Bioaugmentation in domestic and organic wastewater for plant fertilizers

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Abstract. Water pollution is one of the world’s main concerns today. Governments in various countries have tried to find solutions for reducing this problem. Indonesia has a great diversity of local microorganisms. Pichia kudriavzevii UMJ-L, Trichosporon asahii UMJ-A2, Burkholderia ambifaria UMJ-A1, Burkholderia stabilis UMJ-R, Gluconacetobacter saccharivorans UMJ-K, and Pseudomonas putida are local microorganisms that can be used for bioaugmentation in wastewater. The results of bioaugmentation of domestic and organic wastewater not only can be used for waterering plants but also expected to have a positive value as fertilizer for the growth or production of some plants. Microorganisms in this study were the result of isolation, which is made of pellets from rice flour/bran. The pellet after mashed is used as an inoculant which acts as bioaugmentation of incubated wastewater for 4 days. The results showed that bioaugmentation fertilizer from domestic wastewater was better than other wastewater when used as fertilizer on plants. Better results, all wastewater still requires the addition of inorganic fertilizers between 25% to 50% because of the low nutrients content.

1. Introduction
Water pollution is one of the main concerns of the world today. Governments in various countries have tried to find solutions for reducing this problem [1], so have Indonesia. Water pollutants will threaten the water supply. Liquid waste that is not properly managed can cause environmental problems related to living things that grow in it and human health. Limited water supply is an impetus for recovery and reuse of limited resources [2]. Prevention of the onset of environmental pollution needs to be continued because it has been known that the dangers of it will cause social, economic, and environmental losses. Therefore, there must be a special management of the waste in order to eliminate or reduce its hazard. Management methods also need to be endeavoured to be environmentally friendly with proper and careful supervision by various parties [3].

Industrial waste has a lot of standard treatment with a good system, but at the community level, public awareness about the danger of wastewater is still lacking. The wastewater disposal system commonly used by the community is wastewater from the toilet (Blackwater) flowed into the septic tank, the runoff then grounded or flowed into the public channel (sewer). Non-toilet wastewater (greywater), which originating from bathrooms, washing clothes and kitchen waste, is usually channeled directly into drains and rivers. This is the same with home industry businesses such as tofu making. Wastewater of rice, domestic wastewater (greywater) and organic wastewater from this tofu factory will...
be discussed further. Pollution caused by wastewater has shown a quite serious level due to the lack of municipal wastewater treatment facilities (sewerage system) [4].

Study of Environmental Health Risk Assessment (EHRA) in the city of Bogor [4] the majority of household waste originating from kitchens, bathrooms, washing clothes and sinks flowed most into the river (60.24%) and into open channels (15.07%). Some residents channeled it into dug holes (7.22%), closed channels (6.15%), and as many as 0.73% dumped it to the road.

Metropolitan cities also have problems with the high volume of wastewater that pollutes river water. The survey results in Tangerang showed that most (84%) of the water pollution load comes from community domestic wastewater [5]. The results of the Malang City Environment Agency (BLH) study from year to year state that domestic waste contributes 60-70 percent in water pollution [6]. Therefore, one area and city need to take a policy to further emphasize independent management at the household scale, especially the communal scale such as housing, apartments, flats, boarding schools, offices, shops as well as the campus.

Bioremediation according to [7] is one method for cleaning up contamination in the environment through the activity of living organisms. Bioaugmentation strategy is a bioremediation which in some cases, microbial organisms that can reduce contaminants need to be added. Biostimulation Strategy - in some cases, natural populations can be stimulated to reduce contaminants by adding something, such as nutrition and aeration.

Indonesia has a great diversity of local microorganisms. Pichia kudriavzevii UMJ-L, Trichosporon asahii UMJ-A2, Burkholderia ambifaria UMJ-A1, Burkholderia stabilis UMJ-R, Gluconacetobacter saccharivorans UMJ-K, and Pseudomonas putida are local microorganisms that can be used for bioaugmentation in wastewater [8].

Problems related to wastewater and its effects, the development of science and technology becomes crucial that must be utilized as a solution in the field of domestic wastewater treatment. The selected wastewater treatment technology must be able to improve the quality of effluent water chemically, physically, and biologically. Therefore, the communal scale of domestic wastewater treatment can be an option to ease the burden on local / city governments so that the environment is more sustainable, utilizing domestic and organic wastewater to be more valuable as fertilizer for plants.

2. Material and Method

2.1. Microbial
Microbes were obtained from wastewater of rice, cassava tape, and Kombucha, respectively Pichia kudriavzevii UMJ-L, Trichosporon asahii UMJ-A2, Burkholderia ambifaria UMJ-A1, Burkholderia stabilis UMJ-R and Gluconacetobacter saccharivorans UMJ-K[8]. Pseudomonas putida bacteria is obtained from the Indonesian Institute of Sciences (LIPI).

