Mycoscience 35: 95–98, 1994

# Radiation and fermentation treatment of cellulosic wastes

**Mohammed A. Malek[1], Najmul A. Chowdhury[1], Shinpei Matsuhashi[2], Shoji Hashimoto[2] and Tamikazu Kume[2]**

[1] Institute of Food and Radiation Biology, Atomic Energy Research Institute. P. O. Box No. 3787, Dhaka, Bangladesh
[2] Takasaki Radiation Chemistry Research Establishment, Japan Atomic Energy Research Institute, Watanuki-machi 1233, Takasaki, Gunma 370-12, Japan

Accepted for publication 14 January 1994

**The effect of radiation pasteurization of sugar cane bagasse and rice straw and fermentation using various strains of fungi were studied for upgrading of cellulosic wastes. The initial contamination by fungi and aerobic bacteria both in bagasse and straw was high. The doses of 30 kGy for sterilization and 8 kGy for elimination of fungi were required. Irradiation effect showed that rice straw contained comparatively radioresistant microorganisms. It was observed that all the fungi (*Hericium erinacium, Pleurotus djamor, Ganoderma lucidum, Auricularia auricula, Lentinus sajor-caju, Coriolus versicolor, Polyporus arcularius, Coprinus cinereus*) grow extending over the entire substrates during one month after inoculation in irradiated bagasse and rice straw with 3% rice bran and 65% moisture content incubated at 30°C. Initially, sugar cane bagasse and rice straw substrates contained 39.4% and 25.9% of cellulose, 22.9% and 26.9% of hemicellulose, and 19.6% and 13.9% of lignin+cutin, respectively. Neutral detergent fibre (NDF) values decreased significantly in sugar cane bagasse fermented by *G. lucidum, A. auricula* and *P. arcularius*, and in rice straw fermented by all the 8 strains of fungi. Acid detergent fibre (ADF) values also decreased in bagasse and rice straw fermented by all the fungi. *P. arcularius, H. erinacium, G. lucidum* and *C. cinereus* were found to be the most effective strains for delignification of sugar cane bagasse.**

Key Words——cellulosic wastes; fermentation; radiation; rice straw; sugar cane bagasse.

## Introduction

A large quantity of cellulosic agricultural wastes or by-products like sugar cane bagasse and rice straw are totally discarded or under utilized through out the world. Upgrading of these wastes into more useful end-products may be one potential solution to increasing demands of food, feed and fuel for ever increasing population. Utilization of these cellulosic wastes is necessary to recycle the resources and also to reduce the environmental pollution. Over the last 20 years, increasing research efforts have been directed to the enzymatic conversion of cellulose to glucose and subsequent fermentation to ethanol (Chahal, 1991). However, the enzymatic hydrolysis of lignocellulosic materials is very slow, mainly due to compositional heterogeneity and structural complexity. The forest biomasses contain 40-60% cellulose, 10-25% hemicellulose, and 13-18% lignin (Wenzel, 1970); the agricultural wastes contain 30-45% cellulose, 16-29% hemicellulose and 3-13% lignin (Sloneker, 1976). Microbial fermentation of these materials by desired microorganism is greatly interfered by natural contamination during processing and storage. Disinfection of microorganisms by irradiation and effect of storage of feed have been reported (Kume et al., 1983). In solid state fermentation, *Pleurotus sajor-caju* (Fr.) Sing. has been reported (Awang et al., 1993) to be able to degrade oil palm empty fruit bunch (EFB) fibre leaving 70% useful materials.

Combination of radiation under proper conditions and dose may bring easier degradation of lignocellulosic wastes by fermentation, and whereby these wastes can be better utilized. In this study, sugar cane bagasse and rice straw were irradiated and subjected to fermentation by 8 different strains of fungi.

## Materials and Methods

**Substrate** Sugar cane bagasse donated by Okinawa Prefectural Agricultural Experiment Station and rice straw collected from a local paddy field in Takasaki were used in this experiment. All the materials were dried at 40°C for 4-5 days before use. Sugar cane bagasse cut into size of 2 mm and rice straw into pieces of 1-2 cm length, mixed with 3% of rice bran (65% moisture content) were used as substrates for fungal growth.

**Irradiation** For decontamination study, 5 g of samples (dried) were packed in polyethylene bags and irradiated by $^{60}$Co gamma-rays at dose rates of 1-5 kGy/h. For pasteurization or sterilization, irradiation was carried out keeping the substrate in 300 ml conical flask at a dose of 30 kGy with a dose rate of 6 kGy/h.

