogen content is less, the ability of bacteria to self-divide becomes slower. As a result, bacterial growth becomes low. In fact, bacteria can assimilate organic and inorganic nitrogen compounds for their growth. Hence, the nitrogen compound in such form will be reduced or there will be catabolism by bacteria into ammonia [35].

Table 1. Chemical composition of vinasse from Bekonang alcohol industry

Parameter	Method	concentration (%)

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

In growing bacteria, an alternative media to grow microbes by using materials that are cheap and easy to obtain was used, such as vinasse, rice bran, shrimp paste, molasses and urea. The average composition of rice bran according to the quality requirements is presented in Table 2. These materials contain nutrients which can be used by the effective microorganism to grow and multiply appropriately [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 yielded. For the result, the microbial proliferation with the vinasse media is presented in Table 3.

Table 2. The Average Nutrients Composition of the Rice Bran (w/w) [37]

Parameters	%
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

Based on Table 3, it maps out that bacteria *Lactobacillales sp*., yeast, and total microbial increased. *Lactobacillales* is responsible for the production of lactic acid in the early stages of composting and anaerobic digestion process. At the beginning of composting, other bacterial groups may be reduced due to the presence of *Lactobacillales* which produces hydrogen peroxide, bacteriocin and antibiotics [38]. Furthermore, the low amounts of indigenous microorganism inoculums which is less than 20% can cause insufficient sources for the composting [39]. Besides, the number of phosphate solubilising bacteria (PSB) cannot breed well and decrease the number of bacteria. Whereas, PSB plays an essential role in the manufacture of fertiliser because it can increase the phosphate levels in the fertiliser [40,41].

Table 3. Results of microbial analysis in soil fertiliser starter products

Microbial group	Initial (cfu/mL)	Final (cfu/mL)
Phosphate solubilizing bacteria (PSB)	$7,5 \times 10^6$	$2,3 \times 10^5$
Lactobacillales sp.	$8,7 \times 10^5$	$4,9 \times 10^7$
Yeast	$8,5 \times 10^6$	$3,9 \times 10^7$
Microbial total	-	$3,4 \times 10^{10}$

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

Table 4. The result of organic fertilisers (manure) and planting media analysis

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

Table 4 shows the organic materials content, N, P, and K total in the manure yielded was in accordance with SNI 19-7030-2004. In addition, The regulation of the Agriculture Minister 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 for organic matter is 15%, and for nutrient content (N + P_2O_5 + K_2O) is 4% [45]. In this research, the sum of organic matter and nutrient content (N + P_2O_5 + K_2O) is higher than the regulation. The compost produced is better than planting media which is defined as a mixture of manure: soil and charcoal husk. Moreover, compared to pure manure, the compost resulted shows the superiority.

CONCLUSION

The experimental study of recycling vinasse as medium growth for natural beneficial organisms using EM and use it to produce fertiliser from livestock waste in a simple method was successfully conducted. Based on the discussion, it clearly shows that Vinasse could be used as an alternative media of effective microorganisms cultivation and then used as microbial accelerator of organic fertiliser maturation. Organic fertiliser produced from goat livestock waste has high levels of organic materials, N, P and K and in accordance with those set out in Indonesian government regulations.

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

Triastuti Sulistyaningsih[1], Nuni Widiarti[1], Widi Astuti[2], Dewanto Harjunowibowo[3,4]

[1] Chemistry Department, Faculty of Matematics and Natural Science

Universitas Negeri Semarang, Indonesia

[2] Chemical Engineering, Faculty of Engineering

Universitas Negeri Semarang, Indonesia

[3] Physics Education Department, University of Sebelas Maret, Surakarta, Indonesia

[4] Department of Architecture and Built Environment, University of Nottingham, UK

E-mail: triastuti.s@mail.unnes.ac.id, dewanto_h@fkip.uns.ac.id

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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]. Wereas, *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) 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.

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]. 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 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 as the only source of carbon, whereas heterotrophs use organic molecules as a source of carbon for growth [34].