Microbial is made into pellets that have the basis of rice bran/Flour. There are 3 variations of pellets containing microbes used in this study, due to the development of the research over time. The pellets used are as follows:
- Pellet 1 contains Pi. kudriavzevii UMJ-L, T. asahii UMJ-A2, B. ambifaria UMJ-A1, B. stabilis of UMJ-R and G. saccharivorans UMJ-K.
- Pellet 2 contains pellet 1 without B. stabilis UMJ-R
- Pellet 3 contains Pellet 2 + Ps. putida

2.2. Pellet Application in Wastewater
Pellets containing microbes are inoculated into 2-3 g/L waste. Then pellets are incubated for 4 days, shaken with shaker/manually 1-2 times a day.

2.3. Nutrient in Wastewater
The pH analysis was carried out at the Laboratory of the Faculty of Agriculture UMJ. Nutrient content carried out in competent and certified institutions.
2.4. Utilization of Wastewater as Plant Fertilizers

Plants are planted in different locations and times for each plant. The designs used in this study all use the Randomized Complete Group Design (RCGD). The response of plants to the treatment is known from the data analyzed using the F test, then followed by Post-hoc test using the Honestly Significant Difference (HSD) test.

2.4.1. Rice Wastewater Application for Tuberose Plant (Polianthes tuberosa L.).

Bioaugmentation of rice wastewater using Pellet 1 and Pellet 2 according to the treatment. Fertilizers resulting from wastewater bioaugmentation with pellet 1 hereinafter referred to as FWB1, and Pellet 2 called FWB2. Control with recommended fertilizer 5g NPK Grower and 5g Saprodap per plant. Waste that has been incubated for 4 days is poured into plants as much as 100 mL per plant per week. The study consisted of five treatments:

- P0 = 100% inorganic fertilizer (control)
- P1 = FWB1 + 50% inorganic fertilizer
- P2 = FWB1 + 25% inorganic fertilizer
- P3 = FWB2 + 50% inorganic fertilizer
- P4 = FWB2 + 25% inorganic fertilizer

Each treatment was repeated five times so that there were 25 experimental units. Each plot has 20 plants. Five plants/plots were observed so that the number of plants observed in this study was 125 plants.

2.4.2. Tofu Wastewater Application for Shallots.

Tofu wastewater bioaugmentation using pellet 3 hereinafter referred to as FWB3. Waste that has been incubated for 4 days is poured into the plant as much as 1L per plot. Watering is done once a week. Basic fertilizer uses 5 tons/ha of chicken manure. Control with recommended dosage fertilizer (100% inorganic) using Urea 100 kg/ha, Za 200 kg/ha and KCl 100 kg/ha. The study consisted of four treatments:

- P0 = 100% inorganic (control)
- P1 = without Bioaugmentation
- P2 = FWB3
- P3 = FWB3 + 50% inorganic fertilizer.

Each treatment was repeated 3 times so that there were 12 experimental units (plots). Each plot of land contained 20 plants and 5 plants/plot were observed so that the total number of plants planted in this study was 240 plants and the number of plants observed was 60 plants.

2.4.3. Rice Wastewater Application for Tomato Plants.

Bioaugmentation of rice wastewater using pellet 3, hereinafter referred to as FWB3. Control with a recommended dosage of NPK 15: 15: 15 as much as 5g (100%). Liquid Biofertilizer (LB) using Biohouse Gold® 2mL at 4-day intervals. Waste that has been incubated for 4 days is poured into plants as much as 100 mL per plant per week. The study consisted of 5 treatments:

- P0 = 100% inorganic fertilizer
- P1 = LB + 25% inorganic fertilizer
- P2 = FWB3 + 25% inorganic fertilizer
- P3 = PHC
- P4 = FWB3

Each treatment was repeated 5 replications so that there were 25 experimental units. Each experimental unit consisted of 3 plants, so the number of plants studied was 75 plants in polybags.

2.4.4. Domestic Wastewater Application for Banana Plants.

Bioaugmentation of domestic wastewater using Pellet 2, hereinafter referred to as FWB2, Pellet 3 hereinafter referred to as FWB3. Waste that has been incubated for 4 days is poured into plants as much as 100 mL per plant per week. P0 is the control using inorganic fertilizer with recommended dosage of
NPK 16:16:16 as much as 3.15g (100%) and vitamin B1 as much as 1 tablespoon with 5 liters of water (ratio of ± 1: 500). The study consisted of 5 treatments:

- P0 = 100% NPK + Vitamin B1 (Control)
- P1 = FWB2 + NPK 25%
- P2 = FWB3 + NPK 25%
- P3 = FWB2
- P4 = FWB3

Each treatment was repeated 5 times so that there were 25 experimental units. Each experimental unit consisted of 3 plants, so the number of plants studied was 75 plants in polybags.

### 3. Results and Discussion

Microbes used in the bioaugmentation process in wastewater are microbes resulting from isolation and identification carried out by [8]. The inoculants are two yeasts derived from rice wastewater and one bacterium, respectively *Pichia kudriavzevii* UMJ-L, *Trichosporon asahii* UMJ-A2, and *Burkholderia ambifaria* UMJ-A1. Two other bacteria are derived from *tape* yeast and kombucha, respectively *Burkholderia stabilis* UMJ-R, and *Gluconacetobacter saccharivorans* UMJ-K. *Pseudomonas putida* comes from the Indonesian Institute of Sciences (LIPI).