**Fermentation** Irradiated substrates were inoculated by 8 different strains of fungi, namely *Hericium erinacium* (Fr.) Pers., *Pleurotus djamor* (Fr.: Fr.) Boedijn. (=*Pleurotus flavellatus*), *Ganoderma lucidum* (Leyss.: Fr.) Karst.,

*Auricularia auricula* (Hook.) Underw., *Lentinus sajor-caju* (Fr.: Fr.) Fr. (=*Pleurotus sajor-caju*), *Coriolus versicolor* (L.: Fr.) Quel., *Polyporus arcularius* Batsch.: Fr. (=*Favolus arcularius*) and *Coprinus cinereus* (Schaeff.: Fr.) S. F. Gray, and incubated at 30°C with a humidity of 80% for a period of 1 month. The growth of inoculated fungi in the substrates were checked periodically.

**Analysis of the fermented product** Neutral detergent fibre (NDF), acid detergent fibre (ADF) and crude fibre (CF) were analyzed by using the Fibertec system 1010 (Tecator, Sweden) (AOAC, 1990). The contents were measured in triplicate and expressed as weight percentage on a moisture free basis.

## Results and Discussion

**Decontamination of microorganisms by irradiation** Figure 1 shows the decrease in number of microorganisms by gamma irradiation in sugar cane bagasse. Fungi were radiosensitive and eliminated below a detectable level by a dose of 8 kGy. Aerobic bacteria were less sensitive to radiation. A dose of 10 kGy decreased total colony forming units (cfu) to $10^4$ cfu/g from an initial load of $1.3 \times 10^9$ cfu/g in sugar cane bagasse. In case of rice straw, the decrease in number of fungi was small at low dose but the fungi were eliminated below detectable level by 8 kGy. Aerobic bacterial load decreased to $2.5 \times 10^4$ cfu/g from an initial load of $2.3 \times 10^7$ cfu/g by a dose of 12 kGy (Fig. 2). Aerobic bacteria in rice straw were less sensitive to gamma radiation compared to those in sugar

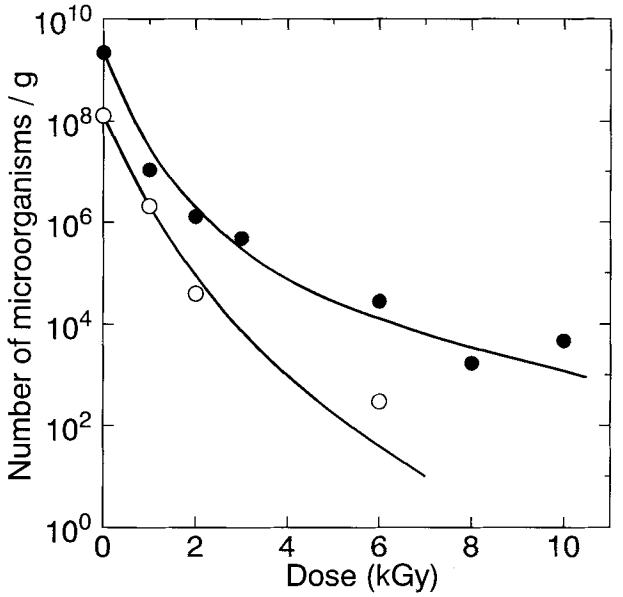


Fig. 1. Decrease in number of contaminating microorganisms in sugar cane bagasse by gamma-ray irradiation.
● Total aerobic bacteria, ○ Fungi.

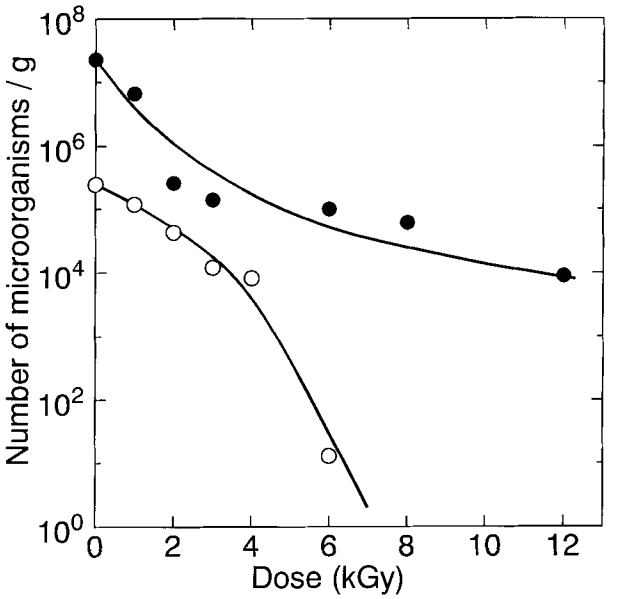


Fig. 2. Decrease in number of contaminating microorganisms in rice straw by gamma irradiation.
● Total aerobic bacteria, ○ Fungi.

