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Effect of Irradiation on Enzymatic Digestion of Cellulosic Wastes

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Combination treatments with irradiation and other methods were examined to enhance the digestion of cellulosic materials such as sugar cane bagasse and rice straw. The amount of crude fiber (CF), acid detergent fiber (ADF) and neutral detergent fiber (NDF) of bagasse and rice straw were changed with various treatments. Alkali treatment (0.2N NaOH) was the most efficient for the enzymatic hydrolysis of bagasse and rice straw. Combination treatments with radiation and alkali or other methods increased their efficiency, and synergistic effect of radiation and alkali treatment was observed. Enzymatic digestion of CF of bagasse and rice straw treated by degassed water yielded high reducing sugar comparable to that of CF treated by alkali. CF of bagasse and rice straw treated by ozone did not show the significant increase in the release of reducing sugar upon saccharification.

ADF and acid detergent lignin (ADL) contents decreased with the fermentation of bagasse by Coriolus versicolor. Electron microscopic observations also revealed the degradation of lignocellulosic

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components of bagasse.

Keywords : Lignocellulosic Wastes, Bagasse, Rice Straw, Radiation Treatments, Enzymatic Digestibility

セルロース質廃棄物の酵素消化性に及ぼす照射の効果

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(1993年3月2日受理)

照射と他の処理方との組み合わせによるセルロース質廃棄物の酵素消化性について検討した。サトウキビバガス及び稲わらの粗繊維（CF），酸溶媒不溶繊維（ADF），中性溶媒不溶繊維（NDF）の量は各種処理により変化した。アルカリ処理が酵素による加水分解に最も効果的であった。照射とアルカリその他の処理法との組み合わせで，分解性はさらに向上した。脱気水ではアルカリ処理とほぼ同様の促進効果が認められた。オゾンでは増大効果は認められなかった。バガス中のADF及びADL（酸溶媒処理リグニン）量は*Coriolus versicolor*による発酵処理で著しく減少した。この分解性の増大は，走査型電子顕微鏡観察でも認められた。

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1. INTRODUCTION

Cellulosic materials such as sugar cane bagasse, rice straw, wheat straw etc., which today are normally wasted or are underutilized, represent one of the largest potential source for future energy and food. Keeping in view of the ever-increasing human population and lack of corresponding increase in food production and dwindling supply of liquid fuel reserves, biomass (wood and crop residues and manures) which is the biggest renewable source of organic carbon in the world has been drawn the attention for its conversion into food, feed and fuel (1).

Recently, it has been estimated that 2.25 billion tons of cereal straw, 560 million tons of leguminous crop residues and 234 million tons of sugar cane bagasse are produced every year in the world (2). Sugar cane bagasse and wheat straw are mainly utilized as fuel and feed for animal, respectively. About one billion tons of crop residues are left in the fields as organic matter, and this residual biomass could yield about 375 million tons of protein-rich animal feed equivalent to 4 times the soybean production in the world. Similarly about 300 million cubic meters of forest residues are available for the production of food, feed and fuel (ethanol) in the world (3).

The forest cellulosic wastes contain 45-56% cellulose, 10-25% hemicellulose and 18-30% lignin (4); the other agricultural cellulosic wastes contain 30-45% cellulose, 16-29% hemicellulose and 3-13% lignin (5).

Over the last 20 years, increasing research efforts have

been directed to the conversion of cellulose to glucose, ethanol and single-cell protein. However, the enzymatic hydrolysis of native lignocellulosic materials is very slow, mainly due to compositional heterogeneity and structural complexity. Association between cellulose, hemicellulose and lignin in the cell walls, cellulose crystallinity and accessibility of surface area to enzymes are generally recognized as the determinants of the extent of degradation by enzymes or microorganisms (6-8). It has become apparent that some kind of pretreatment (physical, chemical, biological or combination) are necessary to improve the susceptibility of cellulose towards enzymes. Enhancement in hydrolysis rates with such pretreatment is attributed to structural modification and/or selective removal of cell wall constituents (9-11).

Many studies on the pretreatment of cellulose by various means have been reported, including the use of chemicals (alkali, per acetic acid, sulfuric acid, etc.) and mechanical milling to reduce size. The use of ionizing radiation in the pretreatment of cellulose and cellulosic wastes for the enhancement of glucose production by acid and enzymatic hydrolysis has recently been interested by various workers (12-14). It is well established that the radiation has a disruptive effect on the cellulose chain, interacting equally with both crystalline and amorphous regions. Studies on radiation-induced degradation of purified cellulose, rice straw, wheat straw, chaff, sawdust, wood etc., have been carried out by several authors (15, 16). Chemical treatments and mechanical milling of waste cellulose materials

coupled to radiolysis have been also tried to improve acid and enzymatic hydrolysis (17).

Most of the works on radiation degradation of lignocellulosic substrates were performed at relatively high doses of radiation. But the use of high doses of radiation is not practical. Radiation treatment is effective for the pasteurization or sterilization of fermentation medium with relatively low doses such as 10 - 30 kGy (18, 19). If the degradation of lignocellulosic wastes is enhanced by such low dose irradiation, it has a benefit for the practical utilization of cellulosic wastes.

In this paper, the effect of low doses for pasteurization or sterilization in combination with other treatments on cellulosic wastes like sugar cane bagasse and rice straw and their subsequent enzymatic digestion is reported.

2. MATERIALS AND METHODS

2.1. Samples

Sugar cane bagasse donated by Okinawa Prefectura Agricultural Experiment Station and rice straw collected from Takasaki area were used in this experiment. Sugar cane bagasse was cut into small pieces having a length of ca. 2 cm and then was ground to particle size of 1.5 mm by Centrifugal Mill ZM-1 (Nihonseiki, Japan). In case of rice straw air dried samples were first cut into small pieces using a straw cutter VL-65

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(Marumasu, Japan). These were then sieved with a wire mesh (Retsch) No. 32 to get rid of any sand or soil that might be present. The rice straw was then cut into small pieces in a Willy Cutting Mill WSX-140 (Kiya, Japan) with a 2 mm screen.

2.2. Irradiation

Dry samples were packed in polyethylene bags and irradiated in air using a Co-60 gamma source. The dose rate used was 10 kGy/h determined by Fricke dosimetry.

In the case of samples in suspension, 3 g of bagasse or rice straw was suspended in 60 ml solution. Various suspensions in distilled water, 0.2N NaOH, 0.5%CH_3COOH, 0.5% detergent or digassed water were irradiated with (oxygen, ozone or N_2O) or without various gas bubbling. After completion of irradiation the substrates were filtered in a cloth and washed thoroughly in running tap water and dried.

2.3. Moisture Content Determination

Moisture content was determined using Infrared Drying Unit LP16 (Mettler, Switzerland) for 120 min (for rice straw) or 180 min (for bagasse) at 135°C.

2.4. Fiber Analysis

Crude fiber (CF), acid detergent fiber (ADF), neutral detergent fiber (NDF) and acid detergent lignin (ADL) of bagasse and rice straw were determined by using the Fibertec system 1010 (Tecator, Sweden) (20) as follows;

a) Determination of CF

Weighing of the crucible
|
Transferring approximately 1 g of the substrate to the crucible
|
Drying at 105°C for 4 hours
|
Cooling in a desiccator and weighing to know the exact amount of substrate (W_0 g)
|
Acid extraction by boiling in 100 ml 1.25% H_2SO_4 for 30 minutes
|
Filtering
|
Boiling for 30 minutes in 100 ml distilled water (single wash)
|
Draining off the water
|
Extraction by boiling in 100 ml 1.25% NaOH for 30 minutes
|
Filtering
|
Boiling for 30 minutes in 100 ml distilled water (single wash)
|
Draining off the water
|
Washing with hot distilled water (3 times)
|
Washing with acetone (3 times)
|
Drying at 105°C for 4 hours
|
Cooling in a desiccator and weighing (W_1 g)--------- Crude fiber
|
Ash at 500°C for 3 hours
|
Cooling in a desiccator and weighing (W_2 g)

Calculation : $$\% \text{ CF} = \frac{W_1 - W_2}{W_0} \times 100$$

Reagents used for CF analysis :

1. Sulfuric acid - 0.128 M (12.5 g of H_2SO_4 diluted to 1 liter and mixed)
2. Sodium hydroxide - 0.223 M (12.5 g of NaOH dissolved in distilled water and diluted to 1 liter)
3. Acetone

b) Determination of ADF

Weighing of the crucible
|
Transferring approximately 1 g of the substrate to the crucible
|
Drying at 105°C for 4 hours
|
Cooling in a desiccator and weighing (W_0 g)
|
Adding 100 ml acid detergent solution from the top of the column
|
Boiling for 60 minutes
|
Filtering
|
Washing three times with hot distilled water using the device sliding on the bar
|
Filtering
|
Washing with acetone (3 times)
|
Drying at 105°C for 4 hours
|
Cooling in a desiccator and weighing (W_1 g) ------ Acid detergent fiber
|
Ash at 500° C for 3 hours
|
Cooling in a desiccator and weighing (W_2 g)

$$\text{Calculation : \% ADF} = \frac{W_1 - W_2}{W_0} \times 100$$

Reagents used for ADF analysis :