The manufacture of pellets used for bioaugmentation of wastewater with a benchmark concentration should reached the number of living cells 10⁷-10⁸ cells/mL according to Minister of Agriculture Regulation No. 70/2011. At the beginning of the study, the benchmark concentration was not reached because per gram of live cell in pellets only reached 10⁵ cells, so the use of pellets is 3g/L wastewater applications. After the concentration of living cells reached >10⁶ cells/g pellets, the use of pellets is only 2g/L.

#### 3.1. Nutrients in Organic Wastewater

Comparison of the provision of pellets 1, pellets 2 and EM4 in wastewater of rice [8] yields a small percentage of NPK nutrient content, which has not been able to meet the fertilizer needs in plants. Likewise with augmentation using 2 microbes, *Pi. kudriavzevii* UMJ-L and *Pseudomonas putida* in tofu wastewater (Whey) Table 1. The fermentation results show that FWB alone cannot meet the needs of plant nutrients. Nitrogen and Phosphorus percentages in tofu wastewater are lower than there in the rice wastewater while potassium is better in tofu wastewater. However, compared with cow manure which has N, P₂O₅ and K₂O contents, respectively 0.29%; 0.17% and 1.5%, the nutrient content in wastewater is relatively low. So did when it compared with chicken manure which has higher nutrients than other animal manure [9].

| Parameters      | Unit | Bioaugmentation Results |
|-----------------|------|-------------------------|
|                 |      | Tofu wastewater         | Wastewater of rice (4 days) [8] |
|                 |      | (3 days) 2 microbes     | 5 microbes | 4 microbes | EM4 |
| Nitrogen (N)    | %    | 0.05                    | 0.12       | 0.09       | 0.12 |
| Phosphorus (P)  | %    | 0.03                    | 0.05       | 0.04       | 0.03 |
| Potassium (K)   | %    | 0.11                    | 0.04       | 0.04       | 0.01 |

#### 3.2. Utilization of Wastewater as Plant Fertilizers

**3.2.1. Rice Wastewater Application for Tuberose Plant (Polianthes tuberosa L.).**

The fermentation results of rice wastewater with microbial bioaugmentation mentioned above are used as fertilizer in tuberose plants. The results of bioaugmentation were used as fertilizer, 25 - 50% of inorganic fertilizers added to the treatment in Table 2. Overall bioaugmented rice wastewater (FWB1 and FWB2) and control were not significantly different in all treatments based on the Honestly
The results of augmentation with 4 microbes (FWB2), namely B. ambifaria, G. saccharivorans, P. kudriavzevii, and T. asahii plus inorganic fertilizer 50% is faster flowering time, the most number of flowers, and better harvest amount.

**Table 2. Fertilization Response to Tuberose (Polianthes tuberosa L.) Crop Growth and Production [8].**

| Treatment                  | Flowering time (days) | Length of stalk (cm) | Stalk diameter (cm) | Number of florets per panicle | Harvest time (days) | Amount of harvest |
|----------------------------|-----------------------|----------------------|---------------------|-----------------------------|---------------------|-------------------|
| P0=100% inorganic          | 91.50±               | 108.56±              | 0.712±              | 34.50±                      | 108.42±             | 2.33±             |
| P1=FWB1+50% inorganic      | 95.17±               | 105.06±              | 0.644±              | 29.39±                      | 108.89±             | 2.33±             |
| P2=FWB1+25% inorganic      | 87.60±               | 108.70±              | 0.661±              | 32.10±                      | 102.40±             | 2.00±             |
| P3=FWB2+50% inorganic      | 84.25±               | 112.33±              | 0.740±              | 36.33±                      | 101.02±             | 3.00±             |
| P4=FWB2+25% inorganic      | 95.50±               | 113.75±              | 0.760±              | 32.50±                      | 116.50±             | 3.00±             |

*±The numbers followed by the same letters in the same column are not significantly different based on the Honestly Significant Difference (HSD) test at the 5% level.*

In economic terms, the results of augmentation with 4 microbes (FWB2) added with 25% of inorganic fertilizer can significantly save the operational costs of tuberose farming on land [8], [10] also showed that fermentation of rice wastewater with EM4 for 4 days and natural fermentation for 2 weeks was not significantly different in some types of vegetables and orchids. Therefore, the provision of these microbes can be used as a microbial substitute for EM4 in rice wastewater used as fertilizer. EM4 is widely used to make organic fertilizer [11] - [13].

**3.2.2. Tofu wastewater Application for Shallots.**

In this research, Pseudomonas putida was tried to be added in pellets (FWB3), so that the benefits of pellets were better. The ability of Ps. putida is known, among others, as a biocontrol of disease in the soil and produces growth hormones [14]. Pseudomonas putida and Trichoderma atroviride produce indole acetic acid (IAA) as a possible mechanism for stimulating plant growth [15]. Pseudomonas putida H-2-3 effectively increases the length of shoots and the fresh weight of plants that are stressed by salt and drought. Chlorophyll content is lower in abiotic stress conditions and bacterial inoculants Ps. putida H-2-3 reduces stress effects with evidence of a higher amount of chlorophyll content in plants exposed to salt and drought [16].