Table 1. Growth of various fungi in sugar cane bagasse and rice straw substrates during 30 days of fermentation.

| Name of fungi | Sugar cane bagasse | | | Rice straw | | |
|---|---|---|---|---|---|---|
| | Incubation period (days) | | | Incubation period (days) | | |
| | 7 | 14 | 30 | 7 | 14 | 30 |
| *Hericium erinacium* | +++ | +++ | +++ | ++ | +++ | +++ |
| *Pleurotus djamor* | +++ | +++ | +++ | +++ | +++ | +++ |
| *Ganoderma lucidum* | +++ | +++ | +++ | +++ | +++ | +++ |
| *Auricularia auricula* | ++ | +++ | +++ | +++ | +++ | +++ |
| *Lentinus sajor-caju* | +++ | +++ | +++ | +++ | +++ | +++ |
| *Coriolus versicolor* | ++ | +++ | +++ | ++ | +++ | +++ |
| *Polyporus arcularius* | +++ | +++ | +++ | ++ | +++ | +++ |
| *Coprinus cinereus* | + | ++ | +++ | ++ | +++ | +++ |

+: Little growth, ++: Fair growth, +++: Good growth, +++: Very good growth.

Table 2. NDF, ADF and CF contents in sugar cane bagasse and rice straw after 1 month fermentation by various fungi.

| Name of fungi | Sugar cane bagasse | | | Rice straw | | |
|---|---|---|---|---|---|---|
| | % NDF | % ADF | % CF | % NDF | % ADF | % CF |
| Control | 81.9±0.4 | 59.0±0.3 | 39.4±0.5 | 66.5±2.3 | 39.8±0.6 | 25.9±1.4 |
| *Hericium erinacium* | 78.9±1.0 | 56.6±0.1 | 49.9±2.6 | 62.2±0.4 | 35.5±0.6 | 25.2±0.8 |
| *Pleurotus djamor* | 79.7±0.8 | 57.3±0.4 | 40.6±0.6 | 61.3±0.8 | 37.6±1.6 | 25.6±0.1 |
| *Ganoderma lucidum* | 73.0±1.3 | 56.6±1.5 | 45.3±0.2 | 57.2±1.2 | 36.6±1.3 | 25.6±0.5 |
| *Auricularia auricula* | 74.3±0.2 | 57.8±0.2 | 38.8±0.4 | 62.3±1.6 | 37.2±1.9 | 25.1±0.6 |
| *Lentinus sajor-caju* | 78.0±0.8 | 58.7±2.6 | 38.9±0.5 | 61.4±2.0 | 36.9±0.8 | 25.0±0.8 |
| *Coriolus versicolor* | 75.0±0.1 | 54.3±0.1 | 34.6±0.8 | 52.3±1.0 | 34.2±1.4 | 22.5±0.7 |
| *Polyporus arcularius* | 68.6±0.6 | 50.8±1.1 | 45.3±1.1 | 55.2±0.4 | 34.9±1.4 | 23.7±0.5 |
| *Coprinus cinereus* | 81.8±0.4 | 54.8±0.6 | 42.3±0.4 | 58.3±0.4 | 34.7±0.1 | 23.9±1.3 |