1. Conc. Sulfuric Acid, reagent grade
2. Cetyl trimethylammonium bromide (CTAB), technical grade
3. Acid Detergent Solution (ADS); 49.04 g conc. sulfuric acid was weighed into a volumetric flask (1000 ml) and was made up to the volume with distilled water at 20°C. 20 g CTAB was added and stirred.
4. Acetone

c) Determination of NDF

Weighing of the crucible
|
Transferring approximately 1 g of the substrate to the crucible
|
Drying at 105°C for 4 hours
|
Cooling in a dessicator and weighing (W_0 g)
|
Adding 100 ml neutral detergent solution from the top of the column
+
0.5 g of sodium sulfite from the top of each column
|
Boiling for 60 minutes
|
Filtering
|
Washing three times with hot distilled water using the device sliding on the bar
|
Filtering
|
Washing with acetone (3 times)
|
Drying at 105°C for 4 hours
|
Cooling in a dessicator and weighing (W_1 g)-------- Neutral detergent fiber
|
Ash at 500°C for 3 hours
|
Cooling in a dessicator and weighing (W_2 g)

$$\text{Calculation : \% NDF} = \frac{W_1 - W_2}{W_0} \times 100$$

Reagents used for NDF analysis :

1. Sodium lauryl sulfate ($C_{12}H_{25}OSO_3Na$) 30 g
2. Disodium ethylenediamine-tetraacetate (EDTA)
3. Sodium borate decahydrate ($Na_2B_4O_7\text{-}10H_2O$)
4. Disodium hydrogen phosphate, anhydrous (Na_2HPO_4)
5. 2-ethoxyethanol ($C_4H_{10}O_2$)
6. Neutral Detergent Solution (NDS). 18.61 g of EDTA and 6.81 g of $Na_2B_4O_7\text{-}10H_2O$ were weighed together in a beaker. Some distilled water was added in it and heated until dissolved. Then 30 g of sodium lauryl sulfate and 10 ml of 2-ethoxyethanol was added. Another 4.56 g of Na_2HPO_4 together with some distilled water was added and heated until dissolved. The two solutions were then mixed and diluted to 1 l. pH was checked to range between 6.9 to 7.1.
7. Sodium sulfite (Na_2SO_3), anhydrous, reagent grade
8. Acetone

d) Determination of ADL

Placing the crucibles with ADF sample in small beakers
|
A glass rod was put into each crucible for stirring
|
Approximately 25 ml of 72% sulfuric acid cooled to 15°C was added in each crucible
|
The sample was then extracted cold for 3 hours, stirring once/hour
|
Filtering off the acid by washing first with cold water and then by hot water until free from acid
|
Drying of the crucibles with the extracted samples at 105°C for 4 hours
|
Cooling and weighing of the samples (W_3 g)
|
Ashing the samples in the crucible at 500°C for 3 hours
|
Cooling and weighing of the ash (W_4 g)

$$\text{Calculation: \% ADL} = \frac{W_3 - W_4}{W_0 \text{ (sample weight)}} \times 100$$

Reagents used for ADL analysis :

72 per cent sulfuric acid:

$$\frac{100 \times 98.08 \times 12 \text{ moles}}{H_2SO_4 \text{ assay (\% on the label of flask)}} = \text{grams acid needed to 1 liter solution}$$

(1000 x 1.634) - grams acid = grams water needed
1.634 = density of 72 per cent H_2SO_4 solution

Acid was carefully added to the water with occasional swirling. The acid solution was allowed to cool down to approximately 15°C before the extraction.

2.5. Hot Water Extract

Bagasse and rice straw samples were washed and dried after various pretreatments. Then the substrates were boiled in water for 30 minutes, filtered and sucked dry with acetone. The percentage of extractives was calculated from the weight loss after the pretreatment.

2.6. Enzyme Digestion

Samples (0.2 g) in 10 ml of 0.05M acetate buffer (pH 4.5) including 0.5% commercial enzyme of cellulase Onozuka 3S (Yakult, Japan) were incubated at 40°C with shaking for 18h. Two drops of toluene were added in each tube to prevent any microbial growth during incubation. Reducing sugar was analyzed by the Somogyi-Nelson method (21).

2.7. Fermentation

Each bagasse samples (20 g) with ca. 65% of moisture in 300 ml conical flasks were prepared with 0%, 1% and 3% rice bran. The substrates were sterilized by irradiation of 30 kGy. Various mushroom strains namely *Pleurotus sajor-caju, Pleurotus flavellatus, Coriolus versicolor, Pleurotus flavellatus* (red mushroom), *Auricularia aurecula, Ganoderma lucidum, Volvariella volvacea* and *Hericium erinaceum,* and some fungal strains isolated from compost were used for this fermentation study. The substrates were inoculated with these strains and were incubated at 30°C with 85% RH.

2.8. Scanning Electron Microscopy

Samples of 2 mm size were used for this study. The surface and cross sectioned cellulosic particles of bagasse and rice straw were observed by Scanning Electron Microscope JXA-733 (JEOL, Japan).

3. RESULTS AND DISCUSSION

3.1. Change in Fiber Components after Various Treatments

Cellulosic components such as crude fiber (CF), acid detergent fiber (ADF) and neutral detergent fiber (NDF) are commonly analyzed for the evaluation of fibrous diets for animal. Negligible change in the CF contents of rice straw and bagasse by irradiation in air was observed (Table 1 and 2). As the results suggest that radiation alone has little effect in degrading the cellulosic materials, the radiation in combination with different chemical and gaseous treatments was examined. For this purpose bagasse and rice straw were irradiated in combination with alkali (0.2N NaOH), acid (0.5% CH_3COOH), oxygen, ozone, nitrous oxide, 0.5% detergent and degassed water. The results indicate that the values of CF have increased with various treatments compared to the values when irradiated in air without any combination treatment. The increase in CF values is an indication of the removal of lignin and hemicellulose from the lignocellulosic substrates which suggests the effectiveness of the particular

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treatment used. The most appreciable increases in the CF contents of both bagasse and rice straw were observed with NaOH alone. However, radiation doses from 10 to 50 kGy coupled with different chemical or gaseous treatment methods cannot produce the synergistic effect. Only CF in bagasse irradiated in combination with NaOH though very small.

Tables 3 and 4 show the ADF values and Tables 5 and 6 show the NDF values of bagasse and rice straw after various combination treatments with radiation. ADF of bagasse and rice straw increased markedly with NaOH treatment whereas the increase with other treatments were small. By the combination treatment with irradiation and NaOH, the ADF values increased some extent in bagasse but no effect in rice straw. The increase in NDF was also observed in both case of bagasse and rice straw especially by NaOH treatment. However, the irradiation up to 50 kGy had little effect for increase in NDF. The notable observations from the results in NDF show that rice straw treated by various methods had higher values compared to bagasse. This indicates that rice straw is more easily affected by various treatment than bagasse. Radiation doses up to 50 kGy combined with different chemical or gaseous treatment methods have little effect in increasing the NDF and ADF values of bagasse or rice straw except in case of alkali treatment.

3.2. Estimation of Fiber Compositions after Various Treatments

The appreciable increase in the cellulosic components suggested the removal of extractable materials by various treatment used in this experiment. The amounts of hot water extract (HWE) were analyzed and shown in Table 7 and 8. HWE were specially increased by NaOH treatment in both cases of bagasse (12.0% to 20.9%) and rice straw (11.6% to 33.8%). Irradiation and NaOH treatment further increased the quantity of HWE from both bagasse and rice straw. In the case of other treatments with or without irradiation, there was negligible increase in the quantity of HWE in both bagasse and rice straw.

As the contents of CF, ADF and NDF except the case of air were analyzed using the samples after wash and dry, the values should be calculated with subtraction of HWE contents. Tables 9 and 10 show the estimated values of fiber components in bagasse and rice straw, respectively. Using these values the amount of hemicellulose in a substrate can be estimated by deducting the values of ADF from NDF; similarly the amount of lignin can be estimated by deducting CF value from ADF value. Figures 1 and 2 show the composition of cellulose, hemicellulose, lignin, neutral detergent extracts (NDF) and HWE in unirradiated, irradiated (50 kGy) with various combination treatment of bagasse and rice straw, respectively. It shows clearly that the irradiation in combination with CH_3COOH, detergent and degassed water had very small effect on each fiber components in bagasse and rice straw. On the other hand, NaOH treatment increased the HWE fraction and

decreased the hemicellulose fraction effectively. In the case of rice straw NDF fraction was bigger than that of bagasse and NaOH treatment increased markedly the HWE fraction. The combination of radiation with NaOH showed the similar effects of increase in HWE fraction and decrease in hemicellulose of rice straw. These results suggest that the combination treatment of irradiation and NaOH is effective specially for hemicellulose degradation.

3.3 Change in Enzymatic Digestibility

3.3.1. Lignocellulosic Wastes

Reducing sugars (RS) released after the enzymatic digestion of bagasse and rice straw with various pretreatment is shown in Fig. 3 and 4. RS released after saccharification with unirradiated bagasse was 83.7 mg/g, whereas that with 50 kGy irradiated bagasse was 90.2 mg/g; with rice straw the release of RS was 73.7 mg/g and 88.1 mg/g, respectively. There seems to be a gradual increase in the RS released with the increase in radiation doses though not that much significant. When bagasse and rice straw were irradiated in combination with alkali (0.2N NaOH), the release of RS after saccharification was increased quite appreciably. Bagasse, when treated only with alkali, produced 218.0 mg/g of RS upon saccharification and when treated in combination with 50 kGy, yielded 273.8 mg/g of RS. Similarly, with rice straw also, the values of RS with alkali were 465.5 mg/g and with combination treatment of 50 kGy with alkali these were 513.5 mg/g. Intermediate doses of 10 and 30 kGy in

combination with alkali also showed gradual increase in reducing sugar release upon saccharification. The combination of radiation with alkali was effective because some lignin was removed by alkali treatment. The other various combination treatments also had some effect but compared to alkali treatment those were insignificant.

The enhancement of enzymatic digestion of rice straw (RS 465.5 mg/g) with alkali treatment was much higher than that of bagasse (RS 218 mg/g). It suggests that alkali treatments were effective to remove lignin, hemicellulose or other extractives present in rice straw than in bagasse. This confirms the earlier finding (Fig. 1 and 2) on the effect of removal of extractives from these substrates.

