Bio-decomposer of seaweed composting

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Abstract. The potential for seaweed in agriculture has not been utilized optimally. The existence of growth-stimulating activity from the seaweed-based formulation can be used as a biostimulant to increase crop productivity. The research objective was to find bio-decomposer isolates and formulas for composting seaweed. The research was conducted at the Biology Laboratory of ISRI at April-September 2018. The research was started from selection of 191 isolates to get the promising isolates as bacterial consortium (BC) inoculation. The second activity was to evaluate the effect of formulation of BC inoculation. The completely randomized design with 6 replications was applied, whereas the treatments tested were inoculation seaweed without BC, inoculation seaweed by BC1, BC2, BC3, BC4. Parameters measured were CO₂ evolution rate, organic C, CN ratio, humic acid, and Seaweed dry weight of seaweed. The results showed that the BC formula consisted of GK5.7, SW2.1, and NP2.4 isolates were the best formula for decomposing Sargassum. The BC formula consisted of NP1.2, SW2.4, and GK 6.4 isolates were the best formula for decomposing Gracillaria. Inoculating of Bacterial Consorsium to the Sargassum and Gracillaria seaweed increase the CO₂ evolution rate, humic acid content, and decrease the substrate dryweight and C/N.

1. Introduction
Indonesia as an archipelago has the longest coastline in the world, after Canada, which is 95,181 km. The potential of marine and coastal resources is very large and has the potential to be explored further to support the welfare of people living on land. Seaweed is a one of superior Indonesian marine commodity that contributed 38% to the global seaweed production in 2015 [1]. Natural or cultivated seaweed acts as blue carbon, which is able to fix CO₂. Seaweed is an abundant source of marine carbon.

The potential for seaweed in agriculture has not been utilized optimally. Seaweed can be used as raw material for making organic fertilizers, soil enhancers and biostimulants. In some countries seaweed-based fertilizers (i.e. compost or organic fertilizer) and seaweed extract have been widely used. The compounds contained in seaweed extract are polysaccharides (i.e. galactan, fucoidan, alginate, and laminarin), proteins (lectins), unsaturated fatty acids, pigments (i.e. chlorophyll, carotenoid, and fucoidan), polyphenols (fenolic acid, flavonoids, cinnamon acid, isofovacyn, benzoic acid, lignan, quercetin), macro nutrients (K, Mg, Ca, and Na), and phytohormone (i.e. citokynin, auxin, gibberellin, and abscisic acid) [2]. The existence of growth-stimulating activity from the seaweed-based formulation, can be used as a biostimulant to increase food crop productivity and soil fertility [3–5].

According to [6], biostimulants are plant extracts that contain a broad spectrum of bioactive
compounds, most of which have not been identified, and function to increase the efficiency of nutrient use and also increase tolerance to biotic and abiotic stress. Extraction is a very important initial step in utilizing bioactive compounds of seaweed, for example humic acid. The complexity of plant cell wall is one constrain in the extraction process. Agro-industrial companies generally use toxic and expensive solvents, for example ethanol, acetone, methanol-toluene methanol, petroleum ether, ethyl acetate, dichloromethane, and butanol [7]. The cell walls of Phaeophyta and Rhodophyta were dominated by polymers like cellulose, alginate carrageenan, xylan, mannan, rhodymenan, fucoidan etc [4].

An environmentally friendly approach of cell wall decomposition by applying microbial splitting enzymes is needed. Biodecomposer is a microbes that has the ability to decompose organic biomass. The mechanism of the biodecomposer by excreting enzymes to catalyze the hydrolysis reaction to the polymers that build plant cell wall into the oligomers. Biodecomposer is applied for the making compost, organic fertilizers, biostimulants and other soil enhancers. The purpose of this research was to obtain a biodecomposer isolates and formulas for making seaweed compost.

2. Materials and methods
Research was carried out at Biology Laboratory, Indonesian Soil Research Institute, from April to September 2018.

2.1. Agar plate-based screening for detection activity of cellulase, alginate lyase and carrageenase enzymes

2.1.1. Rejuvenation of bacterial isolates. The number of bacterial isolates used in present study was 191, isolated from seaweed collected from Nusa Penida beach Klungkung sub-district Bali district, Sepanjang beach and Wonosari village Daerah Istimewa Yogyakarta (DIY), and Sawarna beach Bayah sub-district Banten district. Rejuvenation of bacteria isolates using two kinds of media, nutrient agar and sea salt agar. The isolates that showed the good growth will be selected as candidates isolate for further study.

