A study on biodegradation of coir pith using microbial consortium

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Abstract: *In the present study attempt was made to biodegrade coir pith using suitable microbes and their consortium, isolated from the naturally decayed coir pith, From the total colony forming units isolated from old coir pith, seven bacterial and five fungal strains which showed predominant growth were selected and independently screened for predecomposition of water washed coir pith for a period of 30 days. Pleurotus sajor caju was also tested for predecomposition of coir pith. Based on the predigestion characteristics, two microbes i-e Isolate-6 (bacteria, Pseudomonas spp) and Isolate-8 (fungi, Aspergilus niger) were selected. The two selected isolates and Pleurotus sajor caju were mixed with each other into four types of microbial consortia and were used for predecomposition (30 days).*

***Keywords*:** *Aspergilus niger, Coir pith, Pleurotus sajor caju, Pseudomonas spp and predecomposition.*

I. Introduction

Coir pith is a by-product of coconut industry. The dust left behind after extracting long fibres from the husk of a coconut is known as cocopeat or coir dust. It is a fluffy and spongy material with significant water holding capacity (Meerow, A. 1995). Coir pith will not degrade by itself and will remain over the soil years together. When it is burnt, it is not destroyed completely. It emits smoke continuously for a long time, thereby pollute the environment and disposal problems. However at present the coir pith has now become a money spinner. Coir pith is used as a moisture conserving agent in rain fed agriculture. It is also used as mulch for selected agro crops as it has a very high (400-600 percent) water holding capacity (Savithri and Khan, 1994). In the present study coir pith samples were collected from Solavanthan, Madurai District, Tamil Nadu, India. and subjected for biodegradability using microbes.

II. Materials And Methods

Enumeration of the microbial populations

Samples of old coir pith dumped around the coir industry were separately collected from several points, mixed and transported to the laboratory and immediately used for the analysis of total colony forming units (CFU) of bacteria, fungi and actinomycetes and were enumerated using standard plate count method. (Daniel and Karmegam 2000 ;Parthasarathi and Ranganathan, 1998 and Subbarao, 1995). From among the several microbial colonies of bacteria, fungi and actinomycetes which grew in the respective medium, only seven bacterial and five fungal isolates which showed predominant growth were selected for further studies.

Identification of the selected bacteria and fungi

All the seven bacterial isolates (Isolate 1 to Isolate 7) were identified through morphological and biochemical characteristics. (Apun *et al.*, 2000; Manivanan and Daniel 2008). The test results were compared with Bergey's Manual of Determinative Systematic Bacteriology (Holt *et al.*, 1994) and all the five fungal isolates (Isolate 8 to Isolate 12) were identified based on their macroscopic and culture characteristics i.e., fungal colony growth in agar medium and microscopic characteristics of the hyphae and reproductive structures after staining with lactophenol cotton blue. (Alexopoulos and Mims, 1983; Aneja,2001; Daniel *et al.*, 2008-a).

Screening of selected microbes for predigestion of coir pith

The seven predominant colonies of bacteria and five predominant colonies of fungi were independently screened for predigestion of coir pith. The fungus, *Pleurotus sajor caju* is well known for the biodegradation of lignocelluloses and hence *P.sajor caju* was also selected as a member for the predigestion of coir pith.

Predigestion of coir pith using microbes

The washed fresh coir pith was drained and dried in shade for 24 h and separately mixed with the 12 selected strains of microbes and subjected to predigestion in rectangular plastic troughs of 45 x 35 x 15cm size

for one month. Turning of the substrate was carried out at three days interval. After 30 d the EC, p^H**,** temperature and colour of the predigestion coir pith were recorded and based on the changes, two microbes i-e Isolate-6 (bacteria) and Isolate-8 (fungi) and *P.sajor caju* (fungi) were selected for further study.

Preparation of microbial consortium
        Isolate-6 and Isolate-8 which showed better predigestion ability and *P. sajor caju* were mixed with each other into four types of consortia as given in Table 1. *P. sajor caju* was procured from a local mushroom farm.

Table 1. Formulation of microbial consortia

Formulated consortium	Microbes used for the consortium
C-1	*Asp + Pleu* (1:1)
C-2	*Asp +Pseu* (1:1)
C-3	*Pleu + Pseu* (1:1)
C-4	*Asp + Pleu + Pseu* (1:1:1)

Asp- Aspergillus niger, Pleu- Pleurotus sajor caju, Pseu- pseudomonas spp

III.   Results

        The organic wastes used for the present study include fresh coir pith and aged old coir pith . Attempt was made to predigest fresh coir pith using microbes and their consortium. The results of the enumeration of the microbial populations such as bacteria, fungi and actinomycetes from old age coir pith showed the dominance of bacteria (230 ± 1.05 g^{-1}) followed by actinomycetes and then fungi as shown in Table 2.

