PN-ABK-903
ISN 76522

# ICAITI
## SECOND PROGRESS REPORT

## "SACCHARIFICATION OF LIGNOCELLULOSIC MATERIALS BY BROWN-ROT FUNGI"

This report covers activities from March to August 1990

### 1. Searching and isolation of brown rot fungi

Since the rainy season has started, a place was chosen for attempting the isolation of brown rot fungi. Between the department of Guatemala and neighboring Jalapa we found a place of mountains that used to be forests of coniphers, specially pine. Apparently there has been exploitation of most of the original trees because we encountered a good number of stumps that are very old. Some are in a certain stage of decomposition and are almost soft and covered by mycellium; they smelled like black soil. Others where in a less advanced stage of degradation and were more firm and brownish in color. When touched by hand they left small pieces of wood ( though not in cubical shape). Samples were taken and transported to ICAITI. We have been doing isolations in Malt extract agar and Potato Dextrose agar. Several apparently pure white mycellium has been subjected to the Bavendamm test but have produced discoloration. The place however seems attractive to keep on exploring because of the humidity, cool clime, forests of coniphers and the old age of those stumps.

### 2. Experiments to optimize degradation of sugar cane bagasse

The experimental design chosen for the optimization of sugar cane bagasse (SCB) where as follows. Two levels of supplementary nitrogen were chosen, a relation of 12:1, carbon to nitrogen ratio and a relation of 25:1, the control sugar cane bagasse has a natural relation of 180:1.

The sugar cane bagasse was obtained from sugar mills in the south coast of Guatemala, department of Escuintla. The fraction utilized in this research was prepared by eliminating the outer cover and using mainly the inner part. The material was dried for 24 h in a tray drier using hot air. Then the SCB was milled in a Fitz Mill model with knives and screened to obtain the fraction that is retained in 40 mesh. The SCB was immersed in water to extract any soluble sugars remaining and then dried again. To obtain data in the nitrogen content, SBC was analyzed with the micro-Kjeldahl technique (AOAC, 1975). To find the C:N relation the carbon content was determined as follows (within 2 to 10 % error, de León, 1982)

$$\% C = \frac{100 - \% \text{ of ashes}}{1.8}$$

The experimental setup was prepared by sterilizing the columns at 121° C for 20 minutes with the sugar cane bagasse at a moisture content of 60 %, the inocula was added from a liquid culture of the strains in malt broth (shaken flasks at 35° C), centrifuged, washed with distilled sterile water and resuspended in a solution of mineral salts (Formula in Annex III). The setup was aerated from small air pumps (Challenger I) or from aereation from the laboratory compressed air, by using a rectifying valve to decrease pressure and flow and passing the air trough a sterile stainless steel cylinder stuffed with glass wool and then to a two liter sterile water container to humidify the air. The whole setup was installed inside a room where the temperature is kept at 35° C.

The fungi utilized in the study were **Gloeophyllum trabeum** and **Wolphiporia cocos** . The experiment was made using for the purpose, 48 columns with a level of aereation of 100 $mm^3$ of air per column. Every two weeks, a sample was taken to subject it to the following analysis:

- Degree of polymerization (according to the method Viscosity of Pulp T-230 om-82) Tappi, 1988

- Lignin-acid-detergent, according to USDA, 1979.

- Neutral detergent, according to Tappi, 1988

- Lignin permanganate, cellulose, insoluble ash and silica

The results after eight weeks of incubation has some interesting results; for one thing there is no apparent need to go longer than six weeks since most of the activity is obtained before; second, the supplementary nitrogen seems no neccesary in the case of **G. trabeum** but does have an effect with **W. cocos.** Considering results presented in Annex I, in Figures 1 through 10 for **G. trabeum** and **W. cocos** and the Tables 1 to 5 in Annex II, the addition of supplemental nitrogen did not cause any particular advantage for G. trabeum. The degree of polymerization decreased from 2669, a value for the sugar cane bagasse control, to 1500 in six weeks at a ratio of 180:1, C:N; that is a 43.8 % whereas for the supplemented SCB at 12:1 C:N the reduction was only 17.3 % and at 25:1, C:N, a 11.8 %. For the digestion of cellulose, the non-supplemented SCB with G. trabeum decreased the cellulose from 37.58 g/100 g to 21.13, a reduction of 43.77 % supplemented SCB at 12:1 C:N made only a 17.3 % and at 25:1, a 11.6 %. Other data such as the hemicellulose and the lignin contents show the same tendency.