2.1.2. Detection of cellulase, alginate lyase and carrageenase enzymes activity of isolate candidates. Agar plate-based screening method was used to detection of enzymes activities. CarboxymethylCellulose/CMC (Himedia), Sodium Alginate (Merck) and Carrageenan (Sigma) were used as sole C-source substrate to determine cellulase, alginate lyase and carrageenase, respectively. One microliter of each isolate culture grown on Nutrient Broth (Himedia), was dropped to agar plates containing each substrate. The plates were incubated in 30°C for 5 days. After certain time of incubation, the plates containing CMC substrate were stained by Congo Red (Himedia) to form hydrolysis zone produced by bacterial activity [8]. The plates containing Sodium Alginates (Merck) substrate were stained by Gram’s Iodine Solution (Himedia) [9] The Cellulolytic Index (CI) was measured by the ratio of hydrolysis zone diameter produced by bacteria to the diameter of bacterial colony. Likewise for the value of Alginate Index (AI) and Carrageenan Index (CrI). The isolates showed the high value of AI, CrI and CI were selected as promising isolate for further testing.

2.2. The effect of bacterial consorsium inoculation to the decomposition of Sargassum and Gracillaria
The experiment was conducted at Biology Laboratory of ISRI. Formulation of Bacterial Consorsium (BC) was created by mixing three of promising isolates based on the enzymes screening explained above. Each isolate was grown on nutrient broth liquid media (Himedia) for certain days incubation, depend on the characteritic of the isolate. Subsequent three isolates were mixed for further experiment.

2.2.1. Decomposition assay of bacterial consorsium on Sargassum and Gracillaria. One hundred gram of each dry seaweed, was placed on a tightly sealable jar. The seaweed was moistened with deionize water until the water content is 50%. Completely randomized design with 6 replications was applied.
The treatment consisted of F0 (control) = seaweed without BC, F1= inoculation of seaweed by BC1, F2= inoculation of seaweed by BC2, F3= inoculation of seaweed by BC3 and F4 = inoculation of seaweed by BC4. Total bacteria population of BC formulas were $10^7$ cfu/ml. Inoculant concentration applied were 1% v/w. Parameters measured were CO$_2$ evolution rate applying alkali absorbtion methods follow by Alef (1995). The content of organic C (dry combustion), C/N, and humic acid (gravimetry) follow by Balai Penelitian Tanah (2005). Seaweed dryweight was measured after 7 days incubation.

3. Results and discussion

3.1. Agar plate-based screening for detection activity of cellulase, alginate lyase and carrageenase enzymes

Seaweed collected from Nusa Penida beach belong to genus Euchema, Gracillaria and Ulva. The same genus is also obtained from Sepanjang beach and Sawarna beach. The genus of Gelidium is also obtained from both beaches. Isolates rejuvenation shows that 72 out of 191 isolates grow well on nutrient agar and sea salt agar. Meanwhile, 119 other isolates grow well on nutrient agar media, but the growth on sea salt media was very poor and did not even grow. The viability of isolates on sea salt media indicate isolates ability to adapt on the marine environment substrate. The detection of the alginate lyse, carrageenase and cellulase activity from 73 isolates shown in Table 1.