3.3.2. Crude Fiber (CF)

Saccharification results of CF of bagasse and rice straw obtained after various treatments are shown in Fig. 5 and 6. Saccharification of CF is much higher than that of native lignocellulosic wastes (see the values of RS in air in Fig. 1-4). CF of unirradiated and 50 kGy irradiated bagasse released 413.1 mg/g and 494.4 mg/g of RS, respectively; similarly, 536.0 mg/g and 574.4 mg/g of RS, respectively, were released from rice straw. RS released from CF of bagasse treated by degassed water was 458.5 mg/g and that of combination with 50 kGy radiation was 499.0 mg/g. CF of rice straw treated similarly released 474.5 mg/g and 644.1 mg/g RS, respectively.

Saccharification of CF of alkali with 0 and 50 kGy

irradiated bagasse yielded RS of 452.1 mg/g and 493.6 mg/g, respectively; with rice straw the values were 564.1 mg/g and 613.5 mg/g, respectively.

RS released from CF of bagasse treated by ozone and nitrous oxide were low as compared to the other treatments. CF of rice straw irradiated in combination with ozone showed a negative effect in the release of RS.

Compiling all the saccharification results obtained with various treatments, it can be easily seen that alkali treatment is the most efficient, both in bagasse and rice straw. Combination of radiation with these treatments definitely increases their efficiency but it was not so significant. In case of saccharification with CF of the treated substrates their values increased to some extent. This increase in the saccharification values of the CF is obvious because of the removal of lignin, hemicellulose and other extractives from the substrates during the process of the extraction. Another finding is that rice straw always yields higher RS upon saccharification compared to bagasse.

3.3.3. Effect of Alkali Concentrations

Bagasse and rice straw were irradiated at a dose of 50 kGy in combination with different concentrations of NaOH ranging from 0.01N to 0.2N. The saccharification of these treated substrates shows that alkali in combination with irradiation at 50 kGy was more effective (Fig. 7). There is no effect of radiation with low concentration of NaOH. With higher concentrations of NaOH

more lignin could be removed and more pronounced effect of radiation was obtained.

3.4. Fermentation of Bagasse by Various Fungi

Growth of the different fungi on three sets of substrate after two months of fermentation is shown in Table 11. *Pleurotus sajor-caju, Coriolus versicolor* (Kawara-take), *Auricularia aurecula* (Kikurage), *Ganoderma lucidum* (Mannen-take) and *Hericium erinaceum* (Yamabushi-take) showed good growth.

Table 12 shows the ADF and ADL contents of fermented bagasse. When bagasse with 3% rice bran was fermented with *G. lucidum*, there was no significant change in the ADF value whereas significant decrease in the ADL value was observed. Bagasse fermented by *H. erinaceum* also showed similar results suggesting that these two strains have both cellulose and lignin degrading properties. Bagasse fermented by *A. aurecula* did not show any significant ADF or ADL change. When bagasse with or without 3% rice bran was fermented by *C. versicolor* showed quite significant decrease in the ADF content though the decrease in ADL content was negligible. The low values of ADF content obtained in the fermented bagasse with this strain show that it has good cellulose digesting capabilities. Bagasse with or without 3% rice bran when fermented by *P. sajor-caju* showed low values of ADL but comparatively higher values of ADF suggesting that this strain has only lignin degrading properties.

3.5. Scanning Electron Microscopic Studies

The electron photomicrographs of treated and fermented bagasse, are shown in Fig. 8 and 9. These revealed that the surface of bagasse became smooth with NaOH treatment. CF of NaOH treated bagasse showed more smooth surface. But the electron micrograph of bagasse fermented with *C. versicolor* clearly showed that it has not only made the surface smooth but it has also cellulose digesting properties which can be seen by numerous linear holes producing along the cellulose structure.

The electron photomicrographs of rice straw are shown in Fig. 10. Changes were observed on the surface of rice straw treated by NaOH and enzyme digestion. The surface of rice straw after NaOH treatment showed clear structure because the maretials covered on the surface were removed. The structure of fiber was degradated by enzyme treatment using cellulase Onozuka.

4. CONCLUSION

Irradiation up to 50 kGy combined with various chemical or gaseous treatment has only a small effect on CF, ADF or NDF values of bagasse and rice straw. However, extractability of lignin and other materials were markedly increased by 0.2 NaOH treatment, and irradiation in combination with alkali enhanced the degradation of hemicellulose.

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Irradiation up to 50 kGy combined with various chemical or gaseous treatment has only a small effect on CF, ADF or NDF values of bagasse and rice straw. However, extractability of lignin and other materials were markedly increased by 0.2 NaOH treatment, and irradiation in combination with alkali enhanced the degradation of hemicellulose.

Enzymatic digestion studies of bagasse and rice straw after various combination treatments showed that alkali treatment enhanced the saccharification better than other chemical or gaseous treatments. Enzymatic digestion of CF of bagasse and rice straw treated by degassed water showed comparable results with alkali treatment. Rice straw with any type of treatment yielded higher reducing sugar upon saccharification compared to bagasse.

In the fermentation study, bagasse fermented with *G. lucidum* and *H. erinaceum* showed both cellulose digestion and delignification properties. *C. versicolor* showed good cellulose digesting properties while *P. sajor-caju* had delignification properties. Changes observed on the surface of treated and fermented bagasse and rice straw by electron microscopic studies confirmed all these findings.

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REFERENCES

1. Chahal, D.S., "Food, feed and fuel from biomass", Oxford & IBH, New Delhi (1991).

2. Ishaque, M. and Chahal, D.S., Crop residues, "Food, feed and fuel from biomass", pp. 15-26, ed. by Chahal, D.S., Oxford & IBH, New Delhi (1991).

3. Rawat, J.K. and Nautiyal, J.C., Availability of forest biomass, "Food, feed and fuel from biomass", pp. 27-36, ed. by Chahal, D.S., Oxford & IBH, New Delhi (1991).

4. Wenzel, H.F.J., The chemical technology of wood, Academic Press, New York, N.Y. (1970).

5. Sloneker, J.H., Agricultural residues including feed lot wastes, *Biotechnol. Bioeng. Symp.*, **6**, 235-250 (1976).

6. Cowling, E.B. and Kirk, T.K., Properties of cellulose and lignocellulosic materials as substrates for enzymatic conversion process, *Biotechnol. Bioeng. Symp.*, No. 14, 95-123 (1976).

7. Chesson, A., Effects of sodium hydroxide on cereal straws in relation to the enhanced degradation of structural polysaccharides by lumen microorganisms, *J. Sci. Food Agric.*, **32**, 745-758 (1981).

8. Gharpuray, M.M., Lee, Y.H. and Fan, L.T., Structural modification of lignocellulosics by pretreatment to enhance enzymatic hydrolysis, *Biotechnol. Bioeng.*, **25**, 157-172 (1983).

9. Millett, M.A., Baker, A.J. and Satter, L.D., Pretreatment to enhance chemical, enzymatic and microbiological attack of cellulosic materials, *Biotechnol. Bioeng. Symp.*, No. 5, 21-33 (1975).

10. Mac Donald, D.G., Bakhshi, N.N., Mathews, J.F., Roychowdhury, A., Bajpai, P. and Moo-Young, M., Alkali treatment of corn stover to improve sugar production by enzymatic hydrolysis, *Biotechnol. Bioeng.*, **25**, 2067-2076 (1983).

11. Puri, V.P. and Mamers, H., Explosive treatment of lignocellulosic residues with high-pressure carbon dioxide for the production of fermentation substrates, *Biotechnol. Bioeng.*, **25**, 3149-3161 (1983).

12. Horio, M., Imamura, R. and Mizukami, H., Effect of gamma irradiation upon cellulose, *Bull. Inst. Chem. Res. Kyoto Univ.*, **41** (1), 17-38 (1963).

13. Kunz, N.D., Gainer, J.L. and Kelly, J.L., Effects of gamma radiation on the low-temperature dilute-acid hydrolysis of cellulose, *Nuclear Technol.*, **16**, 556-561 (1972).

14. Kumakura, M. and Kaetsu, I., Radiation-induced decomposition and enzymatic hydrolysis of cellulose, *Biotechnol. Bioeng.*, **20**, 1309-1315 (1978).

15. Ardica, S., Calderaro, E. and Cappadona, C., Radiation pretreatment of cellulose materials for the enhancement of enzymatic hydrolysis - II, Wood chips, paper, grain straw, hay, kapok, *Radiat. Phys. Chem.*, **26**, No.6, 701-704 (1985).

16. Simkovic, I., Mlynar, J., Alfoldi, J., Lubke, H. and Micko, M.M., Increased extractability of irradiated wood meal, *Holzforschung*, **45**, No.3, 229-232 (1991).

17. Kumakura, M. and Kaetsu, I., Radiation pretreatment of cellulosic wastes in the presence of acids, *Int. J. Appl. Radiat. Isotop.*, **35**, 21-24 (1984).

18. Kume, T., Ito, H., Ishigaki, I., LebaiJuri, M., Othman, Z., Ali, F., Mutaat, H. H., Awang, M. R. and Hashim, A. S., 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 (1990).

19. Ito, H., Awang, M. R., Kume, T. and Ishigaki, I., Fungal Degradation of Bagasse after Radiation Pasteurization, *Nippon Shokuhin Kogyo Gakkaishi*, **36**, 643 (1989).

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

21. Nelson, N., A photometric adaptation of the Somogyi method for the determination of glucose, *J. Biol. Chem.*, **153**, 375-380 (1944); Somogyi, M., Notes on sugar determination, *J. Biol. Chem.*, **195**, 19-23 (1952).

