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Co-Composting of EFB and POME with the Role of Nitrogen-Fixers Bacteria as Additives in Composting Process-A Review

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Abstract— This article reviews on Co composting of EFB and POME and the role of nitrogen-fixers to enhance the composting process. Empty Fruit Bunches (EFB) and Palm Oil Mill Effluents (POME) are two major waste streams left by the extraction process of palm oil. Bioconversion of this biomass (EFB and POME) into valuable products and utilization are the main aims of the researchers. Co composting of EFB and POME wastewater that generated from palm oil mill industry was investigated to be an option for the waste utilization and could offer many environments and economics benefits. Meanwhile, nitrogen-fixers play an important role to enhance the composting of EFB and POME. This article also highlighted the role of nitrogen-fixers as enhancer in the composting process. Nitrogen fixers in composting comprise some species of bacteria that developed enzyme systems and able to absorb nitrogen in its elemental form. Bacteria species that live free in the soil are able to extract and fix the nitrogen from air. The bacteria that live in the root nodules have a symbiotic relationship with the plant as both organisms benefit from the association. Cyanobacteria, Azotobacter, and Azomonas are some bacteria that can fix nitrogen and available in aerobic conditions.

Index Terms— Composting, Empty Fruit Bunches (EFB), Palm Oil Mill Effluent (POME), Nitrogen Fixers Bacteria

I. INTRODUCTION

Composting is a biochemical process converting various components in organic wastes into relatively stable humus like substances that can be used as a soil amendment or organic fertilizer [1]. Meanwhile, co composting is the controlled aerobic degradation of organics using more than one material (such Fecal Sludge and Organic Solid Waste) [2]. Fecal sludge has a high moisture and organic content while biodegradable solid waste is high in organic carbon and has bulked properties, because it allows air to flow and circulates [3]. By combining the two, the benefits of each can be used to optimize the process and the product. For dewatered sludge, a ratio 1:2 to 1:3 of dewatered sludge to solid waste should be used. Besides, for liquid sludge, a ratio of 1:5 to 1:10 of liquid waste to solid waste can be used [4]

As a biological process, composting involves a variety of microorganisms. These organisms decompose organic matter and organic compounds. The identification of microorganisms that are capable of degrading specific compounds is very useful in the composting process. The decomposition of organic waste during the composting process is carried out by a chain of microbial communities, which is critical for the utilization of complex substrate such as cellulose, hemicelluloses and lignin [5]. *Phanerocheate chrysospirium* is known to degrade lignin [6]. Hence, as in [7] reported that *Streptomyces* decomposed lignocelluloses. Reference [8] evaluated that several bacteria used the carbon source of lignin-related aromatic compound such as benzoic-p-OH-benzoic, vanillic, veratric, syringic and p-cournaric acids in the composting process. Reference [9] in his study mentioned that, after the biodegradable source of carbon is used up, the compound which takes more time to degrade such as cellulose, hemicelluloses and lignin will undergo biodegradable and part of it will convert to humus form.

Currently, there are more than 3 million hectares of oil palm plantations in Malaysia [10]. And about 80 million tons of palm oil and 57.4 tons of palm oil mill effluent (POME) were generated in the year 2009 [11]. In total, about 90 million tons of renewable biomass such trunks, frond, shells, palm press fiber and the empty fruit bunches are produced each year. Reference [12] had reported that the biomass was made up of 53% empty fruit bunches (EFB), 32% mesocarp and 15% fiber and palm kernel shell. Empty fruit bunches or EFB that produced from palm



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plantation is a suitable raw material for recycling because it produces in large quantities. In the past, EFB was often used as fuel, to generate steam at the mills and also reported that EFB, because it has high content of nutrients, EFB are burnt to produce ash and later used as fertilizer [13]. However, recently burn of EFB is prohibited by regulation in order to prevent air pollutions. Nowadays, most of EFB is mainly applied as mulch in the field [14]. In recent years, there is growing interest in composting EFB, in order to add value and also to reduce the volume to make application easier [15]-[16]. Thus, the treatment of EFB and POME has gained interest from many researchers due to the abundant amount generated in the mills [17].