| No | Isolates | Alginolytic Index | Carrageenolytic Index | Cellulolytic Index |
|----|----------|-------------------|----------------------|-------------------|
| 1  | GK1.2    | 0.14 ± 0.86       | 2.00 ± 0.06          | 92 ± 0.09         |
| 2  | GK1.3    | 2.00 ± 0.06       | 0.14 ± 0.86          | 92 ± 0.09         |
| 3  | GK1.4    | 2.13 ± 0.18       | 2.00 ± 0.06          | 92 ± 0.09         |
| 4  | GK5.1    | 1.75 ± 0.06       | 0.14 ± 0.86          | 92 ± 0.09         |
| 5  | GK5.2    | 3.21 ± 0.65       | 92 ± 0.09            | 92 ± 0.09         |
| 6  | GK5.3    | 5.25 ± 2.47       | 0.14 ± 0.86          | 92 ± 0.09         |
| 7  | GK5.4    | 3.30 ± 0.99       | 0.14 ± 0.86          | 92 ± 0.09         |
| 8  | GK5.5    | 3.30 ± 0.99       | 0.14 ± 0.86          | 92 ± 0.09         |
| 9  | GK5.6    | 4.60 ± 1.98       | 4.33 ± 0.94          | 92 ± 0.09         |
| 10 | GK5.7    | 4.17 ± 0.24       | 3.92 ± 0.59          | 92 ± 0.09         |
| 11 | GK6.1    | 5.00 ± 0.47       | 3.25 ± 1.06          | 92 ± 0.09         |
| 12 | GK6.2    | 4.83 ± 0.24       | 3.96 ± 1.00          | 92 ± 0.09         |
| 13 | GK6.3    | 2.25 ± 0.71       | 4.75 ± 0.35          | 92 ± 0.09         |
| 14 | GK6.4    | 2.67 ± 0.06       | 14.00 ± 0.07         | 92 ± 0.09         |
| 15 | GK6.5    | 2.40 ± 0.85       | 12.75 ± 2.84         | 92 ± 0.09         |
| 16 | GK6.6    | 3.33 ± 0.94       | 3.40 ± 1.78          | 92 ± 0.09         |
| 17 | GK6.7    | 0.14 ± 0.86       | 2.00 ± 0.06          | 92 ± 0.09         |
| 18 | GK7.1    | 8.33 ± 2.36       | 0.14 ± 0.86          | 92 ± 0.09         |
| 19 | GK7.2    | 6.67 ± 0.94       | 0.14 ± 0.86          | 92 ± 0.09         |
| 20 | GK8.1    | 2.46 ± 0.48       | 0.14 ± 0.86          | 92 ± 0.09         |
| 21 | NP1.2    | 0.62 ± 0.01       | 1.52 ± 0.05          | 13.50 ± 2.12      |
| 22 | NP1.3    | 0.35 ± 0.02       | 0.88 ± 0.17          | 3.06 ± 0.63       |
| 23 | NP1.4    | 0.14 ± 0.03       | 1.07 ± 0.06          | 1.87 ± 0.07       |
| 24 | NP1.5    | 0.14 ± 0.03       | 0.35 ± 0.02          | 1.87 ± 0.07       |
| 25 | NP1.6    | 0.88 ± 0.17       | 3.82 ± 0.21          | 1.87 ± 0.07       |
| 26 | NP1.7    | 1.07 ± 0.06       | 1.62 ± 0.11          | 1.87 ± 0.07       |
| 27 | NP1.8    | 1.14 ± 0.02       | 1.09 ± 0.01          | 1.87 ± 0.07       |
| 28 | NP2.1    | 0.35 ± 0.02       | 1.09 ± 0.01          | 1.87 ± 0.07       |
| 29 | NP2.2    | 0.35 ± 0.02       | 1.09 ± 0.01          | 1.87 ± 0.07       |
| 30 | NP2.3    | 0.35 ± 0.02       | 1.09 ± 0.01          | 1.87 ± 0.07       |
The various value of enzymatic index expressed the enzymes activity is very diverse. The scale of alginolytic index, carrageenolytic index and cellulolytic index were 0.64 – 8.33, 0.87 – 15.0 and 1.15 – 13.5, respectively. This diversity, it is suspected, was due to the relatively high genetic diversity of the isolates, compared to their environmental factors. A number of isolates from the same sample produce various enzyme activities. Alginate lyases are group of enzymes wich catalyze depolymerization of alginate into oligosaccharide that exhibit biological activity as a promotion plant growth [9,12]. Carrageenases is an enzymes which degrade carrageenan. They all are endo-hydrolases that cleave the internal β-(1–4) linkages of carrageenans yielding products of the oligo-carrageenans. Cellulases is a group of enzymes that catalyse the hydrolize 1,4-β-D-glycosidic linkages in cellulose and hemicellulose into cellobiose that compose of two glucose unit. Those three enzymes must be expressed by isolates which function as seaweed decomposer, because the polymers that build the seaweed cell wall are dominated by cellulose, alginate, carrageenan and hemicellulose.
3.2. The effect of bacterial consortia inoculation on the Sargassum and Gracillaria decomposition

Based on the agar plate-base screening, 12 promising isolates were selected and partly randomized grouping into 4 formulas. Each formula contained three promising isolates as shown in Table 2.

