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Organic material decomposition capacity of indigenous microorganism communities from different farming systems in Soc Trang province, Vietnam

Le T. Xa[1], Hüseyin B.Tecimen[2] and Nguyen K. Nghia[3,*]

[1]School of Education, Soc Trang Community College, Soc Trang City, Vietnam
[2]Department of Soil Science and Ecology, Faculty of Forestry, Istanbul University-Cerrahpaşa, Istanbul, Turkey
[3]Department of Soil Science, College of Agriculture, Can Tho University, Can Tho City, Vietnam
[*]Corresponding author**:** nknghia@ctu.edu.vn

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Abstract

Indigenous microorganisms (IMO) are widely used in agriculture due to their high ability in bio-degradation. However, their decomposition abilities of different organic materials is still limited. This study aimed to assess fifteen IMOs' sourced from different farming systems in Soc Trang province, Vietnam ability to decompose rice straw, sugarcane bagasse, cocopeat, sawdust, and rice husk. Microbial density capable of decomposing cellulose was determined by the plate counting method on agar medium containing 1% carboxymethylcellulose. Cellulose degradation capacity of IMO was estimated by the Congo red method with determination of a halo zone diameter. Finally, the decomposition capacity of IMO of organic materials was determined by the mass loss method after 30 days of inoculation under laboratory conditions. The results showed that the microbial density of cellulose decomposing microbes in IMO was around 10^5 colony-forming units (CFU)/g and all 15 IMOs had halo zone diameters varying between 3.38-8.63 cm. Results of the laboratory decomposition experiment for five different organic materials revealed that decomposition of rice straw ranged between 52.2-57.9%, and decomposition of sugarcane bagasse changed from 19.03 to 47.72% while the values for the control treatments without IMO inoculation of these two materials were 20.39% and 11.81%, respectively. However, for other materials including coco peat, sawdust and rice husk, all fifteen IMOs exhibited very low capacity in decomposition and there was no significant difference between the treatments with and without IMO inoculation. The results of this study indicated that IMOs can be used to increase decomposition of some agricultural waste products, but varies depending on organic matter.

Keywords: Decomposition, Indigenous microorganism community, Organic materials, Rice straw, Sugarcane bagasse

1. Introduction

Vietnam is faced with more efficient utilization of agricultural harvest residues. The amount of rice straw and rice husk annually reaches approximately ten million tons during the process of harvesting and processing rice, whereas, recycling or re-evaluation of sawdust, coco peat, and sugarcane still requires a solution. Addition of liable residues to the soil by organic material decomposition provides significant carbon input to soil nutrient pool system [1]. Since the degradation of cellulose, hemicellulose, and lignin-rich wastes by physical and chemical methods is complex, costly, and environmentally toxic, treating cellulose and lignin-containing organic wastes with microorganisms represent a potentially efficient and eco-friendly solution.

Microbes play a pivotal role in the decomposition of recalcitrant and complex organic compounds into elemental and plant available forms. Soils are home to microorganisms capable of degrading lignocelluloses material of dead plants. Fungal species are predominately degrading cellulose and lignin into simple sugars and phenolic acids [2], which subsequently are available to other soil microbes. Some soil fungi already economically employed in crop soil amendments are species of *Trichoderma* and *Pleurotus* [3]. Organic

resources play an essential role in soil fertility management in the tropics by their short-term effects on nutrient supply and longer-term contribution to soil organic matter (SOM) formation [4]. The decomposition rate and subsequent nutrient release from organic matter (OM) determine the short-term benefits of organic residues for crop nutrition [5], while the decomposition capacity of indigenous microorganisms (IMO) of raw residue materials is dependent upon the pH [6], C:N ratio [7], and temperature [8] of the environment. To design more efficient agricultural systems, there should be a clear understanding of the determinants of nutrient supply, especially those that reduce the time of organic material decomposition and release of available nutrients.