The analysis result of the treatment with 100% inorganic fertilizer is seen significantly different from other treatments. Tofu wastewater that was fermented with Ps. putida, B. ambifaria, G. saccharivorans, P. kudriavzevii, and T. asahii (FWB3) or not, the results are not significantly different, but the production is better after added with 50% inorganic fertilizer, but still the 100% inorganic fertilizer is better. Fertilizers from rice wastewater which have been bioaugmented and added 25% - 50% inorganic fertilizer, are not significantly different from the control of 100% inorganic fertilizer when applied to the tuberose plants above. This could be due to slightly lower nitrogen and phosphorus tofu wastewater analysis results and or because the content of the elements that were not analyzed better in the rice wastewater (Table 1). The influence of the type and variety of microbes or wastewater certainly also affects the diversity of elements produced and needed by plants. Sumenep variety of shallots yields is low for tons/ha unit (Table 3), because the land is not added with basic fertilizer. This avoids the influence of a more dominant manure. Sumenep variety planted in Bengkayang West Kalimantan dry lowlands can produce shallots 6.34 tons/ha [17].
Table 3. Fertilizing Response to the Production of Shallot Bulbs.

| Treatment                          | Weight of Dry Bulb per sample (g) | Weight of Dry Bulb per plot (g) | Weight of Dry Bulb Per hectare (ton) |
|-----------------------------------|-----------------------------------|---------------------------------|-------------------------------------|
| P0 = 100% inorganic (control)     | 10,00\(^b\)                       | 295,50\(^b\)                   | 2,05\(^b\)                           |
| P1 = without Bioaugmentation      | 8,20\(^a\)                       | 225,25\(^a\)                   | 1,56\(^a\)                           |
| P2 = FWB3                         | 6,57 \(^a\)                      | 222,50\(^a\)                   | 1,55\(^a\)                           |
| P3 = FWB3 + inorganic 50%         | 6,97 \(^a\)                      | 250,25\(^ab\)                  | 1,74\(^ab\)                          |

\(^{ab}\) The numbers followed by the same letters in the same column are not significantly different based on the Honestly Significant Difference (HSD) test at 5% level.

3.2.3. Rice Wastewater Applications for Tomato Plants.
In general, 100% inorganic fertilizer is not significantly different from all treatments. The use of liquid biofertilizer (LB) from the market and liquid organic fertilizer originating from rice wastewater fermented with microbial B. ambifaria, G. saccarivorans, T. asahii, Pi. kudriavzevii and Ps. putida (FWB3) can be used as fertilizer. In general, the application of 25% fertilizer shows better results on biological and organic fertilization compared to not added with inorganic fertilizer. However, compared to the criteria of Lentana variety of Tomato planted in the land that has fruit between 13-15 fruits/ plant, weight per fruit 70-80g, and diameters 4,4 cm, the tomato production in polybags in this study is still too low, this is due to the treatment is not given basic fertilizer as it should. So with the tuberose plant, agronomically, it is better to fertilize with FWB added 50% inorganic fertilizer, although that is not significantly different from 25%. In some studies also proved that biological fertilizers and organic fertilizers still require the addition of inorganic fertilizers around 50% [10] [18] - [20].

Table 4. Comparison of Several Types of Fertilizers in Tomato Fruit Production.

| Treatment                          | Number of Fruits Per Plant (Fruit) | Fruit Weight Per Plant (g) | Weight Per Fruit (g) | Fruit Diameter (cm) |
|-----------------------------------|-----------------------------------|---------------------------|---------------------|--------------------|
| P0 = 100% inorganic               | 5.7\(^a\)                        | 224.09\(^a\)             | 37.84\(^a\)         | 4.06\(^a\)         |
| P1 = LB + inorganic 25%           | 7.4\(^a\)                        | 229.68\(^a\)             | 36.08\(^a\)         | 3.94\(^a\)         |
| P2 = FWB3 + inorganic 25%         | 6.1\(^a\)                        | 190.92\(^a\)             | 29.92\(^a\)         | 3.79\(^a\)         |
| P3 = LB                           | 3.6\(^a\)                        | 99.73\(^a\)              | 27.45\(^a\)         | 3.48\(^a\)         |
| P4 = FWB3                         | 5.3\(^a\)                        | 113.93\(^a\)             | 28.18\(^a\)         | 3.49\(^a\)         |

\(^{a}\) Numbers followed by the same letter in the same column are not significantly different based on the Honestly Significant Difference (HSD) test at 5% level.