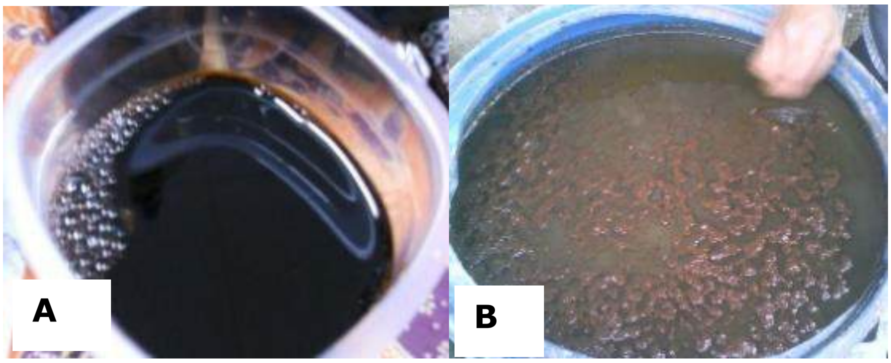

Fig. 1. An appearance of the liquid waste of bioethanol production: (a) a good one; (b) a bad one.

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].

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

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 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

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

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)	$7{,}5 \times 10^{6}$	$2{,}3 \times 10^{5}$
Lactobacillales sp.	$8{,}7 \times 10^{5}$	$4{,}9 \times 10^{7}$
Yeast	$8{,}5 \times 10^{6}$	$3{,}9 \times 10^{7}$
Microbial total	-	$3{,}4 \times 10^{10}$

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. 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

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

Triastuti Sulistyaningsih[1], Nuni Widiarti[1], Widi Astuti[2], Dewanto Harjunowibowo[3,4]

[1] *Chemistry Department, Faculty of Matematics and Natural Science Universitas Negeri Semarang, Indonesia*
[2] *Chemical Engineering, Faculty of Engineering Universitas Negeri Semarang, Indonesia*
[3] *Physics Education Department, University of Sebelas Maret, Surakarta, Indonesia*
[4] *Department of Architecture and Built Environment, University of Nottingham, UK*
E-mail: triastuti.s@mail.unnes.ac.id, dewanto_h@fkip.uns.ac.id

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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) 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.

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

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

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

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]. 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 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 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

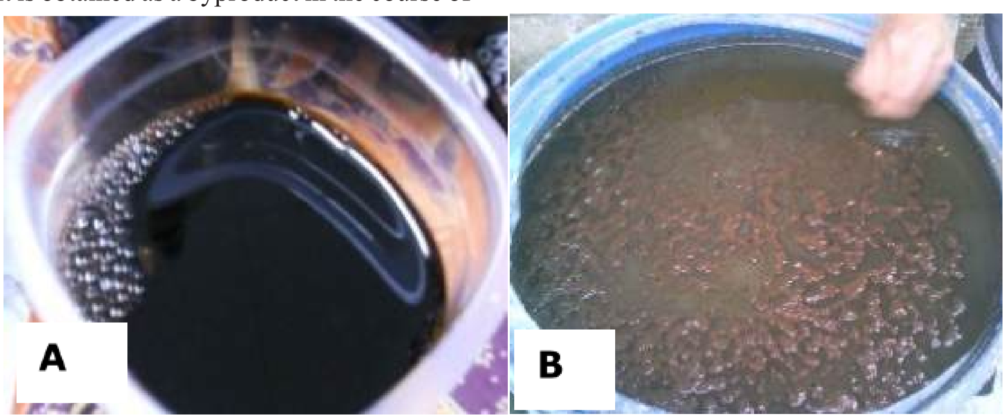

Fig. 1. An appearance of the liquid waste of bioethanol production: (a) a good one; (b) a bad one.

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 x 10^6	2,3 x 10^5
Lactobacillales sp.	8,7 x 10^5	4,9 x 10^7
Yeast	8,5 x 10^6	3,9 x 10^7
Microbial total	-	3,4 x 10^{10}

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 production 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 Min-

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

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

Triastuti Sulistyaningsih[1], Nuni Widiarti[1], Widi Astuti[2], Dewanto Harjunowibowo[3,4]

[1] Chemistry Department, Faculty of Matematics and Natural Science Universitas Negeri Semarang, Indonesia
[2] Chemical Engineering, Faculty of Engineering Universitas Negeri Semarang, Indonesia
[3] Physics Education Department, University of Sebelas Maret, Surakarta, Indonesia
[4] Department of Architecture and Built Environment, University of Nottingham, UK
E-mail: triastuti.s@mail.unnes.ac.id, dewanto_h@fkip.uns.ac.id

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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) 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 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].

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

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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