Multiple beneficial microbes are present in IMO and they have shown implicit potentials of decomposer agents for making compost. IMOs may be sprayed over rice straw before a rice field undergoes tilling to promote the decomposition of rice straw as well as other organic materials. Inoculation of crop residues with beneficial microorganisms [9] has been shown to improve the efficiency of the degradation process. Degradation of organic compounds by IMO without any artificial enhancement is termed as "intrinsic bioremediation" and this is one of the best remedial actions applied without any soil contamination. Kumar and Gopal [10] summarized that, IMOs have been used for natural farming, bio-composting, bio-leaching, bio-remediation, bio-degradation and bio-fertilizer production. Addition of IMOs into compost facilitates the decomposition process. Indigenous microorganisms include filamentous fungi, yeasts and bacteria collected from no cultivated soil. It has a high content of microorganisms on the soil and often found under bamboo trees [11] as well as other cultivation fields like crop rotation, banana, shallot, vegetables, rice, watermelon, grassland, maize, lettuce, oranges, grapefruit, guava and sugarcane [12]. Since microbial communities play an important role in decomposed organic matter and lead to compost production, study upon the ability to decompose organic materials of indigenous microbial communities collected from different farming or soil ecosystems is necessary.

Soc Trang province of Vietnam has a total natural land area of 331,118 ha including 276,958 ha of agricultural land which accounts for 83.64% of the natural area (ranked as the 5th in the Mekong Delta). Land for rice cultivation, perennial crops and forestry is 146,970 ha, 43,000 ha, and 12,156 ha, respectively. There are many diversified and typical crop cultivation systems in Soc Trang province including rice, sugarcane, shallot, watermelon, orange, grapefruit, and vegetables and from the former studies IMO from different farming systems within Soc Trang province were collected and evaluated plant beneficial functions such as nitrogen fixation, phosphate solubilization, IAA synthesis [12] and antagonistic capacity [13]. Moreover, crop residues and other agricultural wastes are often burned by the farmer, leading to less efficient use of residue-derived nutrients for soil and crops and air quality issues. However, deep scientific knowledge about the abilities of IMOs in bio-degradation of organic material is still lacking and should be further elucidated. Therefore, the aim of this study was to assess the organic material decomposition abilities of fifteen IMOs from different farming systems in Soc Trang province, Vietnam.

2. Materials and methods

2.1 Materials

Fifteen different IMOs were collected from different crop systems in Soc Trang province, Vietnam including bamboo, crop rotation (corn-watermelon-courgette), banana, shallot, vegetables, rice, watermelon, grassland, maize, lettuce, oranges, grapefruit, guava, sugarcane by following the method described by Kyu and Koyama [14]. Further information on the sampling sites can be found in Xa and Nghia [15]. At each sampling site, three plastic baskets (25 × 15 × 8 cm) were used, corresponding to 3 replicates. Each basket was filled with 1 kg of steamed rice and covered using cloth and a waist belt. The baskets were buried under ground at a depth of 20-30 cm at each sampling site and covered with leaf litter for three days. After four days of incubation, the fermented rice samples colonized by indigenous microorganisms were harvested. The microorganism-colonized rice was put into a glass jar and transported to the laboratory. This source of microorganisms was called IMO. A Mix IMO was prepared by combining an equal amount of 150 g of each IMO together. All collected IMOs were well mixed with brown sugar with a ratio of 1:1 (w/w) until the mixed material became gooey. These mixed materials were stored in ceramic pots in a cool area and away from direct sunlight for seven days for an additional fermentation time. After seven days of fermentation, these sources of microorganisms were called IMO. The IMO were kept in the refrigerator at 4 °C for further studies.

2.2 Cell counting of microbes in collected IMO capable in decomposing cellulose

An aliquot of 10 grams of each IMO was put into a 250 mL glass bottle containing 90 mL sterilized distilled water on a shaker at a speed of 150 rpm for an hour and left stand for 5 min after shaking. A 1:10 dilution was prepared from each IMO and a 50 µL aliquots of each dilution was spread on carboxymethylcellulose (CMC) agar plates. Each dilution for each IMO was done in triplicate. One liter of CMC medium containing 1 g

$(NH_4)_2SO_4$, 1 g K_2HPO_4, 0.5 g $MgSO_4$, 0.001 g NaCl, 10 g CMC and 15 g agar (pH 7) was used for the IMO inoculation [16]. Samples were placed in incubators at 30 °C for 2 days. Finally, the number of colony-forming units (CFU) developed on agar medium was counted and used to calculate the number of microbes.