| No | Formula | Isolates | Algine Lyase | Carageenase | Celullase |
|----|---------|----------|--------------|-------------|-----------|
| 1  | F1      | SW6.1    | 5.17         | 3.41        | 3.41      |
|    |         | NP2.1    | -            | -           | 3.82      |
|    |         | GK6.2    | 4.83         | 3.96        | -         |
| 2  | F2      | NP2.9    | 0.64         | 1.09        | 3.00      |
|    |         | SW3.5    | 8.00         | 1.06        | 7.00      |
|    |         | GK6.1    | 5.00         | 3.25        | -         |
| 3  | F3      | GK5.7    | 4.60         | 4.33        | -         |
|    |         | SW2.1    | 9.50         | 1.83        | -         |
|    |         | NP2.4    | -            | 1.04        | 4.00      |
| 4  | F4      | NP1.2    | 0.62         | 1.52        | 13.50     |
|    |         | SW2.4    | 4.50         | 15.00       | -         |
|    |         | GK6.4    | 2.67         | 14.00       | -         |

Brown seaweed of Sargassum (Phaeophyta) and red seaweed of Gracillaria (Rhodophyta) used in this experiment, were taken from Mr Nuraji who is seaweed collector from Dunggubah village, Wonosari sub-district, Gunung Kidul district. The chemical characteristic of the seaweed shown in Table 3. Based on the Indonesian National Standard (SNI) 7763: 2018 concerning Solid Organic Fertilizers the two seaweed genus meet the requirements as raw materials or organic fertilizer. But for producing liquid organic fertilizer, other requirements are needed, namely the Ministry of Agriculture regulation 261/2019 [13]. The threshold for Sodium levels is < 2000 ppm. whereas the analysis results show that Sodium content in Sargassum is 7600 ppm, in Gracillaria is 2100 ppm. The use of Sargassum and Gracillaria as an agriculture input must be preceded by washing it first, to remove excess salt. The Ca content in Gracillaria is 16.36% due to the presence of CaCO₃ in their cell walls [4]. Humic acid content and CEC of Sargassum are higher than Gracillaria. Sargassum has a wide potential as raw material for organic fertilizers, soil amendment and soil enhancer or biostimulants.

Measurement of CO₂ evolution rate of Sargassum and Gracillaria inoculated by the BC formulas can be seen in Figure 1. The decomposition process is a reforming of polymer compounds or polysaccharides into the oligomeric or monomeric, carried out synergistically by microbes, which produce CO₂, H₂O. Decomposition that goes well will result in a higher CO₂ evolution rate (Figure 1 and Figure 2), the decrease of organic matter(substrate dryweight (Table 4) and the decrease of organic C and C/N (Table 5). As seen in Figure 1, the CO₂ evolution rates of both Sargassum and Gracillaria after inoculating with BC formulas were higher than that of the control. The average of CO₂ produced per day can be seen in Figure 2. Inoculation with BC formulas increases the number of bacteria capable of decomposing the substrate. The polysaccharide components of Sargassum are dominated by cellulose and alginate, while Gracillaria is dominated by cellulose and carrageenan. The BC F3, which contains a consortium of bacteria with a higher alginitolytic index value, shows better performance for decomposing Sargassum than other formulas, meanwhile BC F4 which has a higher carageenolityc index, shows better performance in Gracillaria (Table 2). This is supported by seaweed dry weight reduction data after 7 days of incubation as shown in Table 4.
Table 3. Chemical composition of Sargassum dan Gracillaria from Dunggubah village, Wonosari sub-district, Gunung Kidul district a)