For **Wolphiporia cocos**, the addition of nitrogen worked better The decrease of the degree of polymerization shows a change from 2669 in control SCB to 2260 after six weeks (15.3%) for a non-supplemented 180:1 C:N. Whereas both for 12:1 and 25:1 C:N supplementary of SCB, the decrease went from 2669 to 1527

and 1437 respectively (42.79 and 46.16 %). Also in relation to cellulose degradation, from an initial value of 37.58 g/100g, the SCB at 180:1, C:N, had in six weeks a change of 15.5 %; a change of 42.8 % for 12:1 and 45.9 % for 25:1. Hemicellulose and lignin were increased as they are fractions not utilized in this particular case of fungal attack.

## 3. Experimental set-up in preparation

The following substrata have been prepared and are now being loaded to study a six week incubation period: oil palm fiber, wheat straw, lemon tea bagasse, pine sawdust and eucaliptus sawdust. we expect to inoculate both G. trabeum and W. cocos and the other additional strains available.

## 4. Materials received during the period.

The requested equipment and materials from Cole-Parmer, an incubator and glassware such as culture tubes, pipets, dilution bottles, and flasks were received. Also, to avoid the costly preparation of the cupriethylendiamine reagent, six liters were requested and received from GFS Chemicals in Columbus, Ohio. Though they were for security reasons not sent by air cargo, they arrived within a reasonable amount of time even shorter than the glassware.

## 5. Bibliography

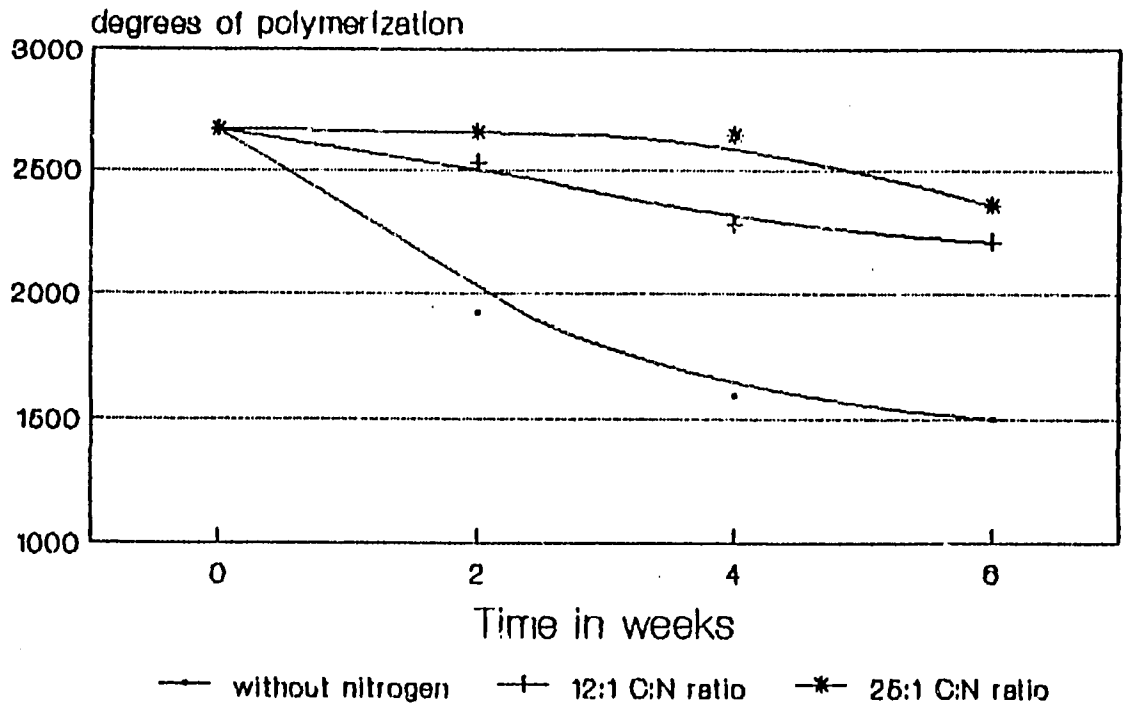

- De León, R. Degradación Aeróbica Termofílica; Su Plicación en el Manejo de Desechos Sólidos. En ICAITI MIRCEN, CID/CONADI. Fermentaciones en Sustratos Sólidos. Tegucigalpa Honduras. pp 33-45. 1982