3.2.4. Domestic Wastewater and Element N Analysis for Cavendish Banana Seedlings.
Based on further testing Honestly Significant Difference (HSD) level at 5% on the seedlings weight gain (seed weight at 12 WAP reduced by weight of seed at 0 WAP), the heaviest is FWB3 plus NPK 25% with 81.38 grams, significantly different from NPK treatment of 100% + vitamin B1 (control). In FWB3, plant biomass increased because the symbiotic frequency of endomycorrhizal fungi in plants increased due to the presence of metabolites produced by Ps. putida [23]. The treatment with FWB plus inorganic fertilizer or not, were significantly different from the controls. Tofu wastewater or rice wastewater compared with domestic wastewater generally shows the best results, because there is no need to add inorganic fertilizer or only add 25% inorganic fertilizer (Table 5).
of microbes and incubated for 4 days, there are greatly increased free ammonia in the shape of NH4+ to NO3− with NH4+ or NO3− (phospholipids), and phosphoproteins [22]. Nitrogen is generally absorbed by plants in the form of ATP and other nucleoproteins), genetic information systems (DNA and RNA), cell membranes (phospholipids), and phosphoproteins [22]. Nitrogen is generally absorbed by plants in the form of NH4+ or NO3−, which is influenced by soil properties, plant types and stages in plant growth. In soil with good treatment, N is absorbed by plants in the form of nitrate ions because there has been a change in the shape of NH4+ to NO3−, conversely, in stagnant soil plants tend to absorb NH4+ [23]. However, N is an element that is mobile, easily leached and volatile, so plants often experience deficiency [24].

Table 5. Weight gain of banana seedlings after 12 weeks after planting (WAP).

| Treatment                          | Banana plant weight gain at 12 weeks |
|------------------------------------|--------------------------------------|
| P0=NPK 100% + Vitamin B1           | 56.06 a                               |
| P1=FWB pellets 2 + NPK 25%         | 78.89 ab                              |
| P2=FWB pellets 3 + NPK 25%         | 81.38 b                               |
| P3=FWB pellets 2                   | 79.73 ab                              |
| P4=FWB pellets 3                   | 76.46 ab                              |

ab Numbers followed by the same letter in the same column are not significantly different based on the Honestly Significant Difference (HSD) test at 5% level.

Nutrient content in domestic wastewater is more complex than tofu wastewater, rice wastewater, or canteen waste. The content of domestic wastewater is more complex because not only consisted of organic wastewater from the kitchen but also bathroom wastewater, dishwashing wastewater, and clothes wastewater. Canteen wastewater usually contains more organic matter, including oil, so the BOD and COD are high compared to domestic wastewater. From the analysis of N content (Table 6), domestic wastewater has higher free ammonia, nitrate and nitrite contents than the initial canteen wastewater. After the addition of microbes and incubated for 4 days, there are greatly increased free ammonia in canteen wastewater, slight increase in nitrite and decrease in nitrate content. Unlike the domestic wastewater case, free ammonia and nitrite content decreases, and nitrate increases (Table 6).

Table 6. N Content in Domestic Wastewater and Canteen Wastewater.

| Parameter for Analysis | Unit | Canteen Waste Water Early | 4 Days a | Domestic Waste Water Early | 4 Days a |
|------------------------|------|---------------------------|----------|---------------------------|----------|
| Free Ammonia (NH3-N)   | ppm  | 0.012                     | 3.09     | 2.46                      | 1.40     |
| Nitrate (NO3-N)        | ppm  | 1.51                      | 0.46     | 3.04                      | 8.58     |
| Nitrite (NO2-N)        | ppm  | 0.012                     | 0.019    | 0.105                     | 0.058    |

a Bioaugmentation

If the element N in domestic wastewater that has become FWB is analyzed, it still much smaller compared to manure [9], so FWB is better if it remains with inorganic fertilizer 25-50%. If the results are better than the control of banana nurseries in this study, it is more due to the availability of basic plant fertilizers and growth hormones produced by FWB. Nitrogen (N) and Phosphorus (P) are nutrients needed by plants in large quantities. Nitrogen is an important element in the formation of chlorophyll, protoplasm, protein, and nucleic acids. This element has an important role in the growth and development of all living tissues [21]. Phosphorus is an important component of compounds for energy transfer (ATP and other nucleoproteins), genetic information systems (DNA and RNA), cell membranes (phospholipids), and phosphoproteins [22]. Nitrogen is generally absorbed by plants in the form of NH4+ or NO3−, which is influenced by soil properties, plant types and stages in plant growth. In soil with good treatment, N is absorbed by plants in the form of nitrate ions because there has been a change in the shape of NH4+ to NO3−, conversely, in stagnant soil plants tend to absorb NH4+ [23]. However, N is an element that is mobile, easily leached and volatile, so plants often experience deficiency [24].

Table 1 and Table 6 show that the nutrient content of wastewater is small, but the application of inorganic fertilizer 25-50% can increase plant growth. This is possible because the microbes used to
produce the growth hormones that plants need. Trichoderma atroviride and Ps. putida can produce IAA [15] while Ps. putida and Ps. fluorescens can produce Plant Growth-Promoting Rhizobacteria (PGPR) [25], also the ability of Pi. kudriavzevii to produce IAA [26, 27].