Otherwise, palm oil mill effluents (POME) are the most polluted organic residues generated from palm oil mills. POME has been identified to be one of the major sources of water pollution due to its high Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) concentrations. In Malaysia, mostly pond system has been adopted in palm oil mill factories for POME treatment technology [18]. Besides, as in [19] in their study, stated that one of the major sources of Green House Gas (GHG) in Malaysia was contributed from palm oil mill wastewater treatment, with estimates at about 12.36 kg of methane was emitted from anaerobic pond. So that, the bioconversion of this biomass (EFB and POME) into valuable products and its utilization is the main aim of the researchers. This is because; the utilization of EFB and POME offers the best prospect of commercial exploitation. Co composting of EFB and POME wastewater that generated from palm oil mill industry was investigated to be an option for the waste utilization and could offer many environmental and economic benefits. These biomass materials (EFB and POME) could be composted and used in oil palm plantation as fertilizer and soil amendment.

II. EMPTY FRUIT BUNCHES

The empty fruit bunches or EFB is a suitable raw material for recycling because it is produced in large quantities in localized areas. An average oil palm plantation can handle about 100 metric tonnes (mt) of fresh fruit bunches daily [20]. EFB contains a high proportion of cellulose matter which is easily decomposed by a combination of physical, chemical and biological processes. The bunch consists of 70% moisture and 30% solids; of which holocellulose accounts for 65.5%, lignin 21.2%, ash 3.5%, hot water-soluble substances 5.6% and alcohol-benzene soluble 4-1% [16]. At the mills, where oil extraction takes place, solid residues and liquid wastes are generated. From the total residues, EFB generates more than 20% of the fresh fruit weight [13], [21]-[22]. In the past, EFB was often used as fuel to generate steam at the mills [13]. EFB was also incinerated or applied directly to the fields. However, incineration of EFB may create environmental problems as incineration and boilers emit gases with particulates such as tar and soot droplets of 20-100 microns and a dust load of about 3000-4000 mg/nm [23]. Besides, indiscriminate dumping of EFB may cause additional methane emission into the atmosphere [24]. It was reported that EFB contain 42% C, 0.8% N, 0.06% P, 2.4% K and 0.2% Mg [25]. Because of high nutrient contents and in order to minimize pollution, a new usage of this waste ought to be looked into. Table I shows the properties characteristic of EFB gathered from different literature. Meanwhile Table II shows the composition of nutrient content in EFB.

Researchers are now trying to utilize this raw material in different applications. Composting of EFB is being extended to farmers by the Department of Agriculture of Malaysia [27]. Currently, most of the EFB is used as mulch in plantations, almost wholly replacing incineration, which is now prohibited by the Department of Environmental. The usual application rate of EFB is 40-70 tonnes per hectare [28]. However, due to escalating labour, transportation and distribution cost of EFB in the field, utilization as mulch is becoming more expensive [29]. There is a growing interest in the low and cost attractive solid state bioconversion of EFB into value added products such as compost, citric acid and enzymes [16],[25],[30]-[32]. Reference [33] also has studied about the evaluation of EFB as a fuel for power generation. According to their study, at 65% moisture content, EFB has a calorific value of 6028 kJ/kg. Considered, in their analysis, the overall thermal efficiency is assumed to be only 25%. Thus, 1 tonne of EFB with 65% moisture content should deliver 1207000 kJ of energy (6028000 x 0.25 kJ).