- Tappi. Test Methods. Atlanta, GA, 1988

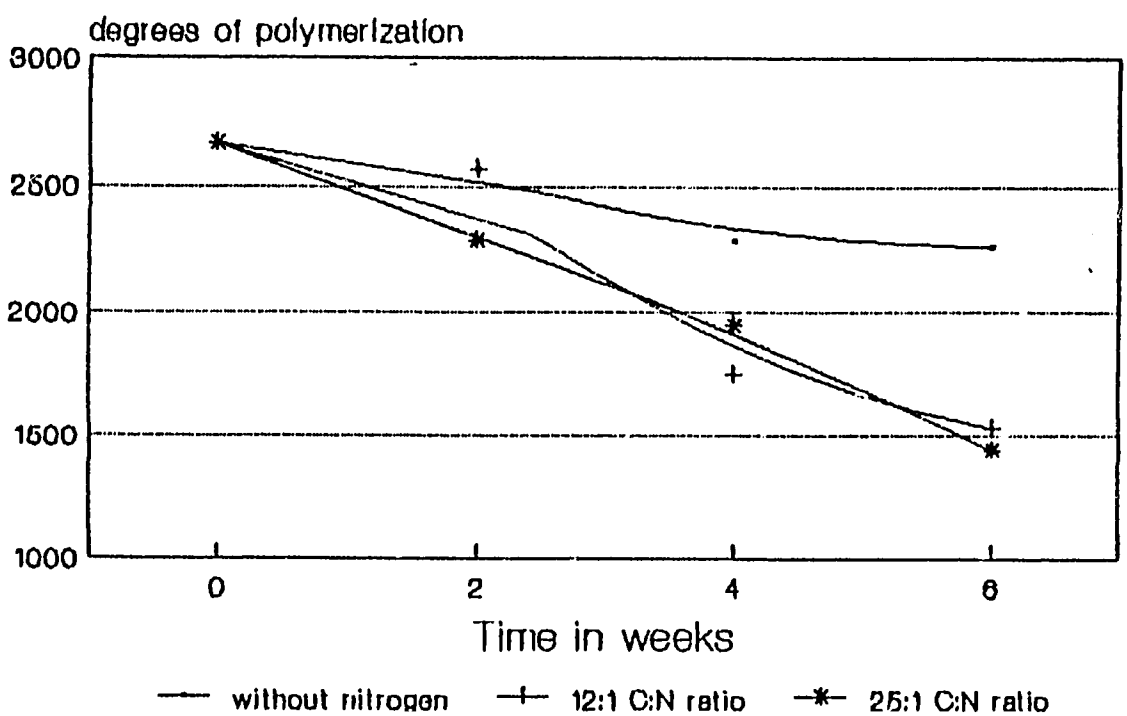
- USDA. Agriculture Handbook 3-79., 1979

- Browne, C.A. & F.M. Zerban. Physical and chemical methods of sugar analysis. New York, John Wiley & Sons Inc. 3d Ed. pp 1955

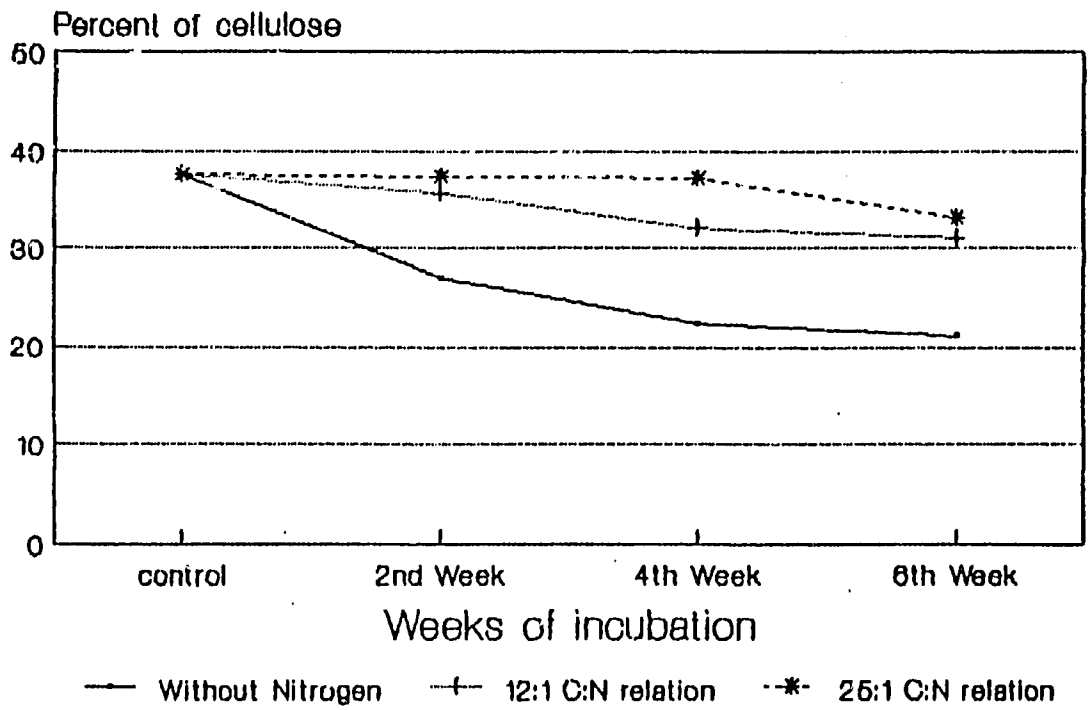
# Figure 1
## Gloeophyllum trabeum
## Sugar cane bagasse