2.3 Determination of cellulose degradation capacity of IMO

Cellulose degradation capacity of IMO was qualified by Congo red method with a halo zone diameter determination. An aliquot of 10 grams of each IMO was put into a 250 mL glass bottle containing 90 mL sterilized distilled water on a shaker at a speed of 150 rpm for an hour and left standing for 5 minutes after shaking. An aliquot of 10 µL IMO dilution was dropped on the central of CMC agar plates and left stand for 30 minutes. Samples were incubated for 3 days at room temperature (30 °C). The plates were incubated at room temperature (30 °C) for five days to allow microbial growth. Each IMO was tested in three replicates. After incubation, 10 mL of 0.5% Congo red staining solution was added to the plate and was shaken for 15 minutes. The Congo red staining solution was discarded and added 10ml of 1M NaCl to destain the plates by shaking for 15 minutes. Finally, 1M NaCl was discarded and the stained plates were analyzed by observing the formation of clear zone (halo zone) around the IMO growth. The halo zone diameter was determined by the average perpendicular diameter of the halo zone.

2.4 Evaluation of decomposition capacity of IMO for organic materials

Decomposition capacity of IMOs for organic materials was determined by the mass loss method after 30 days of inoculation under laboratory conditions. Rice straw, rice husk, sugarcane bagasse, coco peat and sawdust were subjected to decomposition in this study. Rice straw and sugarcane bagasse were cut into 2 cm-short pieces. All organic materials were washed with distilled water, gently spread and allowed to air-dry in laboratory conditions. To incubate the organic materials, 20 g (dry weight) of air-dried organic residue was put into a 250 g round plastic box, 2 ml of IMO solution corrected to a density of 10^7 CFU/g was then added and the mixture made up to a moisture content of 80%, mixed, and left to stand at 30 °C for 30 days. Three replicates were repeated for each IMO. Finally, the percentage of decomposition was calculated based on the residue dry mass.

$$\text{Decomposition rate (\%)} = (\text{initial dry mass} - \text{residual dry mass}) * 100/\text{initial dry mass}$$

2.5 Data analysis

The data were analyzed by ANOVA by MINITAB version 16.2 software (Tukey test).

3. Results and discussion

3.1 The number of microbes in collected IMOs capable of decomposing cellulose

The results of microbial counts showed no significant difference between IMOs derived from different cropping systems. Microbial counts including bacteria and fungi in the IMO ranged from 1.80 x 10^5 to 6.53 x 10^5 CFU/g IMO (data not showed). The highest microbial CFU count was observed in IMO collected from vegetable grove soil and, secondly, the IMO collected from grassland field had the number of microbes 6.13 x 10^5 CFU/g IMO. The lowest number of bacteria was found in IMO collected from sugarcane soil, with 1.8 x 10^5 CFU/g IMO, while the remaining number of IMO microbes including bamboo, crop rotation, banana, shallot, rice, watermelon, maize, lettuce, oranges, grapefruit and guava varied from 2.27 x 10^5 to 5.33 x 10^5 CFU/g IMO. The numbers of IMOs may vary at a high range based on the collection source. In the study by Thuy et al. [17], the bacteria CFUs were ranged from 0.36 to 4.61 x 10^7 CFU/g of soil sample.

These results indicated that there were high numbers of viable microbial organisms capable of cellulose decomposition across all fifteen studied IMOs and this result implies that almost all IMOs could be considered as a good source of beneficial microbes for soil improvement as well as plant growth promotion [18]. They can survive better under the extreme climatic conditions of the local environment than under artificial cultures and environments. Since they have resided and already adapted with the local conditions, they are considered as the best survival source of microbes for soil and plant improvement effectively [18].

3.2 Determination of cellulose degradation capacity of IMO

Cellulose degradation capacity as measured by halo diameters varied widely among IMO systems (Figure 1) and was significantly different amongst each other (p<0.05) (Table 1). An IMO from guava fields had the

highest halo zone (8.63 cm), closely followed by the IMO collected from vegetable and shallot fields 7.70 and 7.47 cm, respectively which were significantly larger than other IMOs. Meanwhile, the remaining IMOs including bamboo, crop rotation, banana, lettuce, rice, watermelon, grassland, maize, oranges, grapefruit and sugarcane exhibited halo zones varying from 3.38 to 6.23 cm. It was noteworthy that when mixing all the collected IMOs together, the average diameter of the halo zone produced by this microbial community was significantly increased. Therefore, a combination of several IMOs from different ecosystem habitats is another approach and is very essential to have better function of IMO [18,12].

