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Biodegradation of Agro-Industrial Wastes by Microorganisms

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Different plant tissue constituents, viz., cellulose, lignin, carbohydrate of jutestick and sugarcane bagasse were differentially degraded by *Trichoderma viride*, *Chaetomium globosum* and *Pleurotus sajor-caju*. Of the three organisms, *T. viride* proved to be the most efficient degrader of cellulose which *P. sajor caju* of lignin. Degradation of the polymers increased with time of incubation.

Key words : Biodegradation, Sugarcane bagasse, Jute stick, Cellulose, Carbohydrate, Lignin, Nitrogen, *Trichoderma viride*, *Chaetomium globosum*, *Pleurotus sajor-caju*

INTRODUCTION

Agroindustrial wastes from the world's most abundant renewable source of organic carbon and contain significant amounts of cellulose, lignin and other carbohydrates.

The ratio of lignin to polysaccharides in these materials ranges from 1 : 2 to 1 : 4, which together from 70-90% of the biomass of vascular plants (Sarkanen and Ludwig, 1971). Different nations are working on the possibility of utilizing the agroindustrial wastes in a more purposeful way like mycoprotein production. The feasibility of producing mycoprotein through efficient utilization of the lignocellulose crop residues largely depends on the types of organisms involved (Mitra and Nandi, 1989 ; Kundu *et al* , 1990) and the degree of understanding of the factors promoting the microbial bioconversions of these polymeric components. With the exception of the report of bacterial conversion of lignin to a water-soluble polyphenol (Crawford *et al.*, 1984), most other bioconversions

involve polysaccharides directly or carbohydrates resulting from solubilization of polysaccharides together with some degradation of lignin (Mukhopadhyay and Nandi, 1979 ; Chatterjee and Nandi, 1981a, 1981b). Benner and Hodson (1985) reported an enhanced rate of anaerobic degradation of polysaccharide components of wood and lignified substrates to methane, carbondioxide and low molecular weight aromatic compounds at elevated temperatures.

The present investigation was designed to study the degradation of holocellulose, lignin, total carbohydrate, soluble carbohydrate, soluble nitrogen in jutestick and sugarcane bagasse using a strong cellulolytic mould, *Trichoderma viride*, an efficient soft rot fungus, *Chaetomium globosum* and a common edible white rot fungus *Pleurotus sajor-caju* which commonly grows on compost. In the present study the former two were isolated from soil in and around Burdwan and the latter procured from W. B. State Agricultural Farm at Chinsurah.

MATERIALS AND METHODS

Dried pieces (1—1.5 cm long) of jute stick (25 g) and sugarcane bagasse (15 g) were taken separately in Erlenmeyer flasks (250 ml,) soaked in distilled water (200 ml) for 1 h and then the excess water was drained off. To each flask, 50 ml and 30 ml distilled water was added in jutesticks and sugarcane bagasse respectively. The flasks were plugged, sterilized at 20 lbs p.s.i. pressure for 20 minutes. These were then inoculated with a spore suspension of more or less equal potency (4.4×10^7 spores ml spore suspension added to 10 g material) in the case of *Trichoderma viride* Pers. ex Fr. and *Chaetomium globosum* Kunze and 9 day old spawn (50 grains/flask) in case of *Pleurotus sajor-caju* (Fr.) Singer. The inoculated flasks were then incubated at $27 \pm 1°C$ for 15, 30 and 45 days in complete darkness. The flasks were occasionally moistened with sterilized distilled water. The degraded materials were taken out from the flasks periodically and dried to constant weight at 60°C. These were then ground to powder (40 mesh) and different constituents of its tissue viz., holocellulose, lignin, total carbohydrate, soluble carbohydrate, soluble nitrogen were estimated.

Holocellulose was determined following mainly Seifert (1983). Powdered decayed tissue (0.4 g) was taken in 50 ml Erlenmeyer flasks containig 7 ml of a buffer solution (pH 3) [consisting of glacial acetic acid (60 ml) and sodium hydroxide (1.3 g) per 100 ml distilled water]. An aqueous solutlon (3 ml) of 20% sodium chlorite was added and the mouth of the flasks were wrapped tightly with polythene and kept under constant at 40°C for 40 h. After the incubation period, the flasks were then placed in an ice bath to stop the reaction. The content in each flasks was mixed with 100 ml acetic acid (1%) and passed

passed through G_2 sintered glass filter. The residue was then washed with diethyl ether (5 ml) three times and oven dried at 65 C to constant weight.