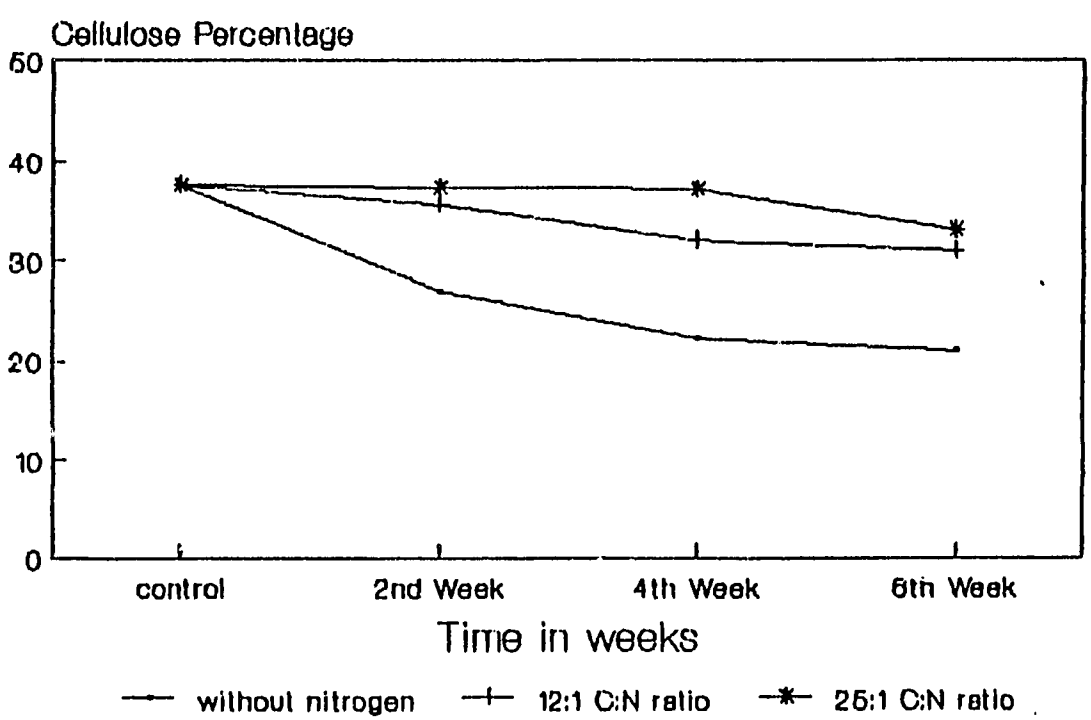

# Figure 2
## Wolphiporia cocos
## Sugar cane bagasse



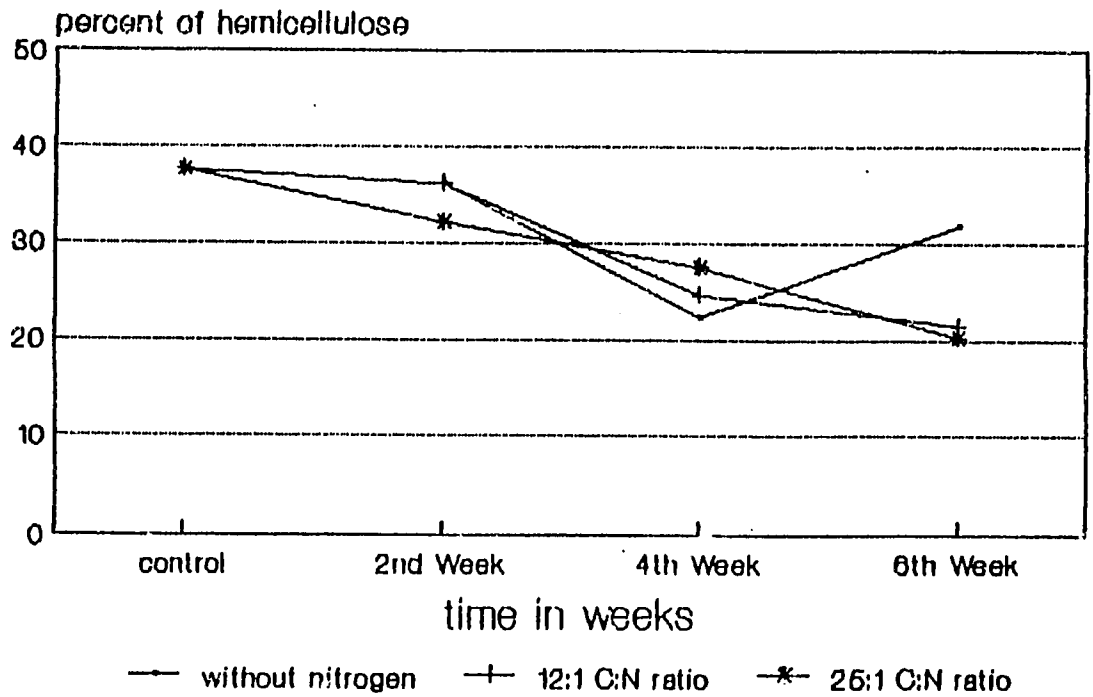
# Figure 3
## G. trabeum
## Sugar cane bagasse