Table 1. CF content of bagasse after different treatments with radiation

Treatment	CF (%)			
	0 kGy	10 kGy	30 kGy	50 kGy
Air	39.2	39.0	38.0	38.3
0.2N NaOH	50.8	49.8	51.3	51.7
0.5% CH_3COOH	44.8	44.7	44.1	44.7
Oxygen	44.0	41.5	41.6	41.3
Ozone	41.8	41.4	41.1	40.4
Nitrous oxide	40.3	40.9	40.3	39.6
0.5% Detergent	43.6	43.2	42.2	42.7
Degassed water	42.4	41.2	42.2	41.0

Table 2. CF of rice straw after different treatments with radiation

Treatment	CF(%)			
	0 kGy	10 kGy	30 kGy	50 kGy
Air	31.6	31.0	30.5	30.1
0.2N NaOH	43.2	43.2	43.9	43.1
0.5% CH_3COOH	33.3	33.2	32.4	33.0
Oxygen	34.3	33.9	33.3	33.6
Ozone	32.9	32.9	32.9	32.3
Nitrous oxide	34.1	33.0	32.1	32.0
0.5% Detergent	36.0	34.6	34.0	34.0
Degassed water	35.1	34.3	33.4	33.2

Table 3. ADF content of bagasse after different treatments with radiation

Treatment	ADF(%)			
	0 kGy	10 kGy	30 kGy	50 kGy
Air	53.2	52.9	52.8	53.2
0.2N NaOH	67.1	67.7	68.7	69.3
0.5% CH_3COOH	60.2	60.5	60.7	60.8
Oxygen	60.1	59.5	59.7	59.6
Ozone	57.1	56.3	56.4	56.7
Nitrous oxide	55.6	54.4	54.4	55.0
0.5% Detergent	60.2	60.3	60.7	60.3
Degassed water	59.1	58.7	59.6	59.0

Table 4. ADF content of rice straw after different treatments with radiation

Treatment	ADF(%)			
	0 kGy	10 kGy	30 kGy	50 kGy
Air	41.7	41.5	40.9	41.3
0.2N NaOH	56.2	54.8	57.5	58.1
0.5% CH_3COOH	45.9	46.8	45.4	45.5
Oxygen	46.6	46.2	46.5	46.0
Ozone	46.8	45.9	45.3	46.2
Nitrous oxide	45.5	43.8	44.7	44.3
0.5% Detergent	46.9	46.5	47.1	47.4
Degassed water	46.5	46.8	46.0	45.9

Table 5. NDF content of bagasse after different treatments with radiation

Treatment	NDF(%)			
	0 kGy	10 kGy	30 kGy	50 kGy
Air	77.1	77.2	77.6	76.7
0.2N NaOH	93.8	93.6	92.9	92.5
0.5% CH_3COOH	91.2	91.5	91.7	90.7
Oxygen	91.6	90.9	90.1	89.9
Ozone	90.2	90.6	90.6	90.3
Nitrous oxide	85.9	84.9	83.8	84.1
0.5% Detergent	91.1	91.4	91.6	91.4
Degassed water	90.9	90.2	90.2	89.0

Table 6. NDF content of rice straw after different treatments with radiation

Treatment	NDF(%)			
	0 kGy	10 kGy	30 kGy	50 kGy
Air	65.5	64.8	64.9	64.2
0.2N NaOH	82.3	82.1	82.5	82.6
0.5% CH_3COOH	73.1	73.7	74.2	73.2
Oxygen	73.9	73.2	73.7	73.3
Ozone	69.6	69.2	70.2	69.6
Nitrous oxide	69.0	68.1	68.6	67.8
0.5% Detergent	74.2	73.4	74.0	74.5
Degassed water	73.5	72.8	72.9	72.7

Table 7. Removal of hot water extract (HWE) from bagasse after various treatments

Treatment	HWE (%)	
	0 kGy	50 kGy
Air	12.0	12.4
0.2N NaOH	20.9	26.5
0.5% CH_3COOH	11.1	11.6
0.5% Detergent	12.9	13.2
Degassed water	11.8	11.5

Table 8. Removal of hot water extract (HWE) from rice straw after various treatments

Treatment	HWE (%)	
	0 kGy	50 kGy
Air	11.8	12.0
0.2N NaOH	33.8	37.1
0.5% CH_3COOH	12.2	12.0
0.5% Detergent	15.1	15.1
Degassed water	13.8	13.9

Table 9. Estimation of NDF, ADF and CF contents of bagasse treated with irradiation and various treatments

Treatment	Fiber component (%)	Dose (kGy)			
		0	10	30	50
Air	NDF	77.1	77.2	77.6	76.7
	ADF	53.2	52.9	52.8	53.2
	CF	39.2	39.0	38.0	38.3
0.2N NaOH	NDF	74.2	73.0	70.3	68.0
	ADF	53.1	52.8	52.0	50.9
	CF	40.2	38.8	38.8	38.0
0.5% CH_3COOH	NDF	81.1	81.3	81.2	80.2
	ADF	53.5	53.7	53.8	53.7
	CF	39.8	39.7	39.1	39.5
0.5% Detergent	NDF	79.3	79.6	79.6	79.3
	ADF	52.4	52.5	52.7	52.3
	CF	38.0	37.6	36.7	37.1
Degassed water	NDF	80.2	79.6	79.6	78.8
	ADF	52.1	51.8	52.6	52.2
	CF	37.4	36.3	37.2	36.3

Table 10. Estimation of NDF, ADF and CF contents of rice straw treated with irradiation and various treatments

Treatment	Fiber component (%)	Dose (kGy)			
		0	10	30	50
Air	NDF	65.5	64.8	64.9	64.2
	ADF	41.7	41.5	40.9	41.3
	CF	31.6	31.0	30.5	30.1
0.2N NaOH	NDF	54.5	53.8	53.0	52.0
	ADF	37.2	35.9	36.9	36.5
	CF	28.6	28.3	28.2	27.1
0.5% CH_3COOH	NDF	64.2	64.7	65.1	64.4
	ADF	40.3	41.1	39.9	40.0
	CF	29.2	29.1	28.4	29.0
0.5% Detergent	NDF	63.0	62.3	62.8	63.3
	ADF	39.8	39.5	40.0	40.2
	CF	30.6	29.4	28.9	28.9
Degassed water	NDF	63.4	62.8	62.8	62.6
	ADF	40.1	40.3	39.6	39.5
	CF	30.3	29.6	28.8	28.6

NDF consists cellulose, hemicellulose and lignin,
ADF consists cellulose and lignin,
CF consists cellulose only.

Table 11. Growth of various fungi after 2 months of fermentation with bagasse

Organism	Japanese mushroom name	Bagasse + 3% rice bran	Bagasse + 1% rice bran	Bagasse
Pleurotus sajor-caju		++	++	+++
Pleurotus flavellatus		++	++	++
Coriolus versicolor	Kawara	++++	+++	+++
Pleurotus flavellatus	Tokiiro	+	+	+
Auricularia aurecula	Kikurage	+++	+++	++
Ganoderma lucidum	Mannen	++++	+++	+++
Volvariella volvacea	Fukuro	+	+	+
Hericium erinaceum	Yamabushi	++	++	+
F2*		-	-	-
T - F2*		-	-	+
OC - F*		-	-	-
T - F3*		+	+	+
T - F4*		+	+	+

(++++)Very good growth; (+++) Good growth; (++) Fair growth; (+) Poor growth; (-) No growth,

*These fungal strains were isolated from compost.

Table 12. ADF and ADL content of fermented bagasse

Organism	Substrate*	ADF (%)	ADL (%)
Control	A	56.1	10.5
"	B	54.9	10.4
G. lucidum	B	56.4	8.6
H. erinaceum	B	56.6	8.9
A. aurecula	B	56.8	10.9
C. versicolor	A	52.5	10.2
"	B	52.0	10.1
P. sajor-caju	A	59.5	9.0
"	B	60.4	8.6

The values are means of duplicates,

A; Bagasse, B; Bagasse + 3% Rice bran.