Table 2.Total colony forming units of bacteria, fungi and actinomycetes observed in old coir pith.

S.no	Microbes	CFU g^{-1}
1	Bacteria	230.00±1.05
2	Fungi	190.00±2.10
3	Actinomycetes	220±2.05

        The results of the biochemical characteristics and the identification of the seven predominant bacterial isolates are given in Table 3. These bacterial isolates were identified as *Micrococcus* spp, *Streptococcus* spp, *Alcaligenes* spp, *Staphylococcus* spp, *Proteus* spp, *Pseudomonas* spp and *Bacillus* spp.

Table 3. Biochemical characteristics and identification of the seven predominant bacteria isolated from old coir pith

S.No	Code no.	Indole test	Methyl Red test	Voges Praskauer test	Citrate Utilization Test	Catalase test	Urease test	Gelatin hydrolysis test	Nitrate reductase test	Starch hydrolysis test	Casein hydrolysis test	Glucose utilization test	Identification result (Name of the bacterial strain)
1	Isolate 1	+	+	-	-	+	-	+	-	-	-	+	*Micrococcus* spp
2	Isolate 2	+	-	-	+	-	+	-	+	-	-	+	*Streptococcus* spp
3	Isolate 3	+	+	+	+	+	-	+	-	-	-	-	*Alcaligenes* spp
4	Isolate 4	-	+	-	-	+	-	+	+	-	-	-	*Staphylococcus* spp
5	Isolate 5	-	+	-	-	-	+	+	+	-	-	+	*Proteus* spp
6	Isolate 6	+	-	-	-	+	+	+	+	-	-	-	*Pseudomonas* spp
7	Isolate 7	+	+	+	+	+	-	+	+	+	-	+	*Bacillus* spp

        + = Positive reaction   - = Negative reaction

The morphological characteristics and the microscopic features of the five predominant fungal strains isolated from the old coir pith are given in Table 4. These colonies were identified as *Aspergillus niger*, *Penicillium* spp, *Aspergillus flavus* , *Rhizopus* spp and *Trichoderma* spp.

Table 4. Morphological characteristics of the five predominant fungi isolated from old coir pith

| S.No	Code no.	Colony morphology	Microscopic features	Identification result
1.	Isolate 8	Large cottony colony with scattered black spores	Conidia with spherical spores	*Aspergillus niger*
2.	Isolate 9	Greenish colony with radiated ring	Repeatedly branched conidiospores with long chains of spores	*Penicillium* spp.
3.	Isolate 10	Colony with yellow spore	Septate conidium with spherical spores	*Aspergillus flavus*
4.	Isolate 11	Cotton candy like colony, initially white that turned grey to yellowish brown in time.	Non septate or sparsely septate and broad conidium	*Rhizopus* spp
5.	Isolate 12	Light green colony with center raised and smooth	Conidia in balls	*Trichoderma* spp

Table 5. The changes in electrical conductivity, p^H and temperature of coir pith subjected to predecomposition using seven bacterial (30 d) .

Sl.no	Microbes	Parameters observed								
		EC			pH			Temperature		
		0d	15d	30d	0d	15d	30d	0d	15d	30d
1	*Micrococcus* spp	0.85	1.11	1.40	7.41	7.39	7.33	28.0	39.0	30.0
2	*Streptococcus* spp	0.85	1.05	1.34	7.41	7.37	7.31	28.0	41.1	31.6
3	*Alcaligenes* spp	0.85	1.03	1.32	7.41	7.38	7.32	28.0	40.0	30.8
4	*Staphylococcus* spp	0.85	1.04	1.33	7.41	7.34	7.28	28.0	41.3	30.5
5	*Proteus* spp	0.85	1.09	1.38	7.41	7.33	7.29	28.0	42.2	32.0
6	*Pseudomonas* spp	0.85	1.20	1.54	7.41	7.31	7.26	28.0	44.5	29.0
7	*Bacillus* spp	0.85	1.07	1.36	7.41	7.35	7.30	28.0	41.8	31.0

Table 6. The changes in electrical conductivity, p^H and temperature of coir pith subjected to predecomposition using five fungal isolates and *Pleurotus sajor caju* (30 d).