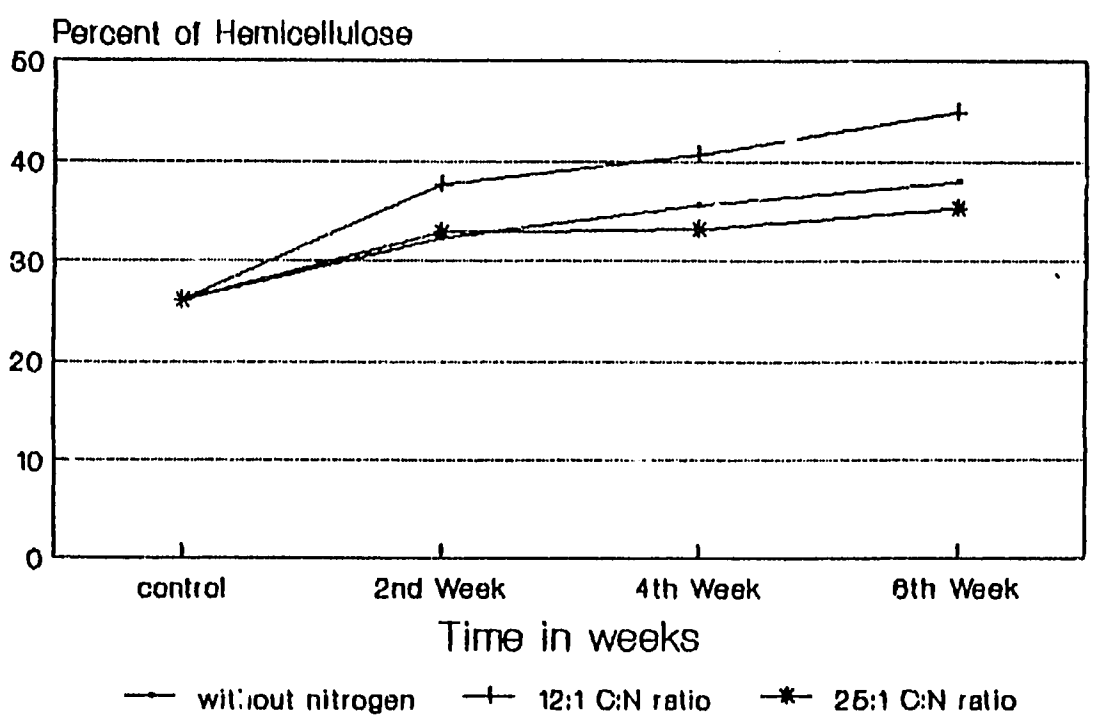

# Figure 4
## W. cocos
## Sugar cane bagasse



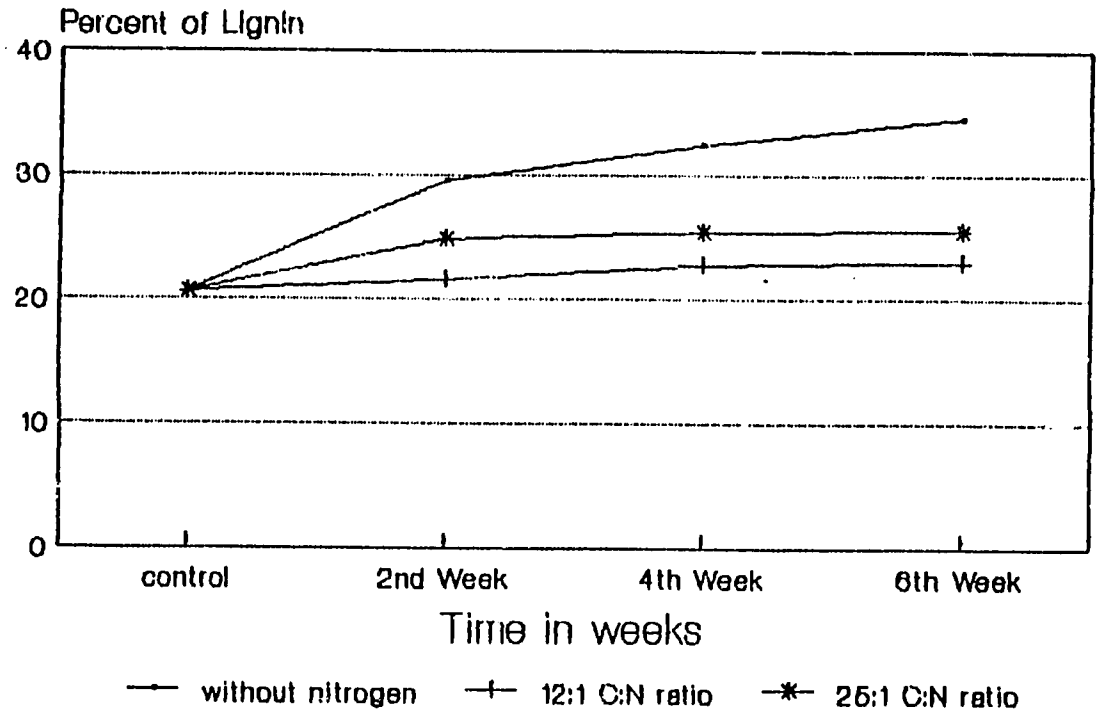
# Figure 5
## G. trabeum
## Sugar cane bagasse