Table I: Properties of empty fruit bunches listed by different literatures

Properties	Reference [34]	Reference [35]	Reference [25]	Reference [36]	Reference [37]
Proximate analysis (wt. %)^a					
Ash	7.54	3.02	-	-	-
Volatile matter	71.2	79.7	-	-	-



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Fixed carbon	18.3	8.65	-	-	-
Lignocellulosic content (wt. %)^a					
Cellulose	23.7	-	42 – 63	42 – 63	42 - 63
Hemicellulose	21.6	-	21.9 – 33	21.9 – 33	21.9 - 33
Lignin	29.2	-	10 – 36.6	10 – 36.6	10 – 36.6
Ultimate Analysis (wt. %)^{a,b}					
C	45.0	48.8	-	-	-
H	6.40	7.33	-	-	-
Continue					
O ^c	47.3	40.2	-	-	-
N	0.25	0.00	-	-	-
S	1.06	0.68	-	-	-

^a Dry basis, ^b Ash free basis, ^c By difference

Table II: Composition as percentage of dry matter in nutrient content of EFB

NUTRIENT CONTENT OF EFB	Composition as a percentage of dry matter
Nitrogen (N)	0.44
Phosphorous (P)	0.144
Potassium (K)	2.24
Magnesium (Mg)	0.36
Calcium (Ca)	0.36

Source: [26]

III. PALM OIL MILL EFFLUENT

In the process of palm oil milling, POME is generated through sterilization of fresh oil palm fruit bunches, clarification of palm oil and effluent from hydro cyclone operations [38]. POME contains many soluble chemical materials that are detrimental to the environment. POME is a viscous brown liquid with fine suspended solids at pH between 4 and 5 [39]. Direct discharge of POME into the environment is not encouraged due to the high BOD and COD value. POME in its untreated form is very high strength waste, depending on the operation of the process that involves informal, semi formal and formal process. More than 85% of palm oil mills in Malaysia have adopted pond system for POME treatment [13]. While, the rest adopted for open digested tank [12]. However, high rate anaerobic bioreactor have also been applied in laboratory-scale POME treatment such as an upflow anaerobic sludge blanket (UASB) reactor as in [40]; upflow anaerobic filtration as in [41]; fluidized bed reactor as in [42]-[43] and upflow anaerobic sludge fixed film (UASFF) reactor as in [39]. Other than anaerobic treatment, POME has been also been treated using membrane technology as in [44]-[45], aerobic activated sludge reactor as in [46] and evaporation method as in [47]

Recently, POME also has used as a fertilizer. However, the application of raw or digested POME as fertilizer on land was initially considered unreasonable because the effluent would kill vegetation and lead of the jamming of percolation and water logging, thus resulting in anaerobic condition [28]. As reported by reference [48], the raw material would readily cause clogging and water logging of the soil, but these problems could be overcome by the controlled application of small quantities of POME at a time. Ground water was tested after 6-12 months of trial applications of raw POME as fertilizer and showed no substantial percolation of oxygen-demanding or other polluting elements without excessive run-off over the surface during wet weather [48]. Reference [49] reconfirmed that a proper use of POME in the land environment would directly improve soil fertility. Their results also showed an enrichment of the soils with regard to phosphorus, nitrogen, calcium and magnesium after the application of POME. Although zinc was particularly in its exchangeable form, copper, iron and lead were predominant in their organic forms. The use of POME as a cheap organic fertilizer may offer an alternative to the excessive application of chemical fertilizers, especially phosphorus, for which cost is a severe economic limitation [50].



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IV. CO COMPOSTING OF EFB AND POME

Empty Fruit Bunches (EFB) and Palm Oil Mill Effluents (POME) are two major waste streams left by the extraction process of palm oil. Unfortunately, they are no attractive application at the contrary of the other streams like mesocarp fibers; kernel shells (PKS) and palm kernel meal (PKM) that are vaporized as boiler fuel and animal feed respectively. It was reported that the EFB contain about 42% C, 0.8% N, 0.06% P, 2.4% K and 0.2% Mg [51]. Previously, the most common practice of EFB treatment is through soil mulching, boiler fuel and incineration, and the rest left unused in the palm oil mills. Reference [51] in their survey stated that most of the EFB and shell at the Thailand palm oil factory are disposed of by the land filling method, which is very costly. And some factories burnt the EFB in the furnace, which will cause air pollution. In Malaysia, the incineration of EFB in the palm oil mills is restricted by the Department of Environment (DOE) [14].