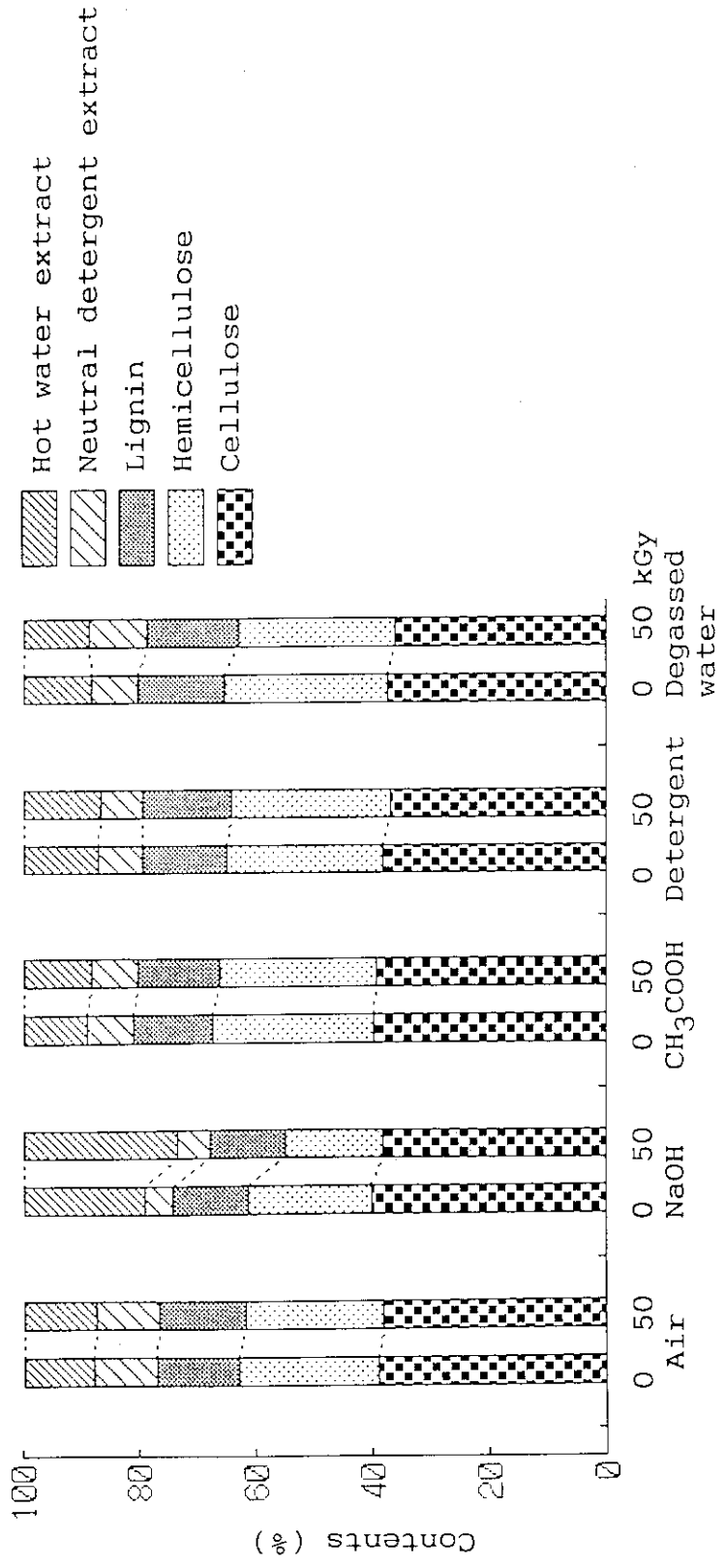


Fig. 1. Fiber components of bagasse after various combination treatments with irradiation

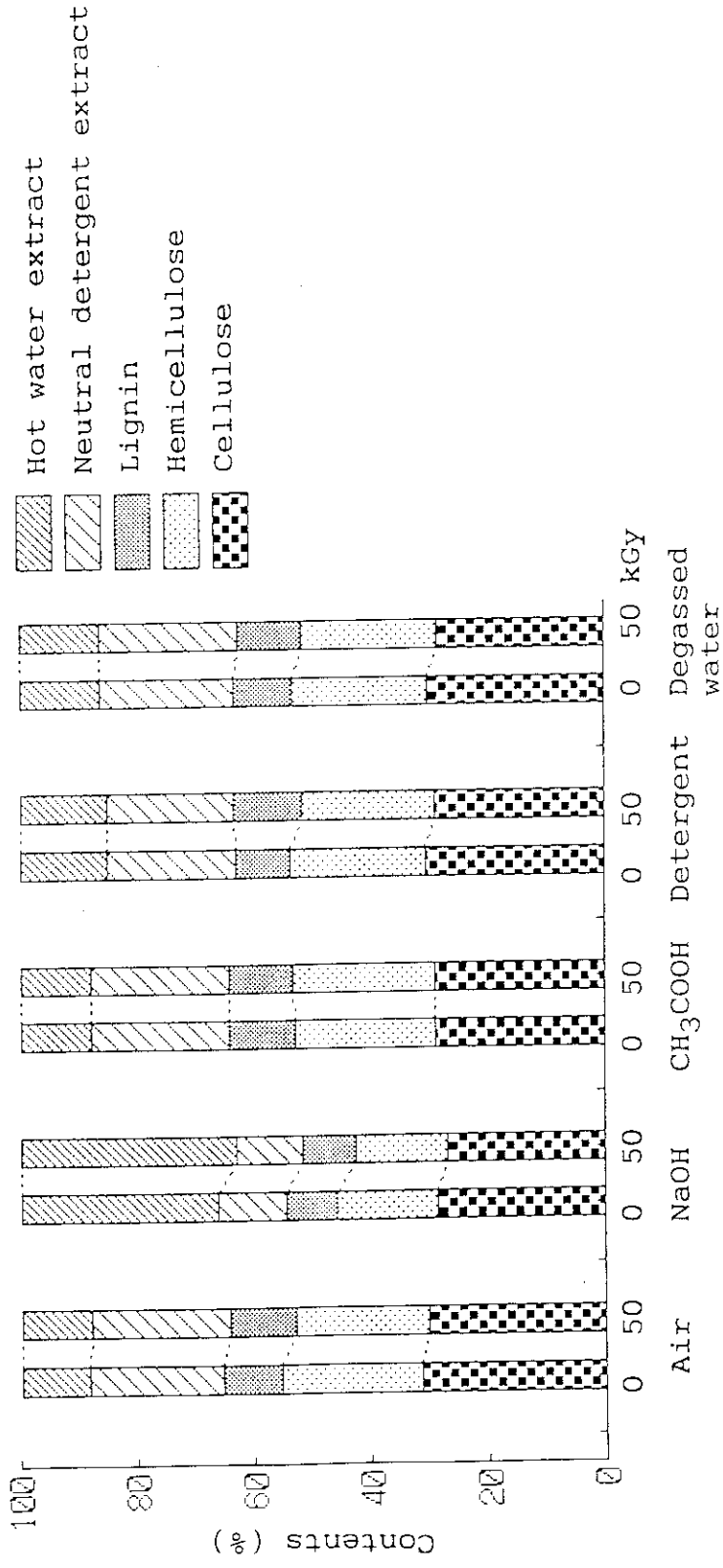


Fig. 2. Fiber components of rice straw after various combination treatments with irradiation

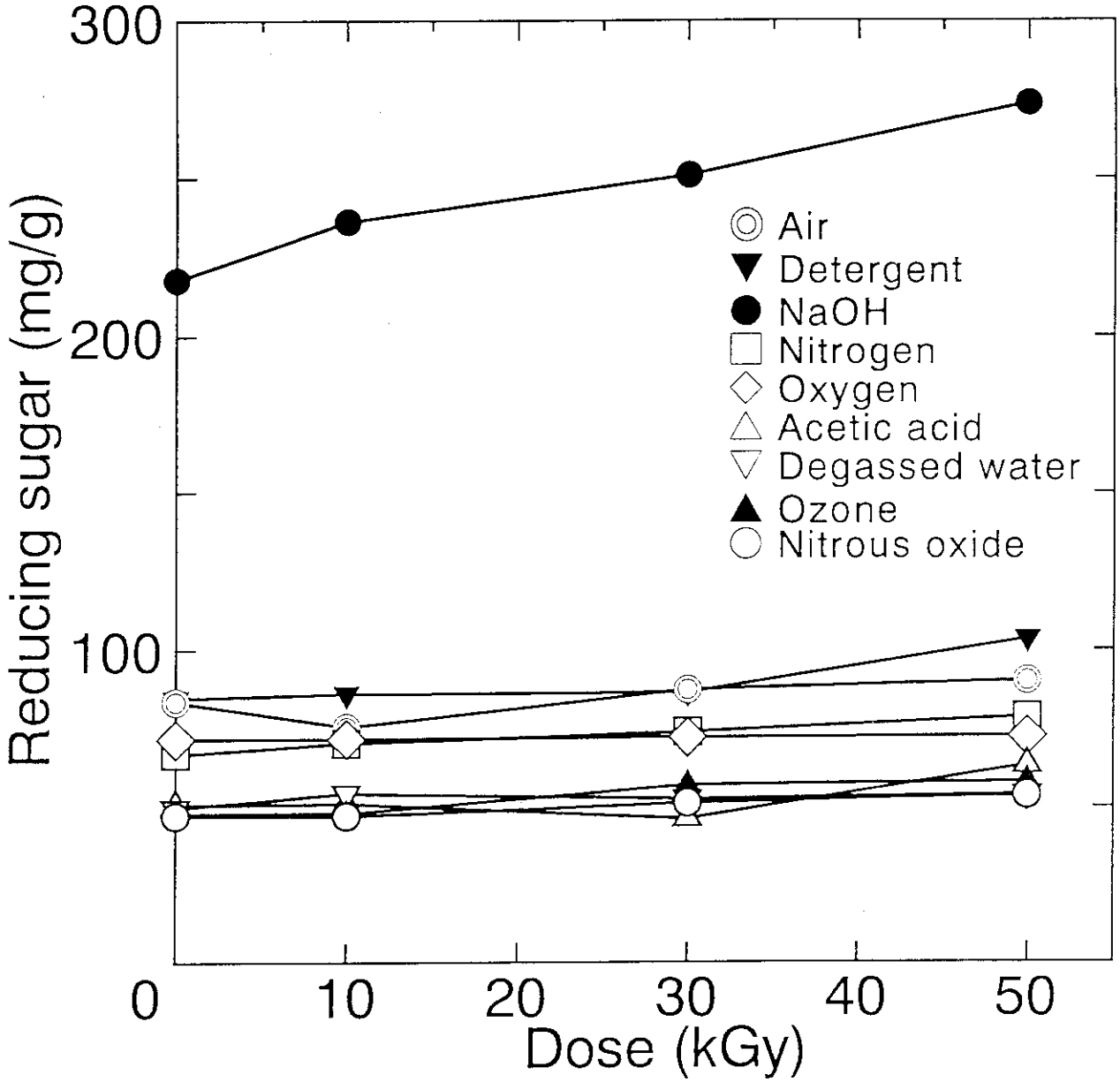


Fig. 3. Enzymatic digestion of bagasse by cellulase after various combination treatments

Enzyme: 0.5% Cellulase Onozuka 3S,
Incubation: 0.2g of sample in 10ml acetate buffer (pH 4.5) at 40°C for 18h.