Lignin content was estimated quantitatively following Saeman *et al.* (1954) by removing the total carbohydrate from the powdered material.

Total carbohydrate was estimated quantitatively following the colorimetric method of Saeman *et al* (1954).

Soluble carbohydrate was estimated quantitatively following Viles and Silverman (1949) and Mc Cready *et al.* (1950).

Soluble nitrogen was estimated colorimetrically following Vogel (1961).

RESULTS

In control, holocellulose was found to be 50% in jutestick and 51% in sugarcane bagasse. As degradation progressed all the test organisms degraded holocellulose considerably. Among the three organisms *T. viride* proved to be best

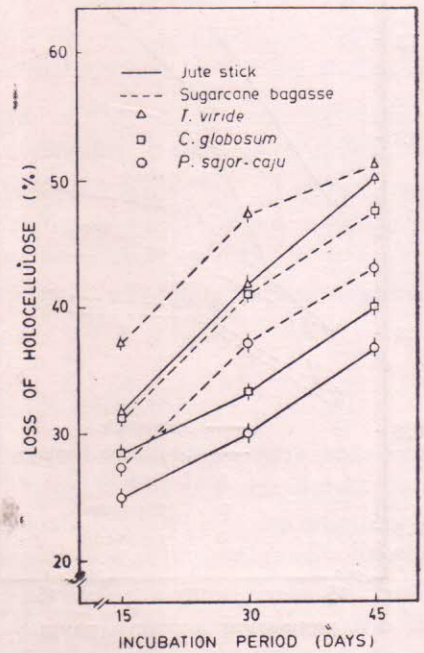

Fig. 1. Loss of holocellulose during degradation of jute stick and sugarcane bagasse powder by test fungi after different periods of incubation

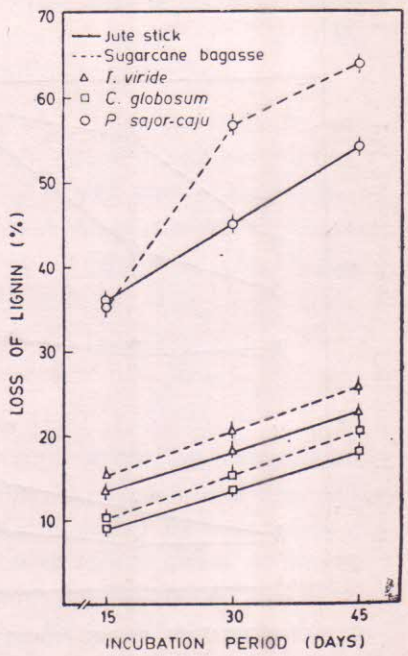

Fig. 2. Loss of lignin during degradation of jute stick and sugarcane bagasse powder by test fungi after different periods of incubation

degrader of holocellulose followed by *C. globosum* and *P. sajor-caju*. The rate of degradation, however, was much higher at the early stage of incubation than the later stage in all fungi-substrates combinations. Of the two substrates, holocellulose was utilized more efficiently from sugarcane bagasse than jutestick by all the test fungi. Loss of the material increased with time of incubation.

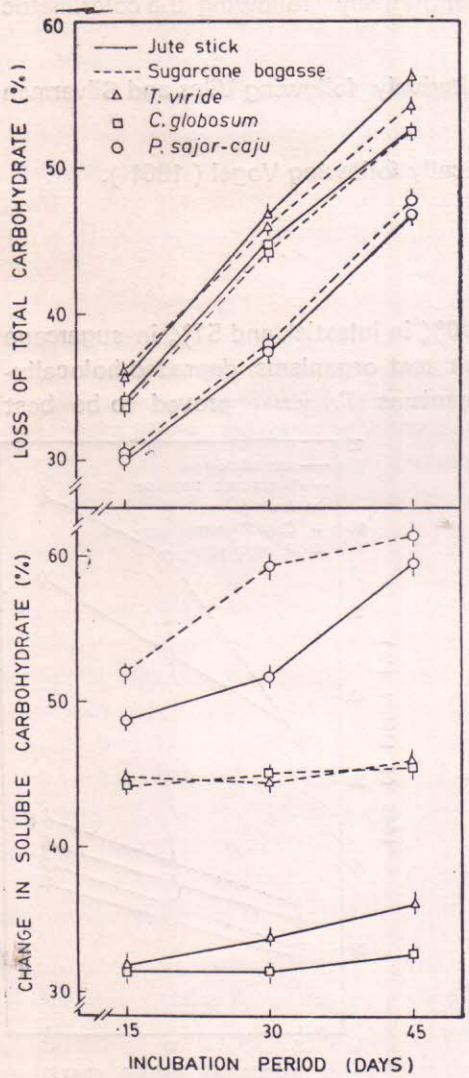

Fig. 3. Loss of total carbohydrate and change in soluble carbohydrate during degradation of jute stick and sugarcane bagasse power by test fungi after different periods of incubation

