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Transformation of Agricultural Wastes into Sugar by *Trichoderma viride*

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The agricultural wastes such as hardwood sawdust, softwood sawdust, banana stems, banana peels were subjected to the cellulytic action of the intact cells of *Trichoderma viride* grown in 5% wheat bran medium in presence of glucose as major carbon source. The central theme of this attempt was their transformation into fermentable sugar for onward conversion into ethanol in energy context. The results indicated that all the wastes showed some tendency to degradation, but banana stem was the best substrate as it exhibited the highest loss in weight, during the fermentation. There was a large consumption of sugar by the organism in the earlier stages of its growth. In the later stages, sugar was produced due to the degradation of cellulose present in agricultural wastes by cellulase produced by the organism

Key words: Transformation, Agricultural Waste, Sugar, *Trichoderma viride*.

In the present techno-economic era, the experts all over the World, particularly in the developing countries, are engaged to work on the transformation of agricultural wastes into sugar for onward conversion to alcohol in order to cope with the food and energy problems. Some activity, in this context, has also been carried in Pakistan.

Agricultural wastes such as banana stalk, straws, grasses, wheat and rice straw, wood sawdust, are the major agricultural wastes encountered in Pakistan. These wastes are rich sources of cellulose that can be converted to fermentable sugars for onward conversion into alcohol in energy context. Alcohol is an important alternative of gasoline that is being used as a motor fuel in some countries. These wastes, of course, have alternative utility also due to presence of cellulose as the major component in them. The alternative uses may be as raw material in the manufacture of paper pulp and animal feed and as conventional fossil fuels.

Van Hertson presented the first significant report on the degradation of cellulose by Fungi in 1904. He used soil as an inoculum instead of using the pure culture. The medium used for growth, contained filter paper moistened with tap water, ammonium nitrate and potassium dihydrogen phosphate.

More active work on this project was started near 1980. Ghose, T.K., reported cellulase biosynthesis and hydrolysis of cellulosic substances (1) Herr *et al.,* in1980 attempted the conversion of cellulose to glucose with cellulase of *Trichoderma viride*[2]. Soon after these events, the experts started thinking in terms of application of cellulase of fungal origin for the transformation of cellulose content of agricultural wastes into

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sugar for onward conversion to alcohol. Thus, a large number of studies have been carried in this context[3-9].

Keeping in view above landmarks, some work was also started on the biological transformation of some agricultural wastes into sugar and alcohol. As the initial results were promising, activity is in progress in Government College University Lahore. Some partial successes have also been achieved[10,11]. The work reported here is one of the primary sequences of events that occurred, on the stage of Government College University, Lahore.

MATERIAL AND METHODS

Collection of Samples

The banana stems and peels of the species *Musa paradisiaca* Lin var. *seplentum* of family Musaceae that are thrown as waste were collected from the local fruit market of Lahore. The wood dust samples were collected from the Timber Market, Ravi Road, Lahore. No difficulty was encountered in sample collection. The samples were processed soon after collection.

Determination of the Moisture Content of Samples

Twenty-five grams of banana stems and peels were placed in an oven at 105°C for 24 hours. The moisture present was thus evaporated to a constant weight of sample. The dried samples were cooled and weighed. By comparison of the two weights the %age of moisture present in the samples was calculated. The moisture contents of sawdust were not determined on the assumption that these are already in the dried state.

Culture and Inoculum

Trichoderma viride was grown on solidified potato-dextrose-agar slants at 28°C on the culture medium with the following composition:

Potatoes	200 g/1
Dextrose	20 g/1
Agar	15 g/1
pH	4.5

For the preparation of culture medium, 200g potato slices were boiled for one hour in about 500 to 700 ml distilled water. The resulting thick syrup was drained through a clean muslin cloth. To this syrup was added glucose and agar. The final volume was made up to 1000 ml. The mixture was cooked for an hour in a water bath. The pH of the medium was maintained at 4.5. Approximately 10 ml of medium was poured in each test tube. All the test tubes were cotton plugged. The medium was sterilized in a pressure cooker at 15 psi for 15 minutes. The test tubes were allowed to set for 24 hours to prepare the slants. The slants were inoculated with a sterilized needle loop and incubated at 30°C. The growth was allowed to occur for 4-6 days. The slants were preserved in a refrigerator.