Ps. putida bacteria used in this study were isolates obtained from the Indonesian Institute of Sciences (LIPI). The leaves of Nicotiana benthamiana necrosis are reduced dramatically during co-infection with Ps. putida and Xanthomonas campestris. Such protection is related to the activity of Ps. putida T6SS. Many ways have been explored in the development of biocontrol agents that can manipulate the composition of the rhizosphere and phyllosphere microbes. A new way of biocontrol for these plants was made by Ps. putida [28]. Pseudomonas putida KT2440 has the potential to induce anthracnose fungal (Colletotrichum graminicola) resistance in corn [29].

Pseudomonas putida is a saprophytic bacterium with exceptional environmental adaptability to tolerate high concentrations of heavy metals. Pseudomonas putida CD001 strains were isolated from soil contaminated with Cd, Zn and Pb [30]. Pseudomonas putida in this study and subsequently is expected to play a role in many things. In addition to the capabilities mentioned above, these bacteria play a role in the process of ammonia formation in wastewater, so the role of nitrifying bacteria is needed to convert ammonia into nitrate which can be used as a nutrient by primary organisms. The process of converting ammonia to nitrate occurs aerobically. Aeration will increase other aerobic bacteria present in wastewater [31].

Burkholderia ambifaria bacteria are closely related to plants, it is not surprising that this strain contains a lot of dioxygenase and oxygenase which can metabolize complex aromatic compounds associated with root exudates, and may also be an important part of an effective phytoremediation system. Utilization of key enzyme diversity in industrial applications, and predicted to be potential in bioremediation as well as catalytic systems for biomass conversion into energy [32]. Burkholderia ambifaria J82 has been used by the US Environmental Protection Agency as a biopesticide and has been used commercially for several years [33]. However, Bcc isolates are now increasingly identified as opportunistic pathogens, especially those that infect people suffering from cystic fibrosis (CF) or chronic granulomatous disease [34]. Therefore, its use as a biocontrol agent is no longer allowed [35]. From unpublished Puspitasari research, B. ambifaria causes hemolysis in the experiment so that henceforth these bacteria are no longer utilized.

Burkholderia stabilis do not show symptoms of chlorosis in the leaves and often located in the roots of plants, the strains do not show pathogenicity to onions (strain inoculation test in onion tissue; J. Jacobs et al., Unpublished) [32]. The genus Burkholderia has the potential to be exploited in biotechnology, biocontrol, and growth control processes and also has species and strains that are beneficial to plants. Ref. [36] [37] study the interactions specifically between plants and Burkholderia will be challenging.

Gluconacetobacter saccharivorans is gram-negative, has the optimum pH 2.5-6 for growth and the optimum temperature 30°C [38]. The cell is Gram-negative and rod-shaped, single or paired, non-motile, and size 0.5-0.8 with 1.0-1.5 μm. Catalase-positive and negative oxidase, and must be aerobic. Lactate and acetate are oxidized to CO₂ and H₂O. Acetic acid is produced from ethanol. The growth of these bacteria in maintenance media is more specific than other isolated microbes so that these microbes cannot be cultured again. From the research [39], it can be understood that the G. saccharivorans media are indeed specific, can grow in the presence of 0.35% acetic acid in AG media, but not on acetic acid 1 or 5% in the AE broth media. Acid is produced from L-arabinose, D-ribose, D-xyllose, D-galactose, D-glucose, glycerol, ethanol, 1-propanol, 1-butanol and 2-butanol, but not from D-fructose, L-sorbose, lactose, maltose, sucrose, raffinose, galactitol, D-mannitol, sorbitol D or starch. D-gluconate and 2-keto-D-gluconate are produced from D-glucose, but 5-keto-D-gluconate and 2,5-keto gluconate are not produced.

The results showed that 69% of the strains isolated were yeast ascomycete and 31% were yeast basidiomycete. One of these ascomycetes is Pichia kudriavzevii. All strains were assessed for their ability to produce indole-3-acetic acid (IAA) and showed that 69 strains could produce IAA when cultured in yeast extract of peptone dextrose broth plus 1 g/L of L-tryptophan. The highest IAA concentration of 565.1 ppm was produced by Rhodosporidium fluviale DMKU-RK253 [26]. Pichia kudriavzevii is one of 9 yeast that can produce IAA with a range of 11.0-332.9 ppm. Nine yeasts from
39 isolated yeasts originated from the surface of the vetiver grass phetoplank in several regions of Thailand. The isolate was cultured in dextrose peptone extract media plus 0.1% L-tryptophan [27].

Pichia kudriavzevii is mainly related to food spoilage, causing biofilms on the surface of the product and having a low pH [40], even in very low pH environments up to pH 2 [41]. Pichia kudriavzevii is also able to survive at very high temperatures up to 95 °C for 2 hours [42]. This yeast lives at a temperature of 46 °C and able to withstand high surface tension, producing phytase, which is an enzyme of the phosphatase group that can break the bonds of orthophosphate groups in the inositol chain of phytate compounds. Phytate compounds are the main form of phosphorus compounds in plants [43]. From the explanation above, besides producing growth hormones and phytase enzymes that are useful for plants, this yeast also has the potential to become a probiotic because it can live at very low pH and high temperatures, so it is interesting to be investigated further.