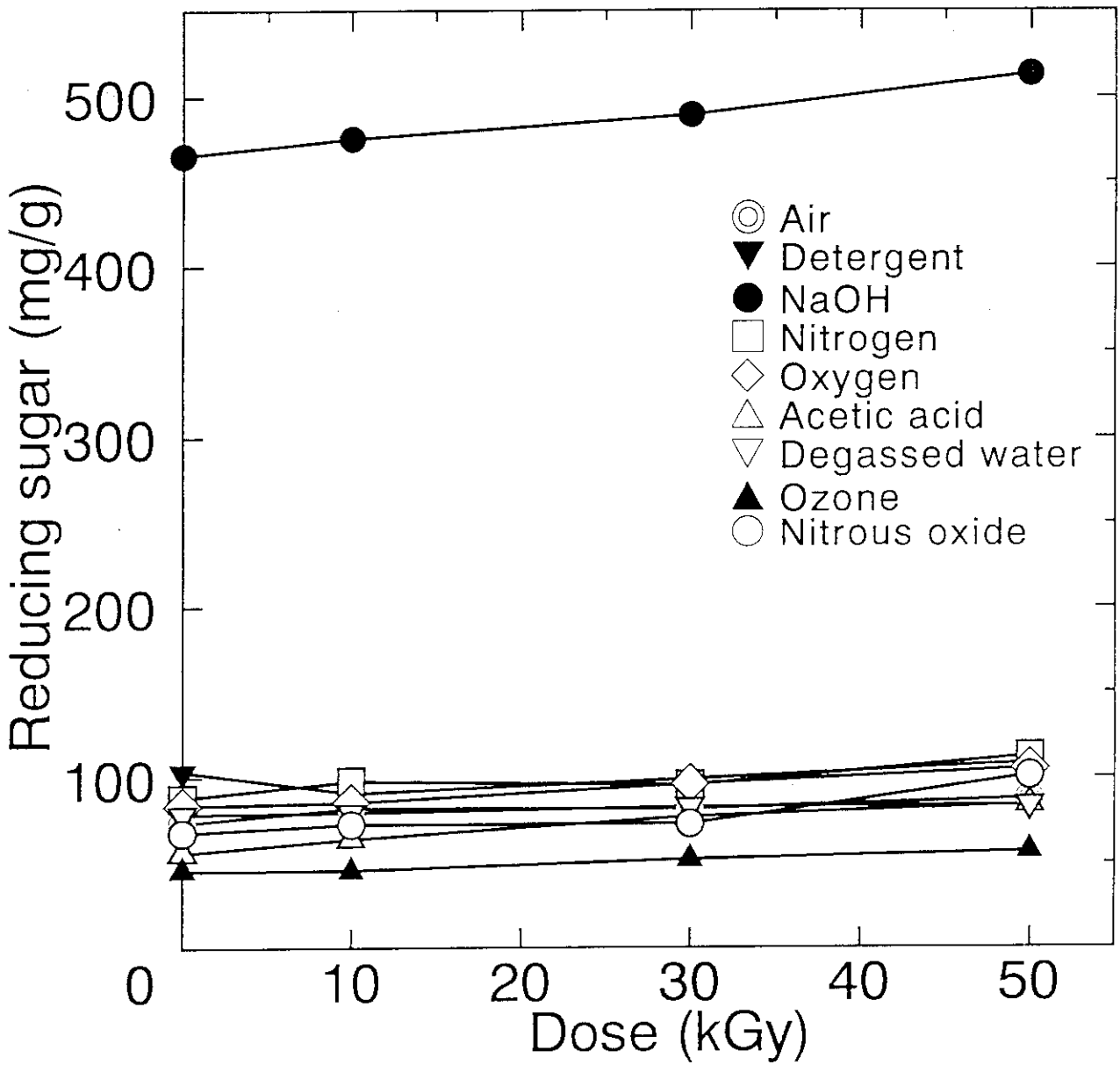


Fig. 4. Enzymatic digestion of rice straw by cellulase after various combination treatments

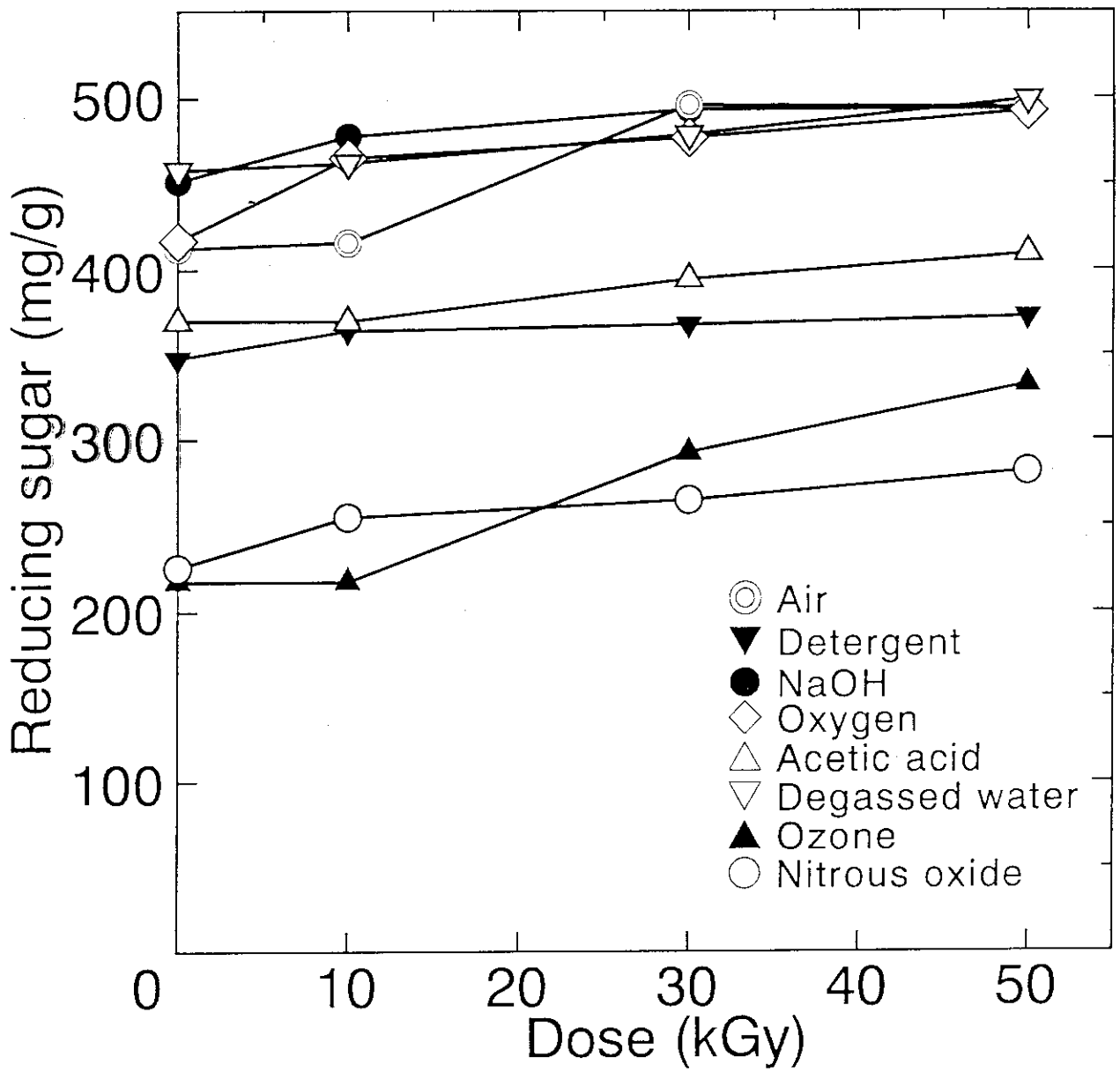


Fig. 5. Enzymatic digestion of crude fiber of bagasse by cellulase after various combination treatments