To prepare the inoculum, the slants were washed carefully with sterilized distilled water and thus a sporal suspension was obtained. The spores were centrifuged at 2500 rpm for 20 minutes in a sterilized centrifuge tube. The supernatant was discarded and the pallet was suspended in an adequate volume of sterilized distilled water. The optical density of the suspension was read in a spectrophotometer. The suspension of the same optical density was transferred each time to keep the total population of spores constant; 10 to 20 ml of sporal suspension was transferred to each of the flasks containing 250ml wheat bran medium and 30 ml glucose solution.

Fermentation Medium

Wheat bran was chosen for the growth of *Trichoderma viride* as it was considered to be a suitable medium for the production of extra-cellular cellulase. The following quantities of ingredients per liter constituted the growth medium for *Trichoderma viride*:

Wheat bran	50 gm
K_2HPO_4	2.0 gm
KCI	0.54 gm
$MgSO_4.7H_2O$	0.5 gm

The ingredients were mixed in distilled water to make one-liter suspension. The pH of the suspension was adjusted to 4.5. The suspension was then sterilized for half an hour at 15 psi. 20 gm glucose was dissolved in 250 ml of distilled water and was sterilized separately for half an hour.

250 ml of wheat bran medium was taken in five different 500 ml conical flasks. One of these flasks was used as a blank. To each flask then was added 30 ml of sterilized glucose solution to make final concentration of glucose 1%. The

flasks were cotton plugged and were ready for inoculation.

Fermentation

Trichoderma viride was grown by surface culture technique. The flasks were inoculated using 10 ml of inoculum and subsequently incubated in an incubator. The growth temperature was 30°C. After three or four days, when the growth of the organism had started, about 5 ml suspension was taken out with a sterilized pipette. The suspension was filtered and the sugar content of the extracellular medium was determined.

Estimation of Reducing Sugar

The reducing sugar, during the course of fermentation was estimated by Nelson's Method[12]. To do so, 1 ml of the test sample was diluted to 100 ml. To 0.5 ml diluted sample was added 2 ml distilled water and then 2 ml alkaline copper sulphate reagent. The tube was cotton plugged carefully to prevent re-oxidation heated in a water bath for 8 minutes and cooled under water tap. Then, 2 ml Phosphomolybdic Acid Reagent was added, the contents of the tube were mixed carefully and diluted finally to 10 ml. The optical density of the color developed was read in a colorimeter at 670nm.

Biological Digestion of Banana Stems and Peels During Growth of Organism

In these experiments, 250 ml of wheat bran medium was taken in three 500 ml flasks. In the first flask, no agricultural waste was added; in the second 20g banana stem (Dry weight 1.85g) and in the third 20 g banana peels (Dry weight 3.10g) were added. All the flasks were cotton plugged and sterilized for half an hour at 15 psi. The flasks were cooled and to each was added 30 ml sterilized glucose solution (20g/250ml). All the flasks were inoculated with 20 ml inoculum and the fermentations were carried as described above. Five ml samples were withdrawn and in each sample, sugar was estimated. The fermentation was carried for about 20 to 25 days.

At the end of the fermentation, the cells formed on the surface, were removed, dried and weighed to assess the biomass formed during the fermentation. At this juncture, the banana stems and peels were also removed, washed, dried and weighed. The initial and final weights were compared to determine the %age loss during fermentation.

Biological Digestion of Plane Newspaper During Growth of Organism

In these experiments, the procedure followed was the same as that in the banana stems and peels except 5 g softwood (*Diyar*) saw dust and 5g, hardwood (*Shisham*) sawdust were added in place of 20g banana stems and peels. 5 ml samples were similarly withdrawn and sugar similarly estimated.

RESULTS AND DISCUSSION

The results of moisture determination are shown in Table 1.

Table 1 shows that the banana stems contain roughly 91% moisture, while banana peels contain about 85% moisture. It seems that the banana peels might have lost some moisture during their travel from banana farms to the fruit markets.

The variation of sugar concentration during the growth of *Trichoderma viride* with the incubation time at 30°C in wheat bran medium containing different agricultural wastes due to sugar consumed by the cells and produced as a result of degradation of cellulosic materials is shown in Fig.1-4. For comparison, the growth of the organism without a waste under same conditions is shown in Fig. 5.

The comparison of profiles indicates that there is a rapid fall in the sugar concentration of the medium in case of all the agricultural wastes. This fall is due to the large consumption of sugar by the organism in the earlier stages of its growth.