Sl.no	Microbes	Parameters observed								
		EC			pH			Temperature		
		0d	15d	30d	0d	15d	30d	0d	15d	30d
1	*Aspergillus niger*	0.85	1.23	1.43	7.41	7.31	7.23	28.0	43.0	29.5
2	*Penicillium* spp.	0.85	1.11	1.33	7.41	7.35	7.29	28.0	40.3	31.4
3	*Aspergillus flavus*	0.85	1.19	1.39	7.41	3.37	7.31	28.0	39.5	31.5
4	*Rhizopus* spp	0.85	1.12	1.31	7.41	7.36	7.30	28.0	39.0	31.3
5	*Trichoderma* spp	0.85	1.18	1.38	7.41	7.33	7.28	28.0	41.0	30.3
6	*Pleurotus sajor caju*	0.85	1.25	1.48	7.41	7.30	7.22	28.0	44.0	29.4

Fig.1. The changes in electrical conductivity of coir pith subjected to predecomposition using four types of microbial consortia (30 d).
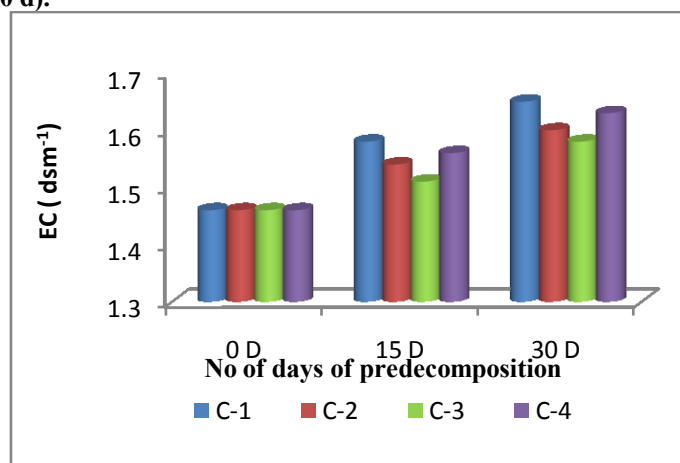
The electrical conductivity of the coir pith subjected to predecomposition using the four types of microbial consortia i.e. C1, C2, C3 and C4 are given in Fig.1. Consortium-1(*A.niger + P.sajor caju*) showed steady increase of electrical conductivity and gave highest value followed by C4, C2 and C3 (C1>C4>C2>C3).

Fig. 2. The changes in the p^{H} of coir pith subjected to predecomposition using four types of microbial consortia (30 d).
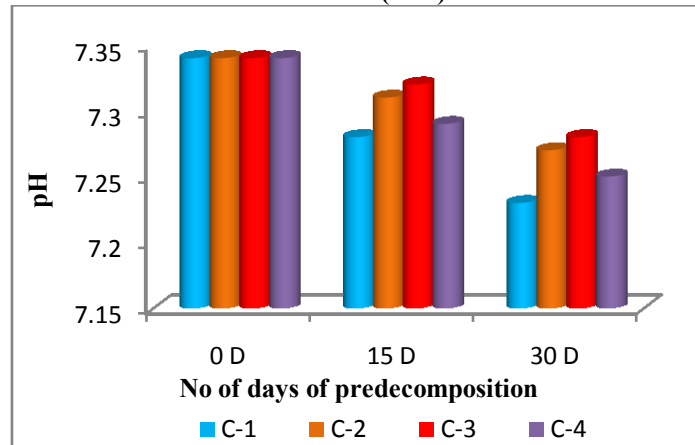
The p^{H} of coir pith subjected to predecomposition using the four types of microbial consortia is shown in Fig.2. The p^{H} steadily decreased with the increase in the number of days of decomposition. The best decrease was observed in C1 i.e. *A.niger* and *P. sajor caju* followed by C4, C2 and C3(C1> C4 > C2 > C3).

Fig.3. The changes in the temperature of coir pith subjected to predecomposition using four types of microbial consortia (30 d).
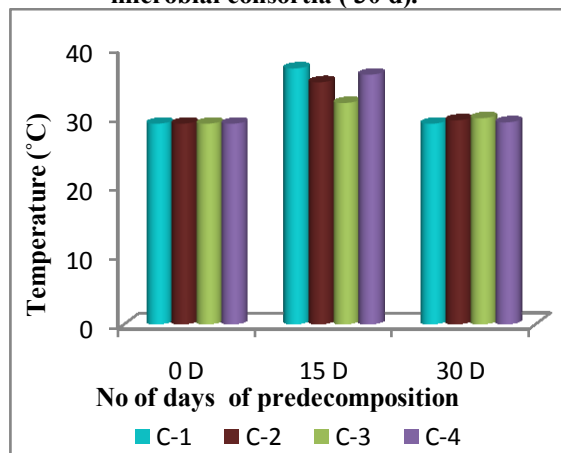
C1= *Aspergillus niger + Pleurotus sajor caju*
C2= *Aspergillus niger + Pseudomonas* spp
C3= *Pleurotus sajor caju + Pseudomonas* spp
C4= *Aspergillus niger + Pleurotus sajor caju + Pseudomonas* spp

The temperature changes in coir pith subjected to predecomposition using the four types of microbial consortia is shown in Fig.3. The temperature increased steadily from 29 ˚C to 37˚C upto 15 day and then the temperature declined to an average of 29 ˚C on the 30 day.