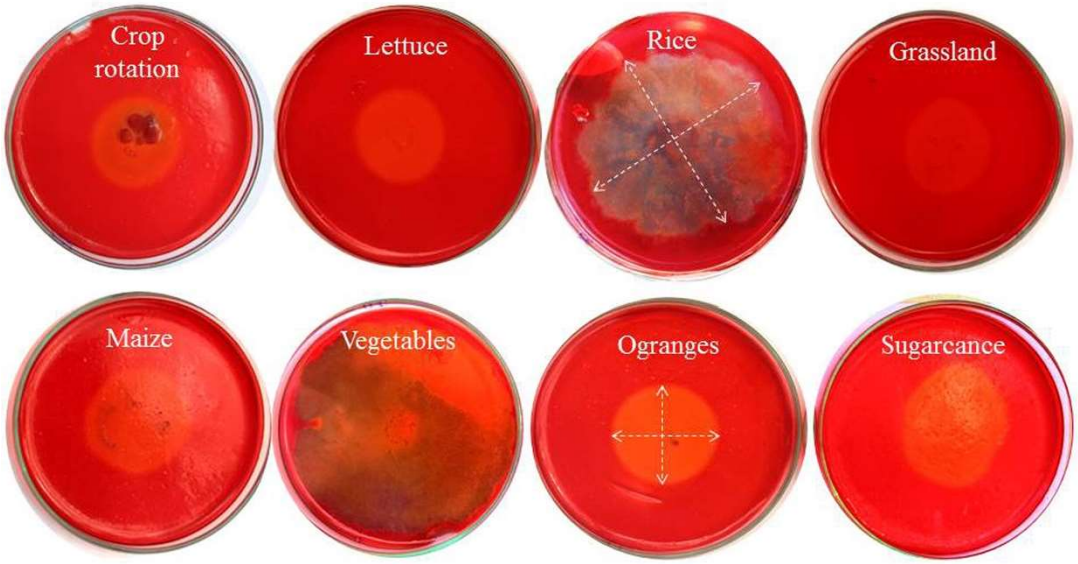

Figure 1 The variety cellulose resolution halo zone diameters of some indigenous microorganism.

Table 1 Diameter of cellulose resolution halo of 15 IMOs.

Number	Origin of samples	Halo zone diameters (cm)
1	Bamboo	3.77^{e}
2	Crop rotation	3.50^{e}
3	Banana	4.03^{e}
4	Shallot	7.47^{b}
5	Lettuce	3.43^{e}
6	Rice	6.23^{cd}
7	Watermelon	4.20^{e}
8	Grassland	3.38^{e}
9	Maize	3.80^{e}
10	Vegetables	7.70^{ab}
11	Oranges	3.76^{e}
12	Grapefruit	3.50^{e}
13	Guava	8.63^{a}
14	Sugarcane	5.57^{d}
15	Mix	6.87^{bc}

*Note: Values in the same column with different letters are significant difference at 5% level by Tukey tested.

3.3 Decomposition capacity of IMO for organic materials

3.3.1 Rice straw

The decomposition capacity of 15 IMOs for rice straw after 30 days of inoculation showed that all IMOs had significantly higher decomposition rates compared to (p <0.05) control treatments. The rice straw decomposition rates of 15 IMOs origins were >50%, with nonsignificant difference amongst each other.