EFB is a common raw material used in composting and POME often added to enhance the composting process. However, POME has a high nutrient content and large oil palm plantations prefer to use it directly as fertilizer [52]. Previous study, reference [53] reported that, shredded EFB and partially treated POME from an open anaerobic pond was used for co composting treatment in open windrow system at pilot field scale. The composting treatment was completed within 60 days with a final C/N ratio of 12.5. The pH of the system at range 7.75 – 8.10 during the treatment period while moisture content was reduced from 65 – 75% to about 60% at the end of treatment periods. In his study also indicate that very low levels of heavy metals were detected in the compost materials product.

Reference [27] from the Department of Agriculture of Malaysia also has done the research in EFB composting. The method that adopted was to mix the EFB with 20% of chicken manure. It took 11 to 12 months to mature, while the maturity of compost materials was determined when the temperature at 30°C and the pH reading was 4.5 – 6.0. Other than that, reference [54] studied about open and closed method of composting the EFB and POME. For open method, a mixture of EFB, fermentation liquid waste and chicken manure were the ingredients for the compost materials. While, for close system EFB, POME and chicken manure has been used as compost materials. A C/N ratio about 16 was achieved in 50 days (open method) and 85 days for close method. Besides, the temperature at final composting treatment has been analyzed more than 35°C.

The quality of final matures compost was difficult to maintain due to variation of POME characteristic in an open pond system that's available in palm oil mill factory. Therefore, it is essential to provide the EFB composting treatment with a consistent nitrogen and microbial source from POME in order to produce the best quality of compost materials. So that reference [55] has improved their previous study by investigating the effect of POME anaerobic sludge from closed anaerobic methane digested tank on the pressed-shredded EFB. This study shows that, high nitrogen and nutrient content were observed in the POME anaerobic sludge. Changes in the physicochemical characteristic of a co composting process also recorded and evaluated. A C/N ratio of 12.4 has reached after 40 days composting treatment. Meanwhile, the pH of the composting pile at range 8.1 – 8.6 and moisture content was reduced from 64.5% to 52.0% after final matured compost. Their study shows that, the addition of POME anaerobic sludge into pressed-shredded EFB composting process could produce acceptable and consistent quality of compost product in a short time.

Otherwise, MG Biogreen Sdn. Bhd. (MGBG) also has developed a small scale project activity of Co composting of EFB and POME. The project that undergoes by MGBG is in line with the development policies of the 3rd Outlook Perspective Plan of Malaysia relating to the improvement of air and water quality, efficient management of solid waste, developing a healthy environment and the conservation of natural resources. The composting technology used in the project is known as “turn windrow composting”. Over the crediting period from 2001-2014, MGBG looking forward the achievements about 75000 tonnes/year co composting of POME and 48000 tonnes/year co composting of EFB with resulting of 14000 tonnes/year of compost product. Reference [56] has studied about the composting process using selected substrate, POME and EFB with the addition of wheat flour as a co substrate in a tray bio reactor. The strains of *P.chrysosporium*, *T.harzianum*, *A.niger* and *Penicillium* that isolated from POME were used in an effective composting process. The composting process was done within 2 months. The C/N ratio and germination index (GI) achieved were 17 and 95% respectively. The other study conducted as in [57] was investigated the efficiency of rice straw and EFB of oil palm compost extracts, either fortified with *T.harzianum* on morpho-physiological growth and occurrence of *Choanephora* wet rot okra. Treatment tested were water (as control), rice straw (RST) composts extract, *Trichoderma*-enriched RST compost extract, EFB oil palm compost



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extract, *Trichoderma*-enriched EFB compost extract, an aqueous suspension of *Trichoderma* and fungicide Dithane M-451. The experimental result were significantly higher in *Trichoderma*-enriched RST composts extract, followed by Dithane M-451, *Trichoderma*-enriched EFB extracts, RST, EFB and aqueous suspension of *T.harzianum* in both Choanephore inoculated and uninoculated (control) plots.