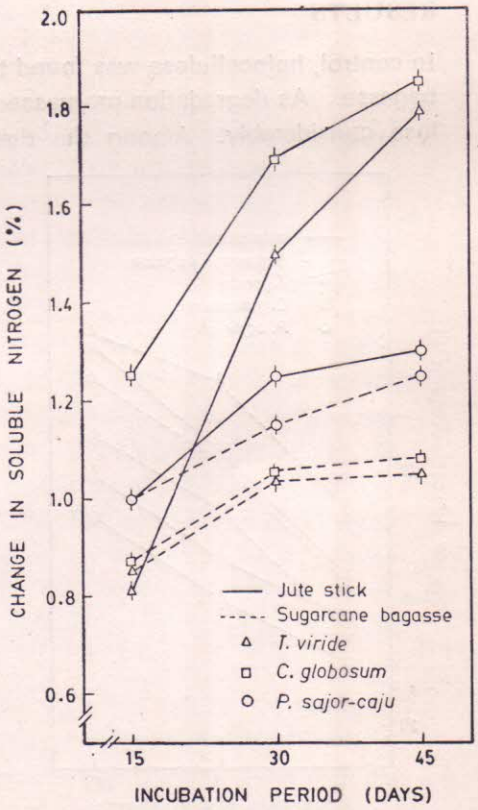

Fig. 4. Change in soluble nitrogen during degradation of jute stick and sugarcane bagasse powder by test fungi after different periods of incubation

In control, lignin content was 22.1% in case jutestick and 19.5% in case of sugarcane bagasse. Lignin of both jutestick and sugarcane bagasse decreased as a result of degradation. Among the three organisms *P. sajor-caju* degraded lignin of both the substrates more efficiently than either *T.viride* and *C. globosum.* It was also evident that the rate of loss of lignin was much higher during the first phase of incubation than the later phases.

Total carbohydrate in jutestick and sugarcane bagasse were 53.3% and 60.8% where as soluble carbohydrate was 0.77% and 19.2% respectively. As degradation progressed total carbohydrate decreased gradually in both jutestick and sugarcane bagasse. Soluble carbahydrathes, on the other hand, increased in the former but decreased in the latter in all cases. Among the three organisms tested *T. viride* showed the highest degradation of carbohydrate followed by *C. globosum* and *P. sajor-caju.* The rate of loss of total carbohydrate was much higher at the first phase of incubation than the later phases of incubation in all fungi and substrates combinations. Soluble carbohydrates increased significantly in jutesticks by all the test fungi, more so as degradation progressed. *P.sajor-caju* showed maximum increase followed by *T. viride* and *C. globosum.* Here also the rate of increase was much higher at the first phase than at the later phases. Soluble carbohydrate in sugarcane bagasse decreased significantly from the initial phases. However, at later phases it increased to a certain extent.

In control, soluble nitrogen content of jutestick and sugarcane bagasse were 0.75% and 0.67% respectively. As degradation progressed soluble nitrogen content was highest in *C. globosum* followed by *T. viride* and *P. sajor-caju* in both substrates. The increase in soluble fraction was highest at the first phase of incubation than at the later phases. Of the two substrates soluble nitrogen content was highest in sugarcane bagasse than jutestick.

DISCUSSIONS

Holocellulose is the most abundant renewable organic carbon sources on earth (Tsao, 1978) and constitutes the major component of jutestick and sugarcane bagasse. Ability of the organisms could thus be determined by their extent of utilization of holocellulose. Maximum degradation of holocellulose at the first phase of incubation probably resulted from abundant growth and activity of the fungus mycelia in presence of some simple carbohydrates initially available in both jutestick and sugarcane bagasse. The amount of simple carbohydrates being higher in the later source resulted comparatively higher mycelial growth. As the simple carbohydrates was gradually utilized with time, growth of mycelia decreased to a considerable extent because of much less simple carbohydrates that originated from the breakdown products of the holocellulose.