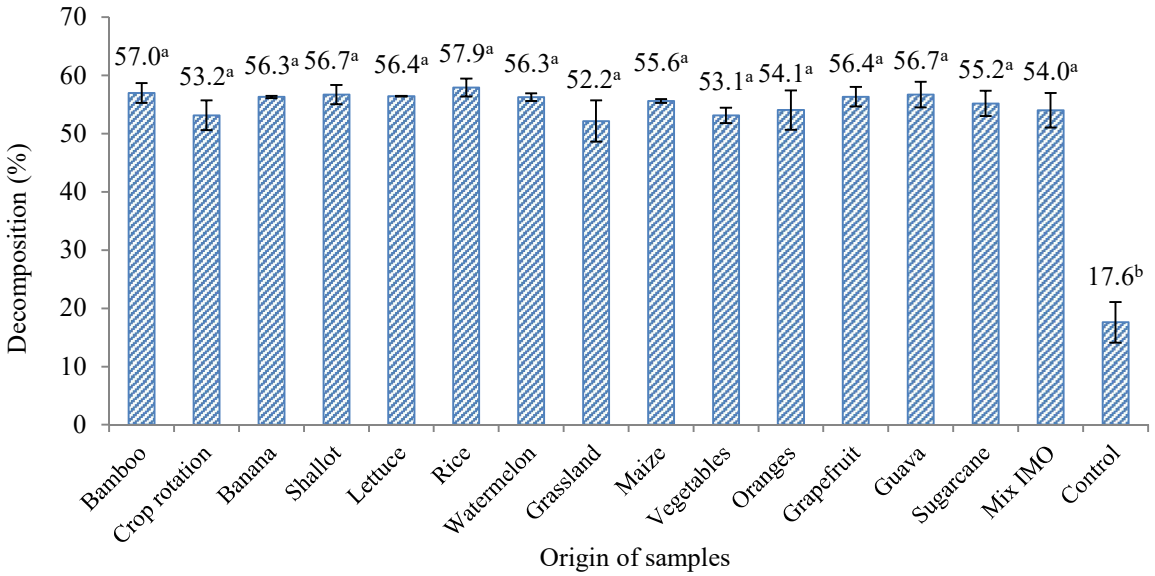

Figure 2 Decomposition percentage of rice straw by fifteen indigenous microorganisms.

We found that rice straw with IMO inoculation exhibited increased decomposition regardless of IMO as compared to the control, with decomposition ranging from 52.2% to 57.9% compared to 17.76% in the control. This confirmed the more effective and efficient biological processes and enzymatic activities exerted by synergistically working microorganisms introduced in a consortium where their performance is higher than microorganisms on an individual population basis [19]. Similarly, Tuerson et al. [20] reported more stable, efficient, and successful microbial activities during their synergetic collaboration. Matthews and Kamal [21] found that co-activity of microorganisms reduced the amounts of hemicellulose to 5.8% compared to chemicals (17%). Microorganisms in a consortium also maintain metabolic and ecological compatibility and stability. Wongwilaiwalin et al. [22] reported that biomass degrading capability is based on the functional and structural stability of a microbial consortium. Some microorganisms are superior to others in degrading different components of rice straw namely cellulose, hemicellulose and lignin. Thus, the most efficient bacteria in cellulose, hemicellulose and lignin degradation were mixed together to function as a strong and competent microbial consortium. The previous study of Bakar et al., [23] suggested that a mixed culture could improve the decomposition process of organic materials. Several researchers also reported that rice straw decomposition processes could be accelerated by using mixed culture of microbial inoculant [24,25].

Compared to a previous study by Cam et al., [26] who isolated 17 strains of fungi which were capable of decomposing rice straw, rice husk, sugarcane bagasse, coco peat and sawdust and showed that all are possible rice straw decomposition was very high, ranging from 37.1% to 47.6% and was significantly higher from control treatment in this study. Que and Diep [27], who made a singular bacteria strain isolation from paddy soils and cow rumen obtained 55% rice straw decomposition on day 10 while they achieved extracellular enzyme activity at only 59 over 96 isolates. These results clearly suggested that IMOs collected in this study are efficiently capable on decomposing rice straw.

3.3.2 Sugarcane bagasse

Results of sugarcane bagasse decomposition after 30-day incubation revealed that IMOs had different decomposition capacities and were all significantly higher ($p<0.05$) than the control treatment. In fact, the decomposition percentage of sugarcane bagasse material varied between 20.6% – 49.6%, while the values for the control treatments without IMO inoculation of this material were 11.81%. The highest decomposition found in tested IMOs came from under maize cultivation at a rate of 49.6%, closely followed by three IMOs collected from cultivation soil of grassland, orange and grapefruit with decomposition of 42.0%, 41.4% and 41.2%, respectively. Surprisingly, the IMO collected from sugarcane field had the lowest decomposing rate at 20.6%, whereas the remaining IMOs collected under bamboo, crop rotation, banana, shallot, lettuce, rice, watermelon, vegetables, guava and mixed IMO had decomposition ranging from 22.7% to 36.6%.

Torkashwand et al., [28] recorded approximately 2/3 loss of sugarcane bagasse (urea added treatment) inoculated with Trichoderma fungi after 10 weeks of incubation. Similarly, Cam et al. [26] obtained sugarcane decomposition rates ranging from 33.2 to 46.9% by 17 isolated strains of fungi which were capable of

decomposing rice straw, rice husk, sugarcane bagasse, coco peat and sawdust. Among those, the strains of PH-L3 had a higher percentage of sugarcane decomposition (32.8%) compared to other strains.