Formation of compost from EFB and decanter cake slurry by the addition of POME with a regular turning operation was investigated by [58]. The addition of decanter cake slurry had speeded up the composting process of EFB. The final C/N ratio after 51 days for the mature compost with decanter cake slurry was 18.65, while the C/N ratio for the matured compost without decanter cake slurry remained high at 28.96. The compost formed from the addition of decanter cake slurry to EFB and POME had 46.4% nitrogen, 17.96% phosphorus, 17.7% potassium and 23.1% calcium which more than compost without adding decanter cake slurry. Table 4.0 shows a summary of the results available in the literature concerned with composting of EFB and POME in different microbial system with controlling parameters.

Table III: Literatures on composting of EFB and POME using different microbial system and controlling parameters

Substrate	Microbial System	Controlling Parameters	References
Palm press fibre, poultry layer deep-litter and broiler floor-litter and urea	Inoculated by bacteria and fungi	Temperature, moisture, C/N, etc.	[59]
EFB, goat dung, cow dung and chicken manure	Inoculated by bacteria and fungi	Temperature, C/N, pH, etc.	[16]
EFB	Natural degradation ^a	C/N	[60]
EFB, fermentation liquid waste and chicken manure and POME	Natural degradation	Moisture, C/N, etc.	[54]
EFB and POME	Natural degradation	Evaporation rate, C/N, rainfall, etc.	[61]
EFB, POME, wheat flour	Inoculated by <i>P. chrysosporium</i> , <i>T. harzianum</i> , <i>A. niger</i> and <i>Penicillium</i>	pH, moisture content, temperature, etc.	[56]
EFB and Rice straw	Inoculated by <i>Trichoderma harzianum</i>	Fortified or unfortified	[57]
EFB and POME	Inoculated by bacteria species	Temperature, moisture content, etc.	[53]
EFB and POME	Natural degradation	With/without decanter cake slurry	[58]
Organic fraction of municipal solid waste (OFMSW)	Inoculated by bacteria originated from MSW leachate	Temperature, pH, OUR, cellulose, nitrogen concentration	[62]
Lead contaminate solid waste	Inoculated by white rod-fungus	With/without inocula	[63]
Rabbit food and organic acid	Inoculated by yeast strain <i>Pichia Kudriavzevii</i>	Temperature and pH	[64]

^a Natural degradation indicates no inoculation of microbes involved in the process

V. NITROGEN FIXER BACTERIA

Nitrogen compounds are essential for many biological processes [65]. Although there is abundant nitrogen in the atmosphere, but just a few organisms are capable of producing ammonia by biological nitrogen fixation, such as the enzyme-catalyzed reduction of nitrogen gas (N₂). Nitrogen fixing microbes has an ability to convert of N₂ into NH₃, which is further being incorporated into amino acids and finally into protein [66]. Generally, nitrogen fixing bacteria can be classified into three groups. First is symbiotic nitrogen fixation. The examples of symbiotic nitrogen fixation are *Rhizobium* (legume association) and *Anabaena* (*Azolla* association). The Second is, non symbiotic and free living nitrogen fixing bacteria such as *Azotobacter*, *Klebsiella* and *Azotobacterium*. Third is nitrogen-fixing autotrophic cyanobacteria such as *Anaabena* [67].



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Through biological nitrogen fixation, 180 x 106 tonnes nitrogen per year is being added to the soil, but still it is not sufficient to replace completely the use of chemical fertilizers [68]. Much research has been attempted to isolate mutants of free living bacteria that can produce ammonia [69]. Nitrogen fixing bacteria have a major role in order to convert atmospheric nitrogen into a chemical form that is usable by plants and provide nitrogen in soil that can reduce or eliminate the nitrogen fertilizer requirement for the next crop [70]. This is because, bio fertilizers are now considered as the most important component of the integrated nutrient system. A group of bacteria is now referred to 'plant growth-promoting rhizobacteria (PGPR)', which participate in many key ecosystem processes such as those involved in the biological control of plant pathogens, nutrient cycling and seedling establishment [71]-[73]. The Effect of plant growth promotion by PGPR, which may be used as the inoculums in bio fertilizers is caused by one or more mechanisms such are, phytohormone production, fungal growth inhibition, nitrogen fixation, increase in nutrient use efficiency, antibiosis against phytophathogen and induction of systematic disease resistance [66].