Trichosporon asahii can assimilate various sources of nitrogen and carbon. This fungus is classified as Basidiomycetes which has a growth temperature of 25 °C, capable of growing at 37 °C - 45 °C [44]. A flexible carbon assimilation strategy is related to its ability to change from commensal to pathogen. Changes in nutrient availability can affect the ability of T. asahii in conditions of commensalism in a niche and then disturbed metabolic homeostasis so that it can increase virulence and can contribute as a disease [45, 46]. From unpublished Puspitasari research, it shows that T. asahii is not pathogenic in plants and humans (hemolysis), so it is expected that it will not cause problems when used.

4. Conclusion

The results show that bioaugmentation with Pichia kudriavzevii UMJ-L, Trichosporon asahii UMJ-A2, Burkholderia ambifaria UMJ-A1, Gluconacetobacter saccharivorans UMJ-K, and Ps. putida in tofu wastewater by adding 50% fertilizer to shallots cannot be as good as 100% inorganic fertilizer. The use of the same microbes for augmentation in rice wastewater and domestic wastewater can save inorganic fertilizers between 50% - 75% when used as fertilizer in tuberose plants, tomatoes, and banana nurseries. Bioaugmentation fertilizer from domestic wastewater was better than other wastewater when used as fertilizer on plants. For better results, all wastewater still requires the addition of inorganic fertilizers between 25% to 50% because of the low nutrients content.

References
[1] Albalawneh A and Chang T K 2015 Int. J. of. Res-Granthaalayah 3(12) 16-35.
[2] Shamabadi N, Bakhtiar H, Kochakian N and Farahani M 2015 En. Proc.74 1337-4
[3] Widodo B, Kasam, Ribut L. and Ike 2013 J. Sains. Tek. Ling. 5(1) 36-47
[4] [EHRA] Environmental Health Risk Assessment. 2014. Survey Penilaian Risiko Kesehatan Lingkungan Kota Bogor.
[5] [PKT] Pemerintah Kota Tangerang. 2011. Laporan Status Lingkungan Hidup Kota Tangerang Tahun 2011. Tangerang.
[6] Christiyaningsih and Nursalikah A. 2016. Limbah Domestik Dominasi Pencemaran Air Kota Malang.
[7] [USGS] United States Geological Survey. Bioremediation. 2013. https://www.usgs.gov/
[8] Puspitasari R Tri, Elfarisna, Suryati Y and Pradana N T 2015 Proc. Int. Conf. and. Workshop on Basic and App Sci, Airlangga Univ, Oct 16th -17th
[9] Roidah I S. 2013. Manfaat Penggunaan Pupuk Organik untuk Kesuburan Tanah. [10]J.Univ.Tulungagung Bonorowo. Vol. 1.No.1
[11] Suryati, Y. 2010. Peluang Pemanfaatan Air Limbah Cucian Beras Sebagai Pupuk Organik. Pros Sem Nas Pert Indonesia Menuju Millenium Development Goals (MDGs). UMY Press. Yogyakarta.
[12] Syafruddin and Safrizal. 2013. The Effect of Concentration and Application Time of EM4 on Growth and Production of Chilli (Capsicum annum L.) in Entisol Soil. J Agrista Vol. 17 (2)
[13] Pinandita A, Biyantor D and Margono. 2017. Pengaruh Penambahan EM-4 dan Molasses terhadap Proses Composting Campuran Daun Angsana (Pterocarpus indicus) dan Akasia (Acasia auriculiformis). J Rek ProsVol. 11 (1) p19-23
[14] Samsudin W, Selomo M, and Natsir MF. 2018. Pengolahan Limbah Cair Industri Tahu Menjadi Pupuk Organik Cair dengan Penambahan Effektif Mikroorganisme-4 (EM-4). JNIK. Vol 1 (2).
[15] Bashan Y and de-Bashan L E. 2005. Bacteria : Plant Growth-Promoting. Encyclopedia of Soils in the Environment. Vol 1. p103-115. 2200
[16] Gravel V, Antoun H and Tweddell RJ. 2007. Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with Pseudomonas putida or Trichoderma atroviride: Possible role of indole acetic acid (IAA). Soil Biol and Biochemis. Vol. 39(8)p1968-1977.
[17] Kang S M, Ramalingam R, Khan A L, Kim M J, Park J M, Kim B R, Shin D H and Lee I J. 2014. Gibberellic Secretion of Rhizobacterium, Pseudomonas Putida H-2-3 Modulates the Hormonal and Stress Physiology of Soybean to Improve the Plant Growth under Saline and Drought Conditions. Plant Physiology and Biochemistry. Vol 85. p 115-124.
[18] Purbianti T, Umar A and Supriyanto A. 2010. Assessment of Adaptation of Shallots Varieties Pest Tolerant Disease on Dry Land In West Kalimantan. BPTP Pontianak.
[19] Bakrie M M, Anas I, Sugiyanta, and Idris K. 2010. Application of inorganic and bio-organic fertilizer on System of Rice Intensification. J. Tanah Lingk., 12 (2). p25-32
[20] Alavan A, Hayati R and Hayati E. 2015. Effect of Fertilization on Growth of Upland Rice Varieties (Oryza sativa L.). J. Floratex 10.p61 - 68
[21] Puspitasari R T, Pradana N T and Sukrianto. 2016. Diversity of culturable yeasts in phylloplane of sugarcane in Thailand and their capability to produce indole acetic acid. Microbiol. Jtrolis. Vol. 30 (6) p1785-1796
[22] Limtong S, Kaewwichian R, Yongmanitchai W and Kawasaki H. 2014. Diversity of culturable yeasts in phylloplane of sugarcane in Thailand and their capability to produce indole-3-acetic acid. WJ Micro Biotech. Vol. 30 (6) p1785-1796
[23] Limtong S, Chamnanpa T, Srisuk N and Limtong P. 2015. Diversity of Yeasts in Vetiver Phylloplane in Thailand and Their Capability to Produce Indole-3-Acetic Acid , a Plant Growth Promoter. 6th Inter Conf on Vetiver. Danang Univ of Tech. Danang City, Vietnam
[24] Bernal P, Allsopp L P, Filloux A and Llamas M A. 2017. The Pseudomonas putida T6SS is a plant warden against phytopathogens.J. The ISME.
[25] Planchamp C, Glaser G and Mauch-Mani B. 2015. Root inoculation with Pseudomonas putidaKT2440 induces transcriptional and metabolic changes and systemic resistance in maize plants. Front. Plant Sci.
[26] Manara A, DelCorso G, Bialiardini C, Farinati S, Cecconi D and Furini A. 2012. Pseudomonas putida Response to Cadmium: Changes in Membrane and Cytosolic Proteomes. J. Proteome Res., 11 (8), pp 4169–4179
[27] Sandri L S. 2011. Peranan Bakteri Pseudomonas putida sebagai Mediator dalam Proses Amonifikasi Limbah Cair Pabrik Tahu. Sarjana thesis, Univ Brawijaya.
[28] Genome. 2017. Burkholderia cepacia complex (Bcc)https://jgi.doe.gov/bura6/bura6.home.html
[29] Parke J L and Guriain-Sherman D. (2001). Diversity of the Burkholderia cepacia complex and implications for risk assessment of biological control strains. Annu Rev Phytopathol 39: 225–258.
[35] Govan J R, Brown AR and Jones AM. (2007). Evolving epidemiology of Pseudomonas aeruginosa and the Burkholderia cepacia complex in cystic fibrosis lung infection. Future Microbiol 2: 153–164.