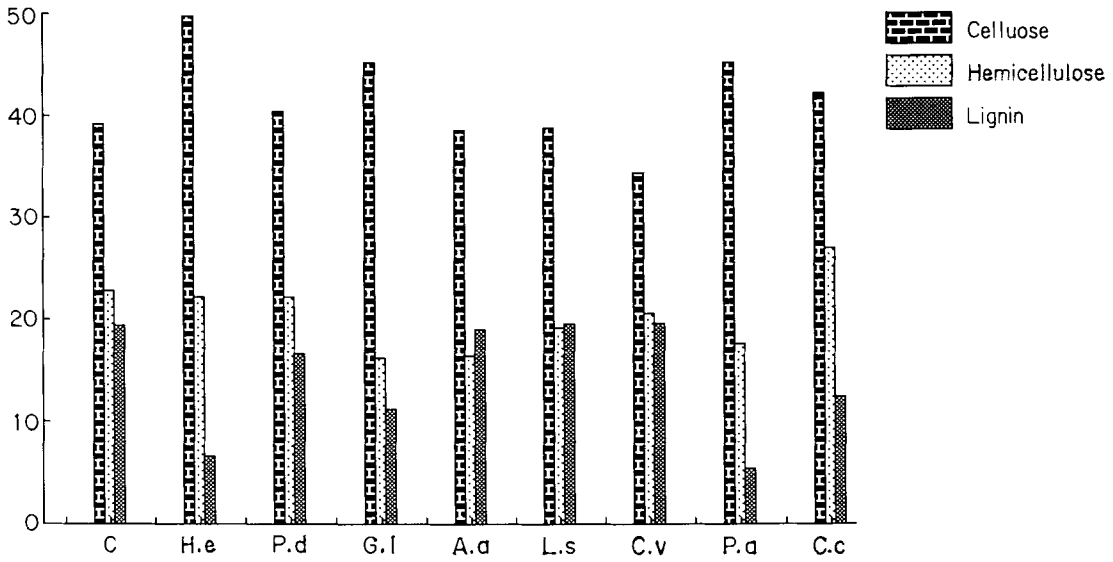


Fig. 3. Cellulosic components of sugar cane bagasse fermented by various fungi for one month.
(C: control; H.e: *Hericium erinacium*; P.d: *Pleurotus djamor*; G.l: *Ganoderma lucidum*; A.a: *Auricularia auricula*; L.s: *Lentinus sajor-caju*; C.v: *Coriolus versicolor*; P.a: *Polyporus arcularius* and C.c: *Coprinus cinereus*).

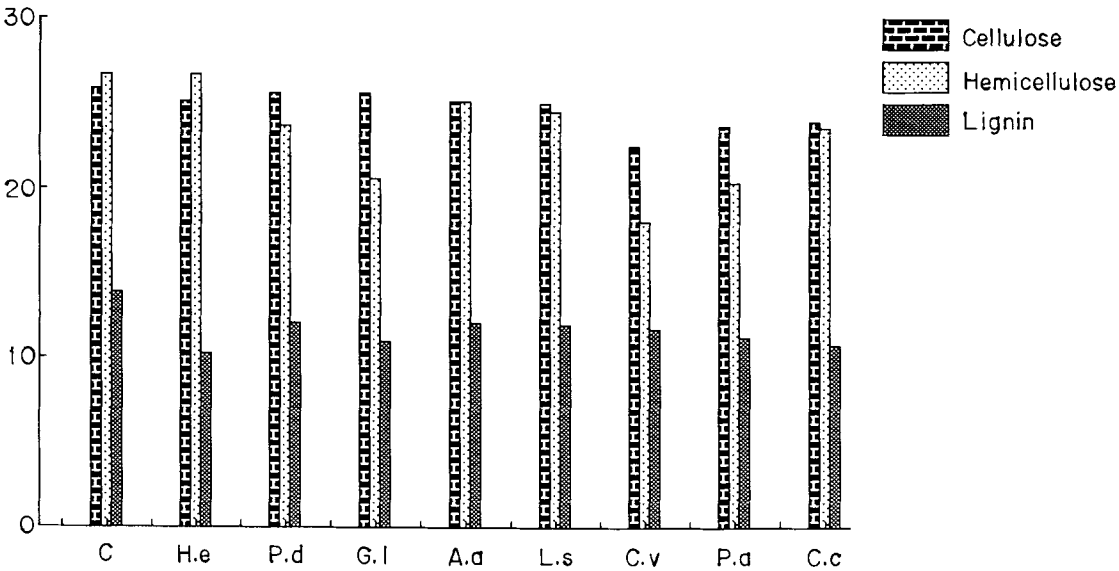


Fig. 4. Cellulosic components of rice straw fermented by various fungi for one month (See Fig. 3).

cane bagasse. With the increase of radiation dose, some small size red colonies appeared on Nutrient agar media. It was considered that the radioresistant bacteria exist in rice straw. Ito et al. (1991) reported that *Deinococcus proteolyticus* and *Pseudomonas radiora* survived at 12 kGy of irradiation in rice straw. Further study is required to identify and radiosensitivity of the red colony forming organism in rice straw. These results suggest that dose of 10 kGy is enough for pasteurization and dose of 30 kGy is required for sterilization. Very negligible change in fibre contents of irradiated empty fruit bunch (EFB) of oil palm wastes (Kume et al., 1990) compared to their control samples suggesting that irradiation at doses up to 50 kGy may have very little effect on degrading lignocellulosic samples.

**Growth of various fungi** The substrates for fungi were sterilized by irradiation of 30 kGy and inoculated with various fungi. Growth of the various fungi on sugar cane bagasse and rice straw substrates during one month period of fermentation is shown in Table 1. *L. sajor-caju* showed very good growth by 7 days after inoculation both in sugar cane bagasse and rice straw. *A. auricula* and *C. versicolor* in bagasse, and *H. erinacium, C. versicolor, P. arcularius* and *C. cinereus* in rice straw showed only fair growth by 7 days while *C. cinereus* showed a little growth in bagasse. However, by 30 days after inoculation all the strains showed very good growth both in sugar cane bagasse and rice straw.