Nitrogen fixation can only be performed by certain strains of prokaryotic microbes [74]. These microbes use the nitrogen fixing enzyme, nitrogenase to reduce atmospheric N₂ to ammonia which is a form that can be utilized by plants [75]-[76]. Many plants from symbiotic, mutualistic relationship with these prokaryotes, which receive carbohydrates and microaerobic environments and supply reduced N for use in amino acids, protein and other biochemical's as mentioned in [77]. Legumes such as *Acacia*, *Fakataria*, *Leucaena*, *Lupinus* and *Robinia* have symbiotic, mutualistic relationship with the bacteria genera *Rhizobium* and *Bradyrhizobium* [74],[77]. Actinorhizal species from genera such as *Alnus*, *Casuarina* and *Ceanothus* are associated with the Frankia genus of actinomycetes [78].

Rates of N₂ fixation depend on the density, age and growth of the host plants, degree of nodulation, the genetics of the host, mycorrhizae, nitrogen fixing bacteria and environmental factors that affect plant growth [79]-[81]. In pot trials, the rate of N₂ fixation and nitrogenase activity of *Acacia* or *Falcataria* seedlings increased with increasing amounts of P fertilizer on soils [82]-[84]. Studied by reference [85] shows that no effect of P fertilizer on *L.leucocephala* seedlings or *Casuarina equisetifolia* seedlings on soil [86]. In other studies, it appeared that N-fertilizer increased N₂ fixation by *Alnus* species [87]-[88]. Table IV shows the estimated average rates of biological nitrogen fixation for specific organisms and associations.

Table IV: The estimated average rates of biological nitrogen fixation for specific organisms and association

Organism or system	Dinitrogen fixed (kg ha⁻¹ yr⁻¹)
Free living microorganisms	
Cyanobacter (blue green algae)	25
Azotobacter	0.3
Clostridium pasteurianum	0.1 – 0.5
Grass bacteria associative symbioses	5 – 25
Continue..	
Plant-cyanobacterial association	
Gunnera	12 – 21
Azolla	313
Lichens	39 – 84
Legumes	
Soybeans (<i>Glycine max</i> L. Merr)	57 – 94
Cowpeans (<i>Vigna</i> , <i>Lespedeza</i> , <i>Phaseolus</i> and others)	84
Clover (<i>Trifolium hybridum</i> L.)	104 – 160
Alfalfa (<i>Medicago sativa</i> L.)	128 – 600
Lupines (<i>Lupinus</i> sp.)	150 – 169
Nodulated nonlegumes	
<i>Alnus</i> (alders, example red and black alders)	40 – 300
<i>Hippophae</i> (sea buckthorn)	2 - 179
<i>Ceanothus</i> (snow brush, New Jearsey tea, California lilac)	60
<i>Coriaria</i> ("tutu" in New Zealand)	60 – 150
<i>Casuarina</i> (Australian pine)	58

Source: [89]



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Reference [26] was studied about the isolation and characterization of nitrogen free fixer bacteria from empty fruit bunches (EFB) oil palm. In his study, the ability of the microorganism to fix nitrogen freely was examined by using the N-free mannitol agar medium. The microorganisms which were able to grow in N-free mannitol agar medium considered as free living nitrogen fixing bacteria [90]. The medium used to contain carbon source without the supplement of nitrogen. Based on C:N ratio, the microorganisms that able to fix the nitrogen were able to grow on the medium. By having the isolates grown on N-free mannitol agar medium, it was observed that the free living N-fixing bacteria possessed slimy, glistening and sticky appearance.