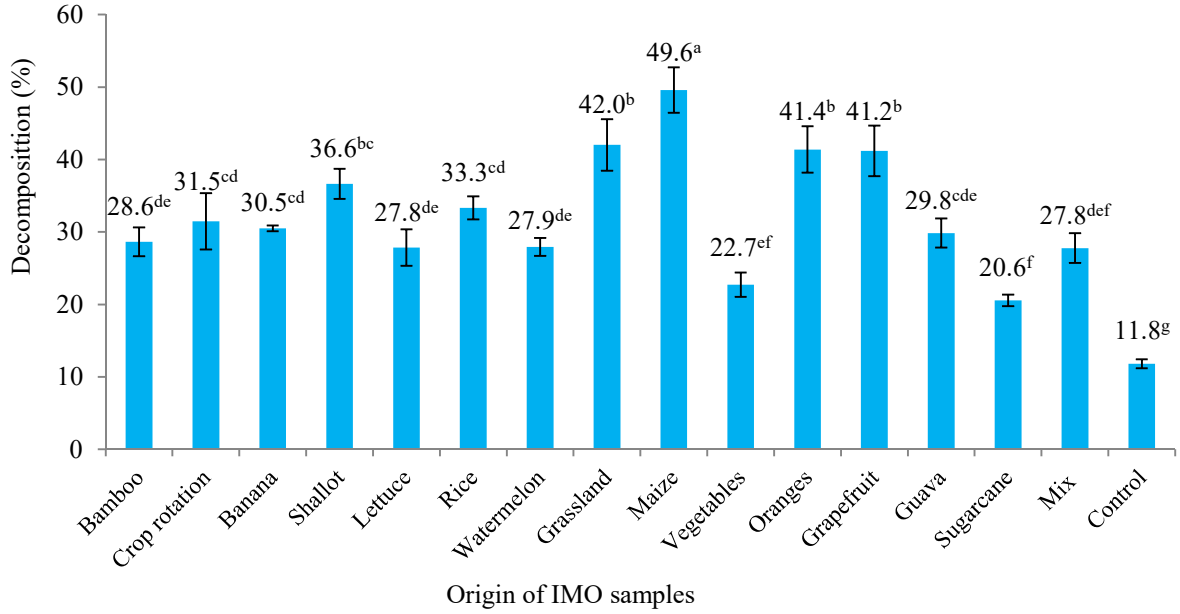

Figure 3 Decomposition percentage of sugarcane bagasse material of fifteen indigenous microorganisms.

3.3.3 Rice husk, coco peat and sawdust

Unlike the relative high rate of rice straw and sugarcane decomposition by all studied IMOs, decomposition of rice husk, coco peat and sawdust was very low. The freshly collected rice husk, coco peat, and sawdust from mills might have retarded and suppressed the decomposition. Moreover, these materials are difficult to decompose, and 30 days period would not be sufficient for microorganisms to degrade them.

Abu-Bakar and Ibrahim [11] considered that addition of IMOs is not the main factor in determining the rate of residue decomposition but the C/N ratio of the residue could be an alternative factor that causes an increase in temperature, thus promoting degradation. In addition, Cam et al. [26] analyzed the components of rice straw, sugarcane, rice husk, sawdust and cocopeat and showed that these residues have high total carbon and subsequently high C/N ratios while total nitrogen, total potassium, total phosphorus are remarkably low (except for rice straw) which leads to decreased decomposition. Thus, our findings of decreased decomposition of rice straw, sawdust and coco peat by IMOs without any additional inputs seems to confirm that N, P and K contents are not sufficient forfor optimal decomposition. In addition to C/N ratio and nutrient content, decomposition rate also depends on lignin and polyphenol contents, and the presence or absence of suitable microbial agents of decomposition [29,30]. Moreover, addition of IMOs enhanced the decomposition efficiency by increased initial microbial community diversity [11]. Therefore, these factors most likely act as limiting effects on the decomposition rate of materials like rice husk, coco peat and sawdust of the fifteen IMOs test.

4. Conclusion

The results from our study showed that fifteen IMO samples collected from different farming ecosystems containing microbial communities with a density of approximately 10^5 CFU/g IMO can synthesize cellulase enzyme to decompose carbon methylcellulose. Addition of IMOs significantly increased the decomposition rate of some organic materials, especially rice straw and the sugarcane bagasse. The results illustrate that IMOs have great potential in the treatment of agricultural cellulose containing waste products and can be used to accelerate decomposition in the composting process for organic fertilizer production to help improve soil, increasing growth, yield, and quality of crops.

5. Acknowledgements

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