The enhanced rate of degradation by *T. viride* might be due to that the fungus was highly cellulolytic mould possessing higher enzyme system.

Next to holocellulose, lignin was the second major component of jutestick and sugarcane bagasse. Lignin being resistant to microbial decay (Kirk *et al.* 1977 ; 1978), its rate of decomposition was slower than that of carbohydrate (c. f. Eslyne *et al.*, 1975 ; Chopra and Somal, 1981). The lignin degrading organisms was reported not to use lignin as sole energy source but to depend on other readily available co substrates for depolymerization of lignin (Ander and Eriksson, 1975 ; Kirk *et al* , 1976). The present observation was in conformity that at the initial phase of degradation when other energy sources were readily available in substrate, the fungi were able to utilize them easily and thus degraded lignin more efficiently. As other easily utilizable co-substrates were gradually exhausted, the rate of lignin degradation decreased at later phases. Mishra *et al.* (1979) also reported *Chaetomium globosum* and *Trichoderma viride* QM 9414 to degrade lignin to some extent. Levi and Preston (1965) reported that *Chaetomium globosum* was similar to brown rot fungi in its effect on lignin causing primarily demethylation. Eslyne *et al.* (1975) showed that all the soft rot fungi utilized the carbohydrate of alder and poplar ahead of the lignin and thus, their action was similar to that of brown rot fungi.

Among the three tested organisms the white rot fungus *P. sajor-caju* was found to be the most effective lignin degrader in both the substrates followed by *T. viride* and *C. globosum*. Higher efficacy of *P. sajor-caju* might be due to the phenol oxidases produced which brought about oxidation and degradation of lignin. The discovery of lignin peroxidase (ligninase) by Tien and Kirk (1983), Glenn *et al.* (1983), Hatakka and Tervilla-Wilo (1985) and Dodson *et al.* (1986) threw some light on lignin biodegradation catalysed by the lignin peroxidase.

Insoluble carbohydrates, which constituted the major part of the jutestick and sugarcane bagasse served as the prime carbon source to the degraders. Changes in the level of carbohydrates during the process of degradation was considered an important index. The maximum rate of decrease in total carbohydrate at the first phases of incubation may be due to the presence of simple carbohydrate initially available in both jutestick and sugarcane bagasse which results the initiation of higher growth by the organisms that leads to decrease in total carbohydrate. But for longer incubation the organisms exhausted the simple carbohydrate resulted the slower growth and lower rate in loss of total carbohydrate. In all fungi and substrates combinations, the efficiency of the organisms was higher on jutestick than on sugarcane bagasse. Because in sugarcane bagasse soluble carbohydrate portion was very much higher than jutestick. So, the organisms can utilize them easily and after exhaustion of simpler carbo-

hydrate, organisms break down the insoluble portion. On the other hand in jutestick where soluble portion was very low, all the organisms break down the insoluble portion from the beginning,

Increase in net amount of soluble carbohydrates in the substrate, however, depended not only on the rate of breakdown of holocellulose but also on the rate of utilization by the organisms. Thus, among the test organisms, although *T. viride* caused maximum degradation of holocellulose than *C. globosum* and *P. sajor-caju* indicating more rapid utilization of soluble carbohydrate by *T. viride* and *C. globosum.*

Although *P. sajor-caju* is white rot fungus it degraded carbohydrate to a considerable extent in conformity with observation of Kirk (1971) who reported white rot fungi which were good degraders of lignin also metabolized carbohydrates well.

In plant materials, nitrogen is normally present in a very limited amount (Waksman, 1927 ; Alexander, 1961). Increase in soluble nitrogen in the substrate, however, depended not only on the differences in the rate of breakdown and rate of utilization of the carbohydrates by the organisms but also on the amount of carbon that was lost as CO_2 during degradation which resulted a relative increase of the nitrogen level. Although *T. viride* and *P. sajor-caju* are good degraders of jutestick and sugarcane bagasse, soluble nitrogen was highest in *C. globosum.* This evidently indicated rapid utilization of the soluble nitrogen by *P. sajor-caju* and *T. viride.* This is in conformity with observation of Rassadi and Amberger (1975) and Zadrazil (1976) that increase in soluble nitrogen may be due to the higher rate of solubilization of nitrogen.

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