There is some nitrogen fixing bacteria that have been studied and most of them giving a good response to the composting process. N₂ fixing bacteria contribute to N increased in the compost product [91]. The potential of using free-living nitrogen fixing bacteria (*Diazotrophs*) as a sources of nitrogen nutrition for crops has not been realized in most parts of the world, largely because of the inability of the organisms to multiply effectively in temperature agriculture soils [92]. Inoculation of compost with cellulose decomposing isolates of *Bacillus Sp.*, *Cephalosporium Sp.* And *Streptomyces Sp.* may increase N₂ fixing activity [93]. Further, compost high in lignocelluloses has potential in N₂ fixing properties [94]. The source of carbohydrate is important to allow N₂ fixation activity, which requires large amounts of energy and reducing equivalent [95]. N₂ fixing bacteria can use carbohydrate (glucose) to fix atmospheric N₂ and use N-derived molecule for their metabolic needs [96]-[98]. Significant nitrogen fixation and plant N availability was stimulated by the glucose treatment of compost, but the mechanisms of these processes require more extensive investigation.

VI. EFFECT OF BIOFERTILIZER CONTAINING N-FIXER BACTERIA

References [99] have reported important differences among free living N₂ fixing bacteria in their ability to fix atmospheric N₂ and found that these bacteria were adapted to their environment. It is well known that various microorganisms fix atmospheric nitrogen such as *rhizobia* in symbiosis with legumes and *Azospirillum* in association with plant roots [100]. In the soil, free living bacteria, including *Pseudomonas* also fix nitrogen [95]. In compost, the presence of *Azomonas*, *Enterobacter Klebsiella*, *Clostridium* and *Bacillus* has been reported after the thermophilic phase [101]. Inoculation of compost with cellulose decomposing isolates of *Bacillus Sp.*, *Cephalosporium Sp.* and *Streptomyces Sp.* also may increase N₂ fixing activity [93].

Reference [94] mentioned that compost which rich in linocellulose has potential N₂ fixing properties. The increase in atmospheric N₂ fixation in soils can readily be shown by the addition of glucose [96]-[98]. There is the possibility of increasing the nitrogen content of compost by inoculation with nitrogen-fixing organisms and the phosphorus content by adding rock phosphate and then inoculate with phosphate solubilizing bacteria [102]. Nitrogen fixing bacteria besides fixing nitrogen in the atmosphere, they also solubilize P due to production of organic acids and enzymes [103]. The nitrogen fixing bacteria most frequently utilized are symbionts associated with the roots of leguminous plants, but the interest shown recently in the use of free living nitrogen fixers (*diazotrophs*) or biological fertilizers [104]-[105]. *Bacillus circulans* has been specifically identified in compost waste as in [106] and this is reported to be a dominant to N₂-fixing organism in temperate soil [107].

For development of sustainable plantation and farming, waste enrichment is interest by researchers. Inoculation of N-fixing and P-solubilizing bacteria would be able to enrich the compost product by changing N and P contents of the end product. References [108] in their research used charcoal-based nitrogen fixing and phosphate solubilizing culture for enriching the vermicompost. For nitrogen fixing bacteria, strains used in their study are *Azotobacter chroococcum* (Mac 27), *A.chroococcum* (54-1), *A.chroococcum* (35-47) and *Azospirillum lipoferum* with 1 phosphate solubilizing bacterium. Phosphate solubilizing bacterium used in their study is *Pseudomonas striata* for enrichment of compost study. In their study, maximum bacterial populations were found between 45-60 days of compost period. The initial N content of vermicompost is 1.40 (g/100g), however N was increased to 2.72 (g/100g) at 60 days by using *A.chroococcum* (Mac 27), 2.53 (g/100g) and 2.50 (g/100g) for strains *Azotobacter* and 2.18 (g/100g) for strain *Azospirillum lipoferum*. Besides, P content was greater after adding 1% MRP (Mussoorie rock phosphate) and *P. striata* combination and the concentration achieved 1.97 (g/100g) at 60 days compost process. However, without adding MRP, the P content is 1.51 (g/100g). Reference [102] mentioned that, the addition of rock phosphate inoculated with *P.striata* led to more availability of P, this is due to the production of organic acids by bacteria which solubilized the rock phosphate. This statement proved that, the result of P content from as in [108] study is greater by adding P than without adding MRP. So that, their study proved that the used of



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strains *Azotobacter*, *Azospirillum* and *Pseudomonas* inoculation helped to increase the N and P content of vermicompost and at once enriched the vermicompost end product.