The sugar concentration subsequently rises in case of all agricultural wastes. This rise may be due to the degradation of cellulose present in the agricultural wastes by the cellulase

Table 1. Moisture Content of Banana Stems and Banana Peels

Substrate	Weight Before Drying(g)	Weight After Drying(g)	%age Loss
Banana Stems	20	1.85	90.75
Banana Peels	20	3.10	84.50

produced by *Trichoderma viride*. The extent of cellulytic degradation during the growth is the highest in presence of banana stems as substrate (Fig. 1). Thus, the highest %age of sugar that is 1.66% is produced in presence of banana stem. The next in order fall the banana peels that is 1.44% (Fig. 2). Sugar produced in presence of hardwood sawdust and softwood sawdust is relatively low (Fig. 3-4).

Maximum sugar produced in different fermentations in presence of different agricultural substrates is shown in Table 2.

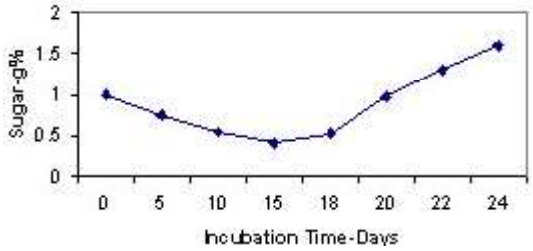

Fig. 1. Variation of sugar concentration during the growth of *Trichoderma viride* in presence of banana stems

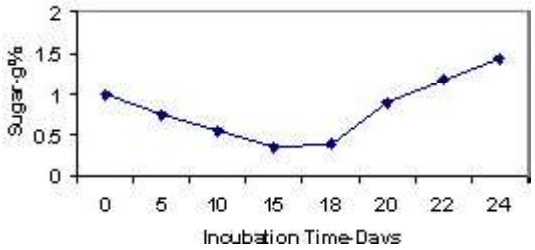

Fig. 2. Variation of sugar concentration during the growth of *Trichoderma viride* in presence of banana peels.

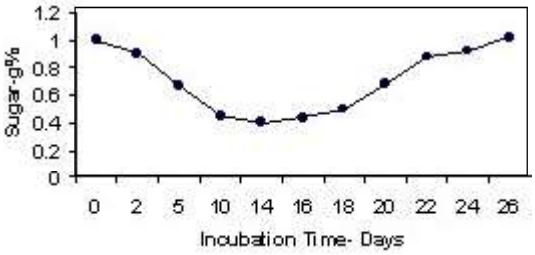

Fig. 3. Variation of sugar concentration during the growth of *Trichoderma viride* in presence of hardwood sawdust

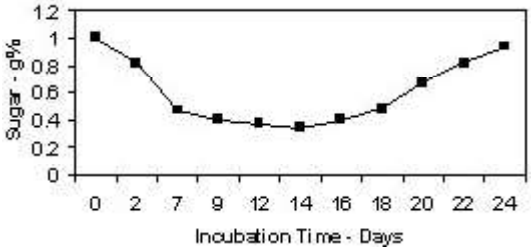

Fig. 4. Variation of sugar concentration during the growth of *Trichoderma viride* in presence of softwood sawdust

Table 2. Maximum sugar produced during different fermentations in presence of different substrates

Substrates	Maximum Sugar Produced
20 g Banana Stem	1.60g %
20 g Banana Peel	1.44g %
5 g Hardwood Sawdust	1.02g %
5g Softwood Sawdust	0.94g %

The comparison of maximum sugar produced indicates that the shredded banana stem is hydrolyzed more than the other substrates studied here.

The shape of the blank containing glucose but no agricultural waste is shown in Fig. 5. The profile suggests that there is a rapid fall in sugar concentration in the earlier stages of fermentation as in case of the agricultural wastes discussed above. That is due to the initial consumption of sugar by the organism to satisfy its nutrition and energy needs. Sugar concentration in blank also rises to some extent and falls again (Two peaks in Fig. 5).

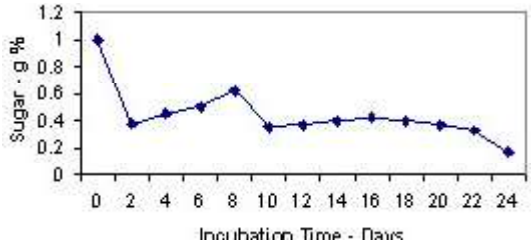

Fig. 5. Variation of sugar concentration during the growth of *Trichoderma viride* in absence of agricultural waste.