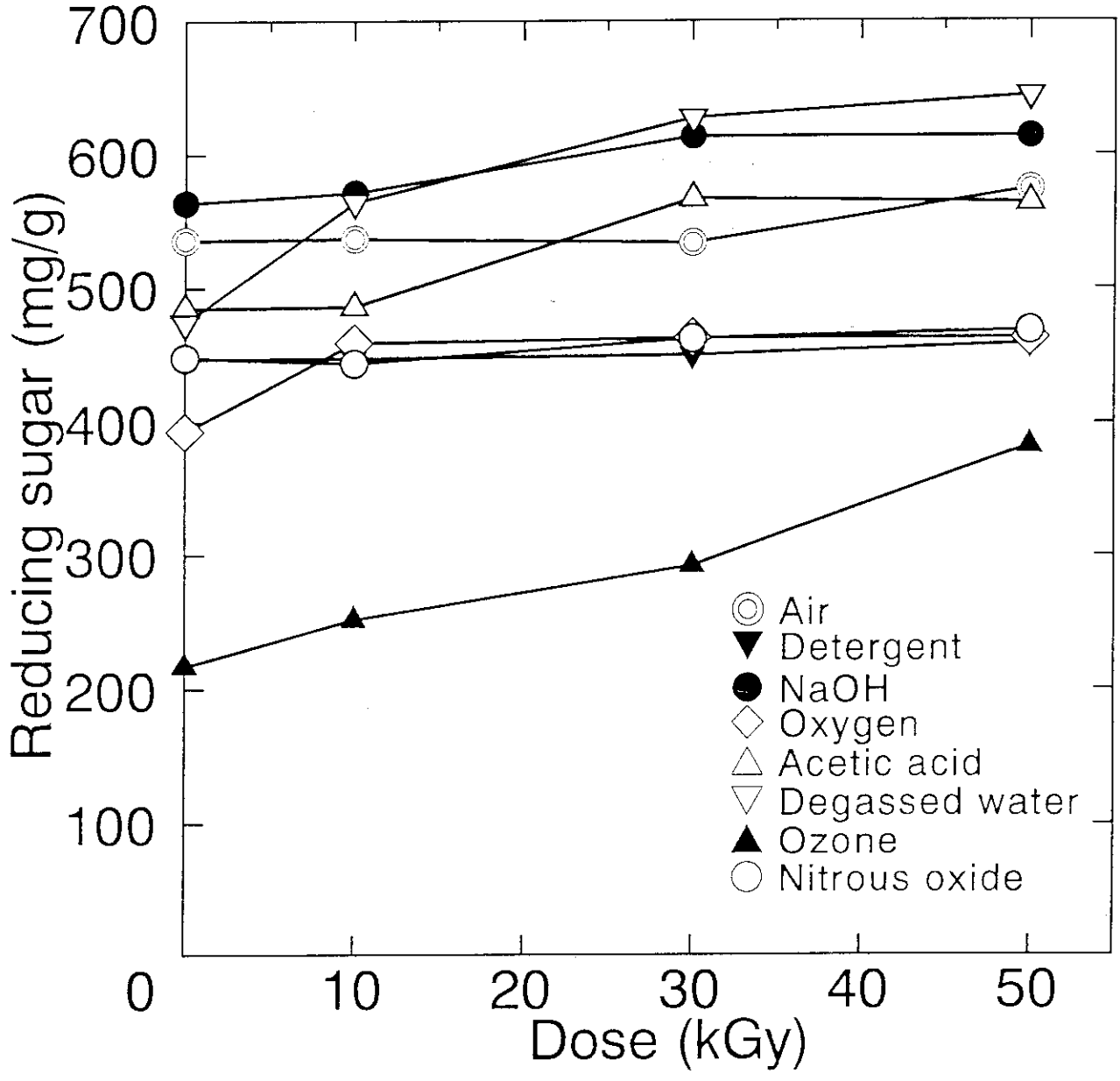


Fig. 6. Enzymatic digestion of crude fiber of rice straw by cellulase after various combination treatments

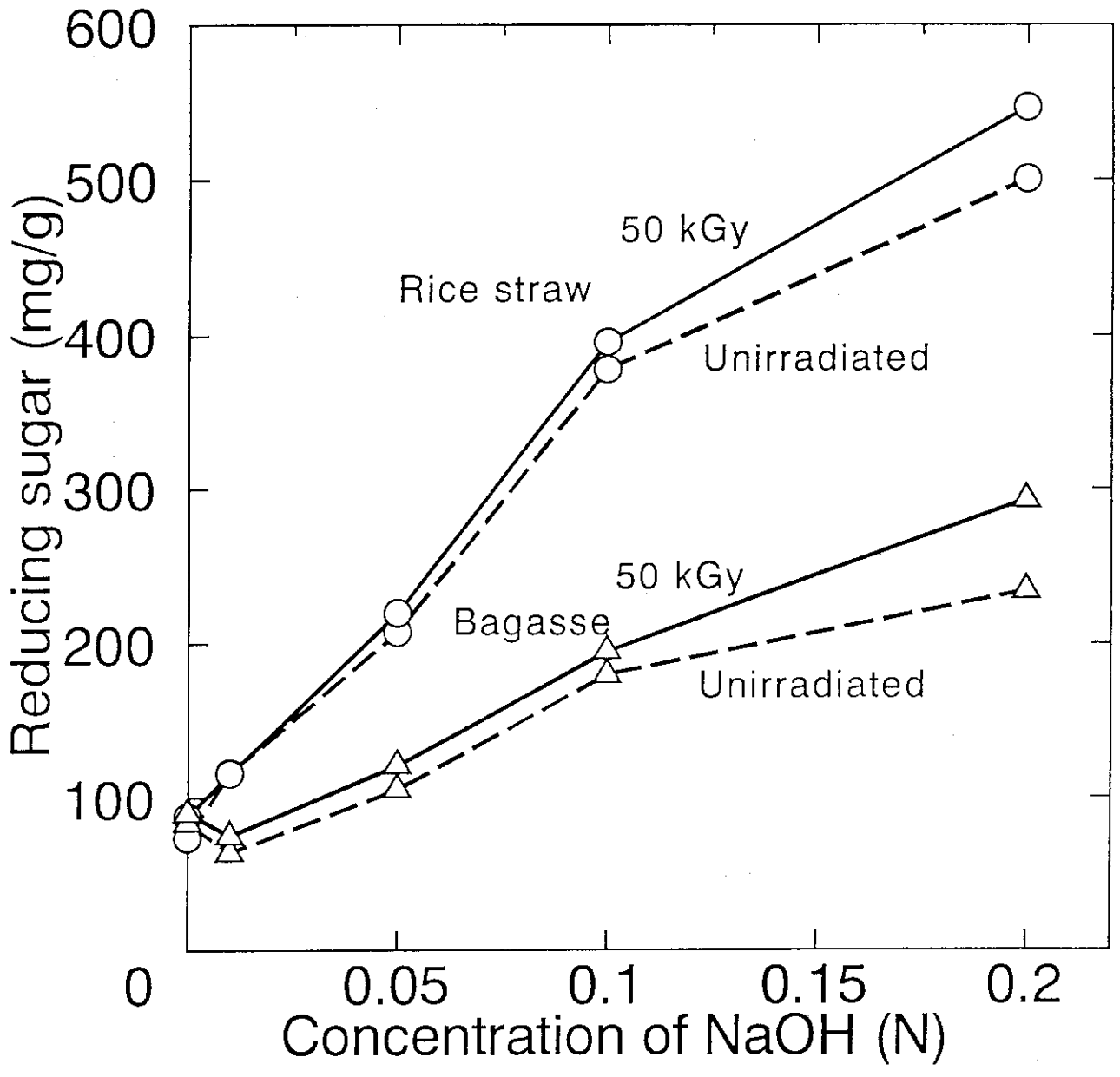
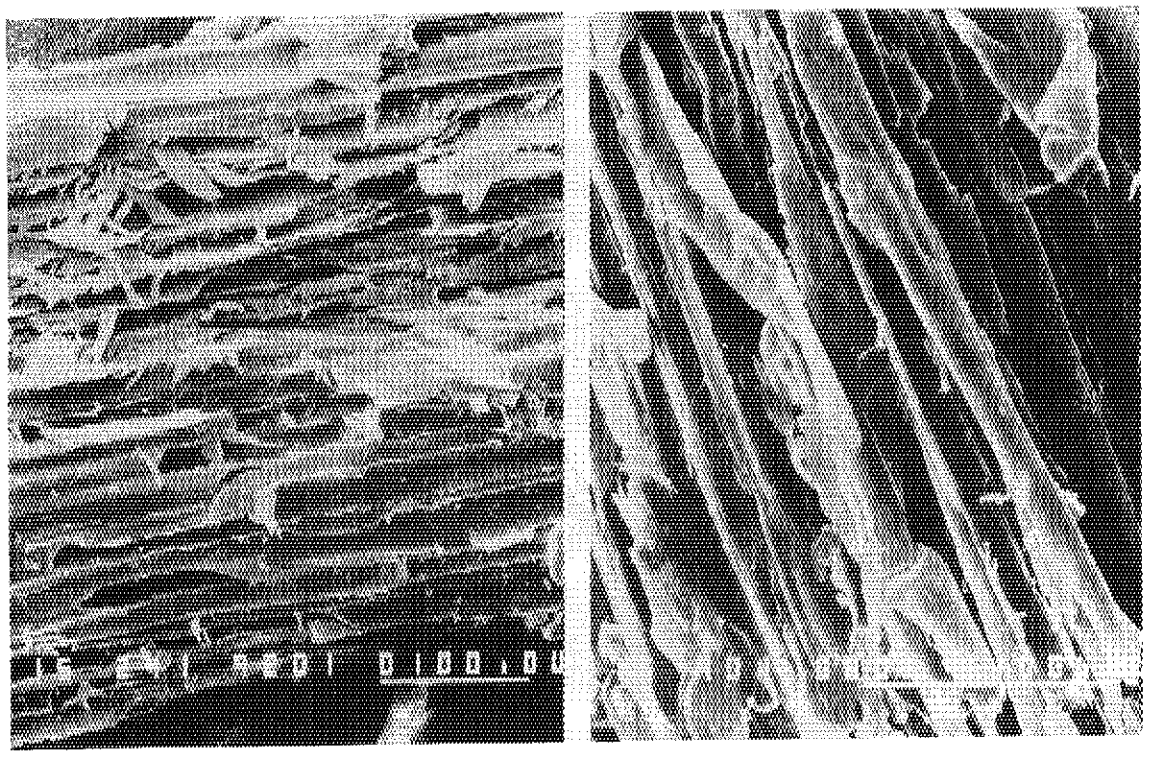
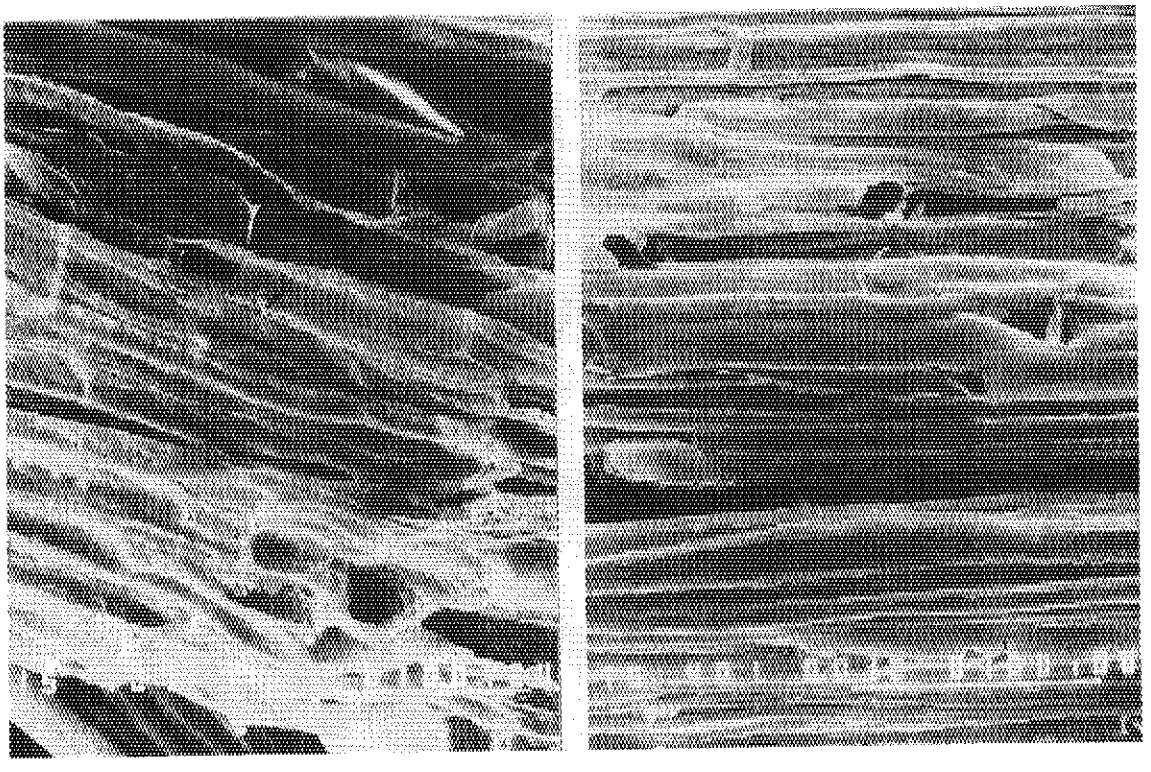