| No | Parameter | Unit | Sargassum | Gracillaria |
|----|-----------|------|-----------|------------|
| 1  | pH        |      | 6.9       | 6.9        |
| 2  | Water content | %  | 15.75  | 6.56        |
| 3  | Organic C | %   | 46.54  | 37.45       |
| 4  | N         | %   | 1.23   | 1.46        |
| 5  | C/N       |     | 38     | 26          |
| 6  | P₂O₅      | %   | 0.13   | 0.14        |
| 7  | K₂O       | %   | 2.45   | 0.36        |
| 8  | Na        | %   | 0.76   | 0.21        |
| 9  | Ca        | %   | 5.84   | 16.36       |
| 10 | Mg        | %   | 1.3    | 3.11        |
| 11 | S         | %   | 0.79   | 0.38        |
| 12 | Fe        | ppm | 1662   | 5169        |
| 13 | Mn        | ppm | 140    | 271         |
| 14 | Cu        | ppm | 6      | 11          |
| 15 | Zn        | ppm | 14     | 28          |
| 16 | Pb        | ppm | 5.9    | 3.6         |
| 17 | Cd        | ppm | 0.4    | td          |
| 18 | Co        | ppm | 1.4    | td          |
| 19 | Cr        | ppm | td     | 27          |
| 20 | Ni        | ppm | 2.1    | 4.7         |
| 21 | Mo        | ppm | 39     | 29          |
| 22 | B         | ppm | 264    | 119         |
| 23 | CEC       | cmol/kg | 101.05 | 15.21       |
| 24 | Humic acid | %  | 13.70  | 4.75        |

a) Analyze was carried out at Chemistry Laboratory, ISRI

Figure 1. CO₂ evolution rate from Sargassum (left) and Gracillaria (right) produced by the treatments during 7 days incubation at Laboratory.

Humic acid is an organic acid found in humus and has long been known as a soil amendment. Humic acid content in Sargassum and Gracillaria inoculated with BC formulas showed higher yields than those without inoculation. Sargassum produced the highest humic acid after inoculation with BC F3, while Gracillaria with BC F4. The humic acid content of the two seaweeds was higher than ordinary compost and manure, namely 2–15%.
Figure 2. CO$_2$ evolution per day from Sargassum and Gracillaria produced by the treatments during 7 days incubation at Laboratory

Table 4. Seaweed dry weight (gram) after the treatments for 7 days incubation in laboratory

| Treatments | Sargassum | Gracillaria |
|------------|-----------|-------------|
| F0         | 83.0 ± 0.3| 85.2 ± 2.1 |
| F1         | 75.7 ± 2.8| 74.8 ± 0.9 |
| F2         | 73.7 ± 2.4| 75.8 ± 2.2 |
| F3         | 70.6 ± 1.1| 80.0 ± 2.0 |
| F4         | 74.0 ± 2.7| 73.0 ± 2.9 |

Table 5. The content of Organic C, humic acid and C/N of Sargassum and Gracillaria after microbial treatment.

| Treatments | Sargassum | Gracillaria |
|------------|-----------|-------------|
| Organic C  | C/N       | Humic acid  | Organic C  | C/N       | Humic acid  |
| (% )       | (%)       | (%)         | (% )       | (%)       | (%)         |
| F0         | 44.69 ± 0.39| 27.00 ± 1.93| 13.48 ± 0.52| 40.27 ± 2.30| 23.00 ± 0.25| 4.41 ± 0.38|
| F1         | 43.49 ± 1.91| 23.00 ± 1.87| 14.70 ± 2.66| 33.76 ± 1.96| 17.00 ± 2.24| 5.79 ± 1.56|
| F2         | 42.28 ± 0.52| 21.00 ± 2.27| 15.51 ± 2.60| 33.79 ± 0.82| 15.00 ± 1.51| 5.04 ± 1.12|
| F3         | 42.82 ± 0.23| 20.00 ± 2.24| 17.51 ± 2.20| 33.53 ± 1.94| 15.00 ± 2.29| 6.44 ± 1.66|
| F4         | 43.88 ± 0.77| 22.00 ± 1.79| 17.28 ± 1.63| 32.68 ± 1.93| 14.00 ± 2.53| 7.03 ± 1.37|

4. Conclusion
Formula F3 consisted of the consorium of isolates bacteri GK5.7, SW2.1 and NP2.4 is the best formula for decomposing Sargassum, while formula F4 consisted of the consorium of isolates bacteri NP1.2, SW2.4 and GK6.4 is the best for decomposing Gracillaria. Inoculating of Bacterial Consorium to the Sargassum and Gracillaria seaweed increase the CO$_2$ evolution rate, humic acid content, and decrease the substrate dryweight and C/N. The F3 and F4 formulas have the potential to be further developed as a seaweed bio-decomposer. Application of bio-decomposer to the seaweed composting is needed to get a good quality of seaweed compost.

Acknowledgments
The authors acknowledge the research grant from The Indonesian Agency of Agriculture Research Development, Ministry of Agriculture.
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