Table 3. Dry biomass formed during the growth of *Trichoderma viride* in presence of agricultural wastes at the end of fermentations

Substrates	Dry Biomass (g)	Excess Biomass (g)
Banana Stems	1.2590	1.2590-0.0012 = 0.2578
Banana Peels	1.9500	1.9500-1.0012 = 0.9488
Hardwood Sawdust	1.2322	1.2322-1.0012= 0.2310
Softwood Sawdust	1.8581	1.8581-1.0012= 0.8569
Blank	1.0012	

Table 4. Comparison of initial and final weights of agricultural wastes before and after fermentation

Substrate	Initial Dry Weight (g)	Final Dry Weight (g)	% age Loss
Banana Stems	1.85	0.10	94.59
Banana Peels	3.10	0.30	90.33
Hardwood Sawdust	5.00	-	-
Softwood Sawdust	5.00	-	-

The height of the first peak is greater than that of second. The rise in the sugar concentration at the later stages may be explained on the basis that it may be the result of the degradation of some cellulose present in wheat bran.

The dry biomass formed during the growth of *Trichoderma viride* in presence of agricultural wastes at the end of fermentations is recorded is recorded in Table 3.

The comparison suggests that the extra biomass formed as a result of consumption of the sugar produced, as aresult of degradation is the highest in case of banana peels. Next in order, in this respect is the softwood sawdust, then falls banana and last comes the hardwood sawdust.
The comparison of the initial and final weights of banana stems and peels before and after the fermentation is made in Table 4.

The results indicate that there is highest loss in case banana stems, while it is also comparable in case of banana peels. As the woods, being in the fine state, were difficult to separate from wheat bran, these were not removed and weighed.

DISCUSSION

The major objective of the project was the techno-economic disposal of the agricultural wastes by their transformation into fermentable sugar for onward transformation into alcohol to meet the energy requirements of humans any where on the Globe. The sugar requirement for effective conversion into ethanol, being 10 to 12%, the target yet seems to be far away, as the yield of sugar from the wastes encountered in the agriculture based economies are like those studied over here and sugar yield being reported can be roughly said to be from 1 to 2%. The misfortune seems to be the organism itself that eats away the sugar formed as a result of the cellulysis of wastes. The major index to this interpretation is the large quantity of biomass formed at the end of the fermentation almost in all cases.

The major indication of the study is that banana stems and banana peels seem to be the most promising substrates. These two may be the research focus of the future investigation. The banana stems and leaves stand even superior to peels, etc, in merit as these are found in bulk in banana cultivation farms and do not involve any cost of collection. The peels, on the other hand, may result from the home eating activity and of fruit processing involved in preparation of fruit salads and other dishes in the restaurants and small shops on the roads or in the streets from where the cost of collection may be very high. The work in this direction may be carried to identify conditions in which the growing

organisms consume less sugar and cause more cellulysis of banana stems. The physiology of different species will also matter a lot in this context. More than 90% degradation of banana stems and peels is definitely the green signal for the experts to engage themselves in this field. Trial of different organisms, different strains of the same organism, changing of growth conditions, etc, constitute fairly good parameters for investigation. *Trichoderma vride* also deserves thorough study in the light of the parameters suggested above.

There are, of course, some abnormalities encountered if the %age loss (Table 4) during fermentations is compared with the formation of biomass (Table 3). The %age degradation of banana stems and peels, being comparable that is more than 90%, the biomass formed in case of peels is almost thrice of that formed in case of banana stem. One of the factors may be that its solid content is almost double of that of banana stem. That means higher cellulose content of banana peels that translates into formation of more sugar that is immediately consumed by the organism to produce more biomass. It is yet understandable to some extent, but the production of more biomass in case of softwood sawdust that is almost comparable to that formed in case of banana peels is alarming. Softwoods have little binding material such as lignins, rosin hemicelluloses, etc, and thus its cellulose fibers are more exposed. There is the likelihood that the exposed cellulose induces production of large quantities of cellulase that degrades the cellulose content of wheat bran and produces high quantity of sugar that is immediately eaten away by the organism to produce high quantity of biomass.

The banana stems and peels re the most favorable substrates for fungi as they don't contain large quantities of binding materials to contribute to their structural compactness. Thus, their cellulose fibers remain exposed and act as better inducers of cellulases as reported by Khan et al (10). After extensive degradation, they produce more sugar that is consumed by the growing fungi. The hard woods are compact due to lot of binding materials to render them compact and hard. Thus, these are not good substrates. They are neither good inducers of cellulase nor degrade it well to produce sugar. Softwoods, of course, being less compact can be investigated, as they seem to be relatively better substrates. Hardwood, of course, may be checked for two-stage transformation. The first stage may be the chemical treatment of hardwoods to remove the binding material and loosening of fibrous structure, while second will be the biological transformation.

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