The colour changes of coir pith subjected to predecomposition using four types of consortia are given in Table 7. The colour changed from creamy brown colour on the initial day to light brown and then to brown colour in all the four consortial treatments as given in Table 7.

Table 7. The colour change of coir pith subjected to predigestion using four types of consortia (30 d).

Formulated consortium	Microbes used for the consortium	Colour of coirpith		
		Initial day	15 d	30 d
C-1	*Asp + Pleu* (1:1)	Creamy	++	++++
C-2	*Asp +Pseu* (1:1)	Creamy	+	+++
C-3	*Pleu + Pseu* (1:1)	Creamy	+	+++
C-4	*Asp + Pleu + Pseu*(1:1:1)	Creamy	++	++++

C1= *Aspergillus niger + Pleurotus sajor caju*
C2= *Aspergillus niger + Pseudomonas* spp
C3= *Pleurotus sajor caju + Pseudomonas* spp
C4= *Aspergillus niger + Pleurotus sajor caju + Pseudomonas* spp
+ = brownish cream, ++ =very light brown, +++ = light brown, ++++ = brown

IV. Discussion

Generally coir pith is dumped around the coir industries, on the roadsides and in no-man's land and allowed to degrade over several years. It takes decades for decomposition because of its pentoson- lignin ratio less than 0.5 (Ramalingam *et al.,* 2005). It is a rich source of potash. Also it helps to retain soil moisture for a longer period (Jothimani,1994). It can retain 5-6 times its weight of water. It is a good mulching agent.In the present study all the seven predominant bacterial isolates and all the five predominant fungal isolates selected from the naturally degraded coir pith and the fungus *P.sajor caju* were independently screened for predecomposition for a period of 30 days . During the 0, 15, and 30 day observation, the electrical conductivity increased with the increase in the number of days of predecomposition (Fig.1) and in all the treatments with the seven bacterial isolates (Table 5) and with the five fungal isolates (Table 6). This observation shows that these microbes carry the necessary enzymes for the biodegradation of coir pith. Nagarajan *et al* (1985)also found that the inoculation of coir pith with *Pleurotus* spp had resulted in drastic reduction in lignin content and cellulose content indicating the degrading of lignocelluloses by *Pleurotus* sp*,* and hence it was included in the present study for biodegradation of coir pith. The p^H steadily decreased with the increase in the number of days of predecomposition (Fig.2) and in all the treatments of seven bacterial isolates and the five fungal isolates .The shifting of p^H to lower levels could be attributed to mineralization of nitrogen and orthophosphates and bioconversion of organic materials into intermediate species of organic acids (Ndegwa *et al*., 2000). Temperature is one of the key indicators for microbiological reactions of predecomposition processes. Elevated temperature is necessary to destroy pathogens and other organic materials.The temperature changes in the all treatments with the selected seven bacterial , the five fungal isolates and with the four types of consortium (Fig 3) has enabled the conditions suitable for the aerobic microbes to proliferate in the present study. The effect of pre-decomposition of various organic wastes before vermicomposting has already been reported by several authors (Karmegam and Daniel, 2000-a, and Gajalakshmi *et al*., 2002-b). Colour has been described as a physical parameter to assess the maturinity and stability of predecomposition. Table 7 shows that 30 day predecomposition was sufficient for further vermicomposting. Matured compost shows a dark brown or almost black color and has a friable structure. The presence of mycelium (fungal growth) is evidence of a poor composting process (Inbar *et al*., 1990). It is generally suggested that as the compost stabilizes, it darkens to a dark brown or black color (Vuorinen and Saharinen, 1999).

V. Conclusion

India is a developing country and plenty of organic wastes are present in India. Animal wastes, agricultural residues, weeds , agroindustrial wastes and bio-municipal solid wastes are abundantly present in India. But most of these wastes are not properly used. There is a possibility of using all these wastes for the production of organic manure by different methods. Coir pith is one such organic waste which is not properly processed and recycled in India. This study is aimed at developing a technology for rapid degradation of coirpith and enrichment using microbes.

Acknowledgement

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