Fig. 7. Effect of alkali concentration on enzymatic digestion of bagasse and rice straw with irradiation

Bagasse



Untreated NaOH + 50kGy



CF(NaOH) CF(NaOH + 50kGy)

Fig. 8. Electron photomicrographs of the surface of bagasse after defferent treatment

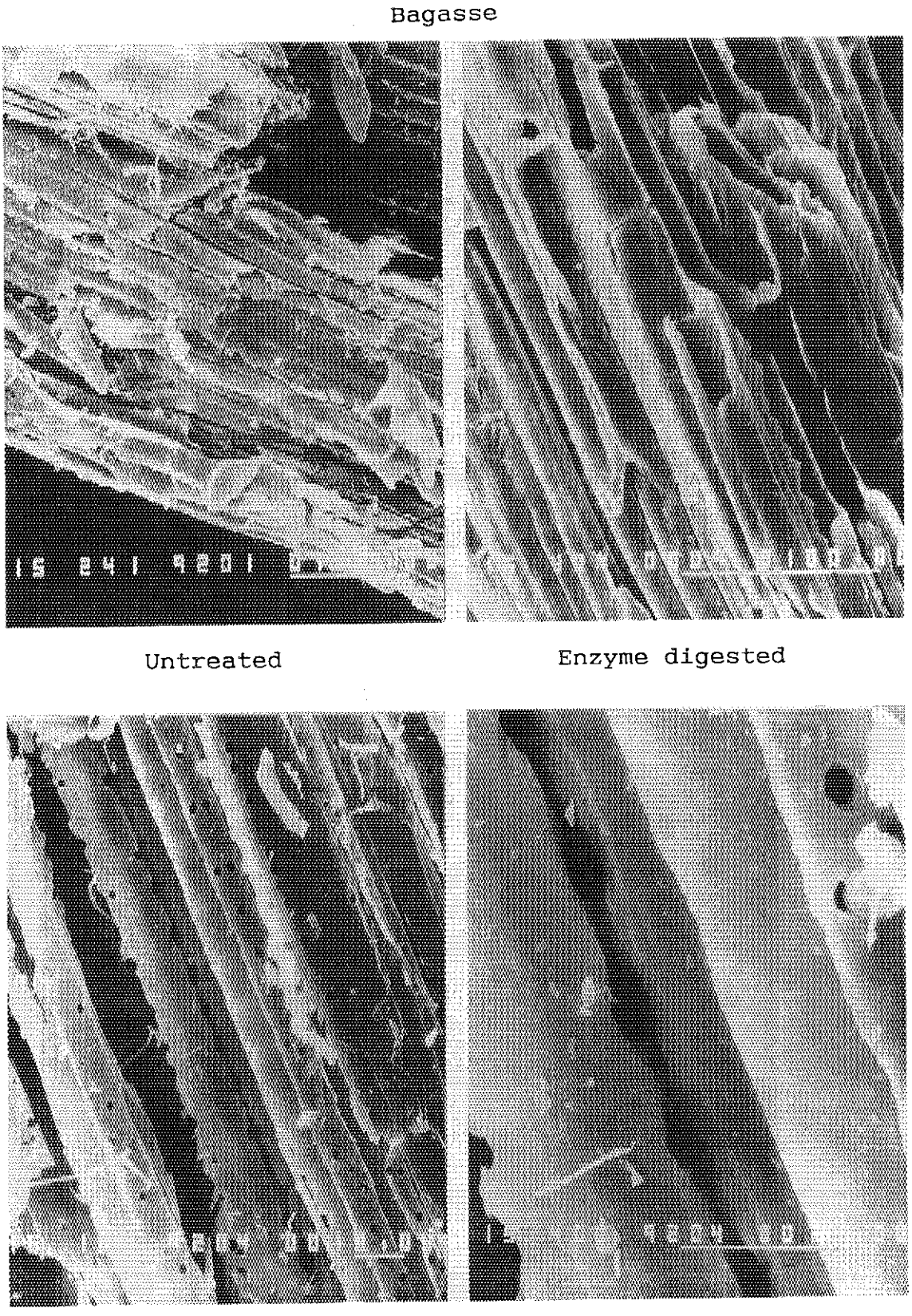


Fig. 9. Electron photomicrographs of the surface of bagasse after microbial degradation

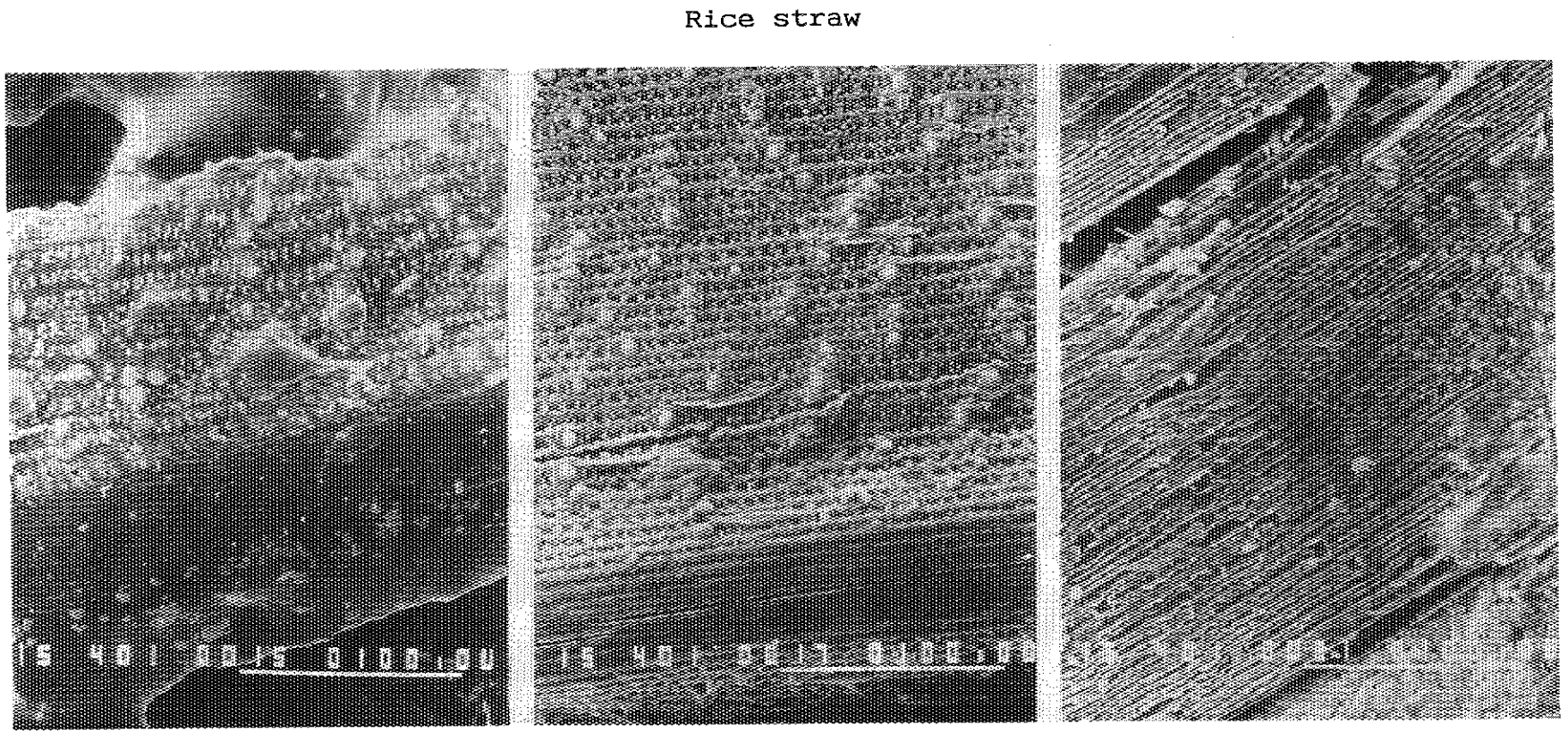


Fig. 10. Electron photomicrographs of the surface of rice straw after defferent treatment