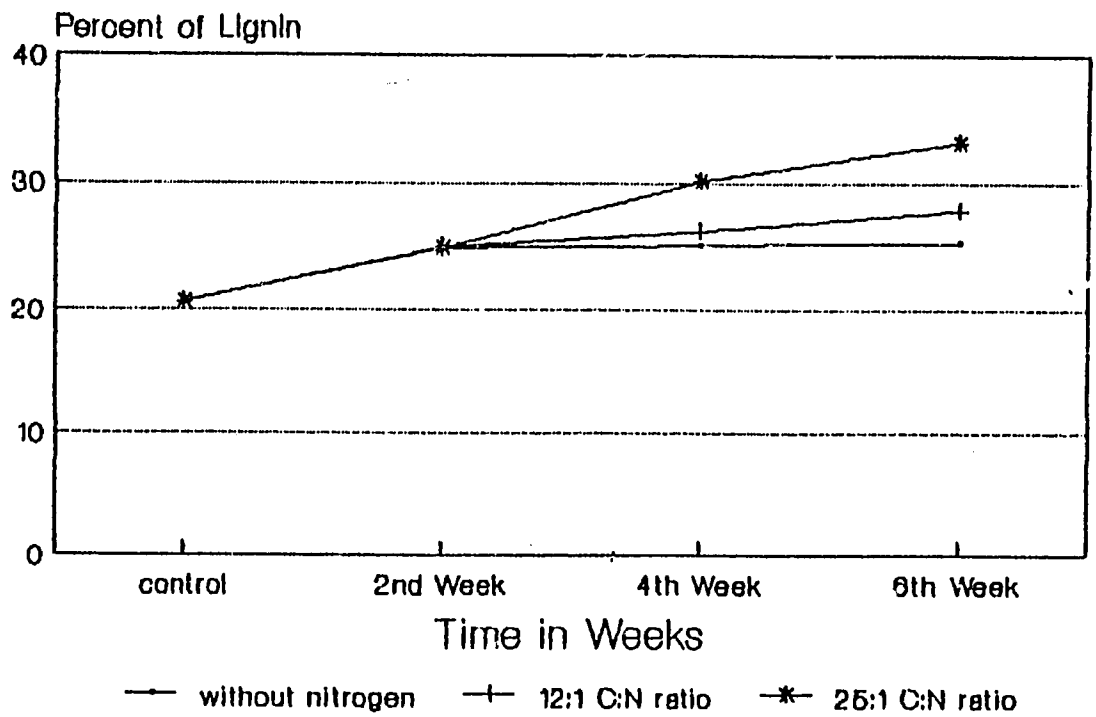

# Figure 6
## W. cocos
## Sugar cane bagasse



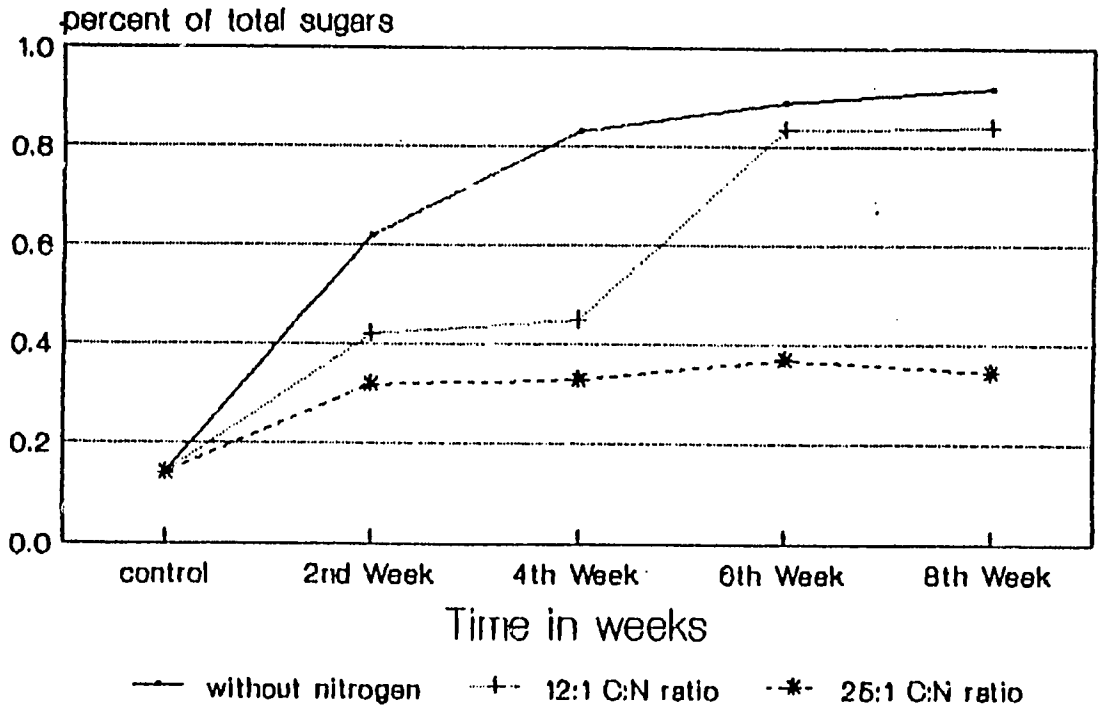
# Figure 7
## G. trabeum
## Sugar cane bagasse




# Figure 8
## W. cocos
## Sugar cane bagasse



# Figure 9