**Changes in fibre components by fermentation** Cellulosic components such as crude fibre (CF), acid detergent fibre (ADF) and neutral detergent fibre (NDF) are analyzed for the evaluation of fibrous diet for animals. Table 2 shows the content of NDF, ADF and CF after one month fermentation by various fungi. It was observed that NDF value significantly decreased in sugar cane bagasse by *G. lucidum, A. auricula, C. versicolor* and *P. arcularius*. In rice straw NDF value is decreased by all the fungi inoculated. Decreased ADF value was also observed in case of all the fungi both in bagasse and rice straw.

NDF value gives the total amount of cellulose, hemicellulose and lignin+cutin present in the sample, similarly ADF value gives the total amount of cellulose and lignin+cutin, and CF represents only the cellulose content in the sample. Figures 3 and 4 represent the calculative values of cellulose, hemicellulose and lignin+cutin in sugar cane bagasse and rice straw, respectively. Sugar cane bagasse substrate contained 39.4%, 22.9%, and 19.6% of cellulose, hemicellulose and lignin+cutin while rice straw substrate contained 25.9%, 26.9% and 13.9%, respectively. It was observed that the cellulose content increased in bagasse fermented by *H. erinacium, G. lucidum* and *P. arcularius* but hemicellulose content decreased in all cases, and lignin+cutin content greatly decreased by *H. erinacium* and *P. arcularius*. Slight decreased value of lignin+cutin content was observed in rice straw fermented by all the fungi inoculated. Lignin is degraded by a number of enzymes like laccase, lignin peroxidase and manganese peroxidase to be produced by various fungi of Basidomycetes and Ascomycetes. Kuwahara (1993) reported that *Pleurotus ostreatus* and *Lentinus edodes* produce all these three enzymes and degraded 40% of the initial lignin in wood meal substrate. Cai et al. (1993) concluded from their experiment that a number of factors accounting for the differing abilities of the mushrooms to grow well on lignin and tannin-rich substrates may be linked to the ability of some mushroom species to produce ligninolytic and other enzymes which degrade and/or detoxify inhibitory substrates.

Decreased value of hemicellulose and lignin content due to fermentation by fungi indicate their degrading capability of these complex fibre components. From Fig. 3, *P. arcularius, H. erinacium, G. lucidum* and *C. cinereus* were found to be the most effective strains for delignification of sugar cane bagasse.

## Literature cited

AOAC, Official Methods of Analysis (15th ed.) 1990. Association of Analytical Chemists, Washington D. C.

Awang, M. R., Mutaat, H. H., Mahmud, M. S., Hussain, W. B. W., Osman, T., Abu Bakar, K., Kassim, A., Mahmud, Z. U. W., Manaf, I., Kume, T. and Hashimoto, S. 1993. Radiation pasteurized oil palm empty fruit bunch fermented with *Pleurotus sajor-caju* as feed supplement to ruminants. Radiat. Phys. Chem. **42**: 611–616.

Cai, Y. J., Buswell, J. A. and Chang, S. T. 1993. Effect of ligninderived phenolic monomers on the growth of the edible mushrooms. World J. Microbio. Biotech. **9**: 503–507.

Chahal, D. S. 1991. "Food, feed and fuel from biomass," Oxford & IBM, New Delhi. 467p.

Ito, H., Ohki, Y. and Ishigaki, I. 1991. Radiation disinfection of rice-straw products. J. Antibact. Antifung. Agents **19**: 577–583.

Kume, T., Ito, H., Ishigaki, I., LebaiJuri, M., Othman, Z., Ali, F., Mutaat, H. H., Awang, M. R. and Hashim, A. S. 1990. Effect of gamma irradiation on microorganisms and components in empty fruit bunch and palm press fibre of oil palm wastes. J. Sci. Food Agric. **52**: 147–157.

Kume, T., Ito, H., Iizuka, H. and Takehisa, M. 1983. Agric. Biol. Chem. **47**: 1065–1069.

Kuwahara, M. 1993. Production of lignin-degrading enzymes by mushrooms. Proc. of the Symp. on Recent Advances in Mycological Research, Dec. 2–3, 1993. Chiba, Japan: 1–3.

Sloneker, J. H. 1976. Agricultural residues including feed lot wastes. Biotechnol. Bioeng. Symp. **6**: 235–250.

Wenzel, H. F. J. 1970. "The chemical technology of wood," Academic press, New York.