[36] Chiarini L, Bevivino A, Dalmastri C, Tabacchioni S and Visca P. 2006. Burkholderia cepacia complex species: health hazards and biotechnological potential. Trends Microbiol 14: 277–286

[37] Compant S, Nowak J, Coenye T, Clément C and Barka EA. 2008. Diversity and occurrence of Burkholderia spp. in the natural environment. FEMS Microbiol.

[38] Tagele S B, Kim S W, Lee H G, Kim H S and Lee Y S. 2018. Effectiveness of multi-trait Burkholderia contaminans KNU17B11 in growth promotion and management of banded leaf and sheath blight in maize seedling. Microbiol Res. 214. p8-18.

[39] Lisdiyanti P, Navarro R R, Uchimura T and Komagata K. 2006. Reclassification of Gluconacetobacter hansenii strains and proposals of Gluconacetobacter saccharivorans sp. nov. and Gluconacetobacter nataicola sp. nov. IJS Vol 56 (9). https://www.microbiologyresearch.org/content/journal/ijsem/10.1099/ijs.0.63252-0

[40] Kurtzman C P, Fell J W, and Boekhout T. 2011. The Yeasts, a Taxonomic Study. Vol 1. Fifth ed. Elsevier.

[41] Greppi A, Saubade F, Botta C, Humblot C, Guyot J P and Cocolin L. 2017. Potential Probiotic Pichia kudriavzevii strains and their ability to enhance folate content of traditional cereal-based African fermented food. Food Microb.

[42] Chelliah R, Ramakrishnan S R, Prabhu P R and Antony U. 2016. Evaluation of antimicrobial activity and probiotic properties of wild-strain Pichia kudriavzevii isolated from frozen idli batter. Yeast. 33(8) p 385-401

[43] Qvirist L, Vorontsov E, Vilg J V, Andlid T. 2016. Microbial Biotechnology: Strain improvement of Pichia kudriavzevii TY13 for raised phytase production and reduced phosphate repression. Sfam.

[44] Munna M S, Zeba Z, Noor R. 2015. Influence of temperature on the growth of Pseudomonas putida. Stamford J. of Microbiol. 5(1)

[45] Childers D S, Raziunaite I, Avelar G M, Mackie J, Budge S, Stead D, Gow N A R, Lenardon M D, Ballou E R, MacCallum D M and Brown A J P. 2016. The Rewiring of Ubiquitination Targets in a Pathogenic Yeast Promotes Metabolic Flexibility, Host Colonization and Virulence. Plos Pathog. 12:e1005566.

[46] Crawford A and Wilson D. 2016. Essential metals at the host-pathogen interface: nutritional immunity and micronutrient assimilation by human fungal pathogens. FEMS Yeast Res. Plos.