## G. trabeum
## Sugar cane bagasse




# Figure 10
## W. cocos
## Sugar cane bagasse

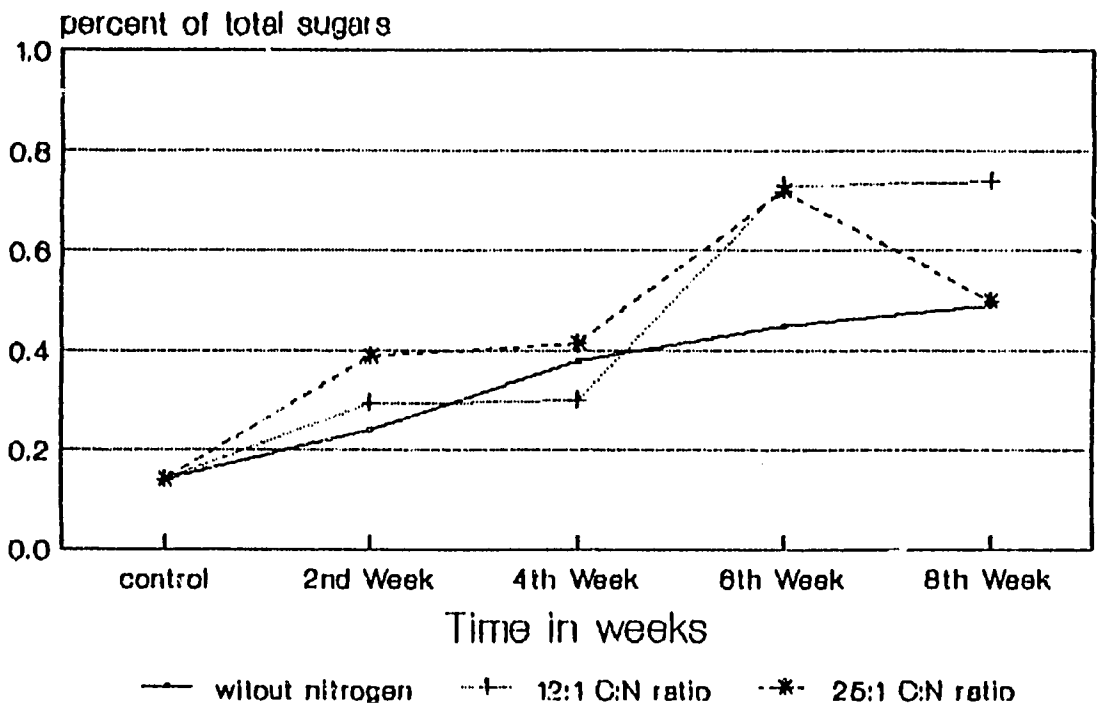


TABLE 1

Results of degree of polymerization in sugar cane bagasse after growth of **G. trabeum** and **W. cocos.**