Besides, reference [109] has studied for effects of N source on the process performance of the plant and animal waste. In their study, they used meat and bone, blood meal and horn and hoof meal as N source to the mixture of carding waste and wheat straw. They found that, composting with using N source from animal waste gave a reliable composting process and good degree of stability and compatible with safe and effective soil utilization. 16rRNA gene strain of N-fixer bacteria also abundantly found in the compost product. The used of N-rich animal waste also gave the consistent increase in the net N content in both compost, which likely to be attributable to atmospheric N fixation.

Reference [110] also studied about the effect of biofertilizer contain N-fixer bacteria. The N-fixer bacterium used in their study is *Azotobacter chroococcum*. The parameter used to indicate the effectiveness of strain is compost properties and plant growth of *Zea Mays*. They found that the application of beneficial microbes likes *Azotobacter* showed promoting effect on the growth of *Z. Mays* and improvement of soil properties through 87 days greenhouse trial. Before, as in [111] reported that *Azotobacter* not only provide nitrogen, but also produces a variety of growth promoting substance such as indole acetic acid, gibberellins and B vitamins. Another important characteristic of *Azotobacter* associated with plant improvement is excretion of ammonia in the rhizosphere in the presence of root exudates [112]. Reference [113] also reported that they used of suitable farmyard manures, green manures and other organic manures and fertilizer may enhance the benefit of N-fixing bacteria (such as *Azotobacter*) inoculation. This is because; N-fixation needs a lot of energy from available organic C to break the bonds between nitrogen atoms [114].

In another study, as in [91] has reported about the isolation of free living dinitrogen fixing bacteria and their activity in compost containing deinking paper sludge (DPS). They found that two gram negative N₂-fixing isolates were identified as *Pseudomonas*. N₂-fixing activity was found to occur between temperatures 18°C to 25°C. After performing a diagnostic test, the N₂-fixing bacteria were grown on TSA (Tryptic Soy Agar). They also found that, the compost successfully showed N₂-fixing activity after carbohydrates amendments both with and without the inoculation of N₂-fixing isolates. The sucrose amendments which combined with *Pseudomonas* isolates also decreased the total C content in the compost product. It will result in compost with lower C/N ratio. In conclusion, their study showed that approximately 5% of the populations of DPS compost consist of free living N₂-fixing bacteria which belong to *Pseudomonas* genus strain.

In this study, empty fruit bunches (EFB) will be used as a compost organic material. Before, reference [26] studied was about the isolation and characterization of nitrogen free fixer bacteria from EFB. In his study, the ability of microorganisms to fix nitrogen freely was determined by using N-free mannitol agar medium. The author conclude that, free living N₂-fixer did present in EFB and the value could be considered good for nitrogen free fixers to be packaged into biofertilizers. It is because; free living N₂-fixer had the potential to be developed as an organic component in biofertilizers.

VII. CONCLUSION

Composting is the highest form of recycling [115]. Organic material is converted into major beneficially used. Empty fruit bunches (EFB) and palm oil mill effluent (POME) are abundantly waste available in our country. Clearly known, EFB and POME are not environmental friendly when they are disposed incongruously. In fact, fresh EFB returns mineral nutrients and organic matter to the soil and helps to maintain soil fertility [116]-[119]. However, EFB may take a very long time to degrade into a small fiber form. Besides, POME is contaminating to the ground and water body with excessive organic load if not well treated. So that, co composting of EFB and POME is the best method to reuse the waste that abundantly available in palm oil mill into potential resource and value added product. Usually, it takes a long time to compost the EFB and POME. Some study stated that, they only achieve a maturation level at 40-60 days compost period. The approach to reduce the composting period and to achieve a low C/N ratio of compost product is the most concern of researchers nowadays. An N-fixer bacterium is a method to enrich the compost product. The utilization of microbial products to enhance the compost product has an advantage over conventional chemicals for agriculture purpose because microbial products are considered safer than many of chemical product. The literatures proved that the utilization of N-fixer bacteria be able to improve the compost product and enriched the soil fertility.



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