| Control | C:N | Two | Four | Six | Eight |
|---|---|---|---|---|---|
| | | **Gloeophyllum trabeum** Time in weeks | | | |
| 2669.68 | 180:1 | 1917.97 | 1590.94 | 1500.36 | 1590.22 |
| | 12:1 | 2529.34 | 2276.78 | 2207.72 | 2413.62 |
| | 25:1 | 2652.04 | 2643.29 | 2354.53 | 2344.50 |
| | | **Wolphiporia cocos** | | | |
| | 180:1 | 2566.16 | 2282.07 | 2260.53 | 1966.55 |
| | 12:1 | 2568.76 | 1748.68 | 1527.40 | 1499.05 |
| | 25:1 | 2286.71 | 1947.57 | 1437.19 | 2008.32 |

TABLE 2

Results of content of % cellulose in sugar cane bagasse after growth of **G. trabeum** and **W. cocos**

| Control | C:N | Two | Four | Six | Eight |
|---|---|---|---|---|---|
| | | Time in weeks | | | |
| | | **G. trabeum** | | | |
| 37.58 | | 26.98 | 22.36 | 21.13 | 22.27 |
| | 12:1 | 35.60 | 32.05 | 31.05 | 32.89 |
| | 25:1 | 37.33 | 37.21 | 33.15 | 33.70 |
| | | **W. cocos** | | | |
| | | 36.11 | 32.06 | 31.75 | 27.65 |
| | 12:1 | 36.14 | 24.59 | 21.48 | 21.03 |
| | 25:1 | 32.19 | 27.54 | 20.32 | 21.46 |

TABLE 3

Results of content of % Hemicellulose in sugar cane bagasse after growth of **G. trabeum** and **W. cocos**

| Control | C:N | Time in weeks Two | Four | Six | Eight |
|---|---|---|---|---|---|
| | | | **G. trabeum** | | |
| 26.12 | 180:1 | 32.38 | 35.60 | 37.91 | 38.11 |
| | 12:1 | 37.68 | 40.64 | 44.88 | 39.38 |
| | 25:1 | 32.88 | 33.23 | 35.34 | 36.18 |
| | | | **W. cocos** | | |
| | 180:1 | 34.66 | 38.35 | 40.02 | 45.93 |
| | 12:1 | 38.06 | 41.76 | 44.81 | 44.23 |
| | 25:1 | 29.83 | 40.22 | 38.46 | 38.64 |

TABLE 4

Results of Lignin content in % in sugar cane bagasse after growth of **G. trabeum** and **W. cocos**

| Control | C:N | Time in weeks Two | Four | Six | Eight |
|---|---|---|---|---|---|
| | | | **G. trabeum** | | |
| 20.62 | 180:1 | 29.54 | 32.43 | 34.60 | 36.15 |
| | 12:1 | 21.53 | 22.75 | 22.91 | 24.61 |
| | 25:1 | 24.81 | 25.33 | 25.43 | 25.70 |
| | | | **W. cocos** | | |
| | 180:1 | 24.86 | 25.08 | 25.25 | 23.99 |
| | 12:1 | 24.92 | 26.18 | 27.79 | 31.04 |
| | 25:1 | 24.89 | 30.11 | 33.19 | 26.12 |

TABLE 5

Results of content of total sugars in % in sugar cane bagasse after growth of G. trabeum and W. cocos

| | | | | | |
|---|---|---|---|---|---|
| | | | **G. trabeum** | | |
| | | | Time in weeks | | |
| Control | C:N | Two | Four | Six | Eight |
| 0.14 | 180:1 | 0.62 | 0.83 | 0.89 | 0.92 |
| | 12:1 | 0.42 | 0.45 | 0.84 | 0.84 |
| | 25:1 | 0.32 | 0.33 | 0.37 | 0.35 |
| | | | **W. cocos** | | |
| | 180:1 | 0.24 | 0.38 | 0.45 | 0.49 |
| | 12:1 | 0.30 | 0.30 | 0.73 | 0.74 |
| | 25:1 | 0.39 | 0.42 | 0.72 | 0.50 |