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STUDY ON UPGRADING OF OIL PALM WASTES TO ANIMAL FEEDS BY RADIATION AND FERMENTATION PROCESSING

March 1998

Tamikazu KUME, Shinpei MATSUHASHI, Hitoshi ITO, Shoji HASHIMOTO, Isao ISHIGAKI, Mat Rasol AWANG*, Muhamad LEBAIJURI*, Zainon OTHMAN*, Foziah ALI*, Wan Badrin Wan HUSAIN* and Hassan HAMDANI*

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Upgrading of oil palm empty fruit bunch (EFB), which is a main by-product of palm oil industry, to animal feeds by radiation pasteurization and fermentation was investigated for recycling the agro-resources and reducing the environmental pollution. The following results were obtained: 1) The necessary dose for pasteurization of EFB contaminated by various microorganisms including aflatoxin producing fungi was determined as 10 kGy. The chemical and biological properties of EFB were changed little by irradiation up to 50 kGy. 2) In the fermentation process, *Pleurotus sajor-caju* was selected as the most effective fungi and the optimum condition for fermentation was clarified. The process of fermentation in suspension was also established for the liquid seed preparation. 3) The digestibility and nutritional value of fermented products were evaluated as ruminant animal feeds and the mushroom can be produced as by-product. 4) The pilot plant named Sterifeed was built at MINT and a large volume production has been trying for animal feeding test and economical evaluation. It is expected to develop the process for the commercial use in Malaysia and to expand the technique to Asian region through UNDP/RCA/IAEA project.

Keywords: Oil Palm, Cellulosic Wastes, EFB, Radiation Pasteurization, Fermentation, Mushroom Fungi, *Pleurotus sajor-caju*, Animal Feeds

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This is the report of Bilateral Research Cooperation between JAERI and MINT.

* Malaysian Institute for Nuclear Technology Research (MINT)

放射線と発酵処理によるオイルパーム廃棄物の飼料化に関する研究

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（1998年2月5日受理）

資源のリサイクルおよび環境汚染防止を目的に、オイルパーム廃棄物の有効利用について検討した。本研究では、主としてパーム油生産工場において大量に廃棄されるオイルパーム空果房（EFB）について、放射線殺菌と発酵処理による飼料化を検討し、以下の結果を得た。1) EFBは、カビ毒アフラトキシンを産生する糸状菌などによって著しく汚染されており、殺菌には10 kGyの照射が必要であった。EFBの化学的、生物学的性状は、50 kGyまでの線量ではほとんど変化しなかった。2) 発酵処理に最も有効な菌として、*Pleurotus sajor-caju*を選抜し、発酵最適条件を明らかにした。また、液状種菌製造のため、懸濁液発酵法を確立した。3) 発酵産物の消化性及び栄養価は、反すう動物用飼料に適していることを明らかにした。また、副産物としてのキノコ生産条件を明らかにした。4) MINT (Malaysian Institute of Nuclear Technology Research) にパイロットプラント(Sterifeedと命名)が建設され、現在動物飼育試験用飼料の大量生産及び経済性の評価試験が行われている。今後、本処理法のマレーシアにおける実用化、UNDP/RCA/IAEAプロジェクトによるアジア地域への発展が期待されている。

本報告書は、「放射線と発酵処理によるオイルパーム廃棄物の飼料化」に関するマレーシア原子力研究所（MINT、旧称UTN）との二国間研究協力で得られた成果をまとめたものである。

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1. INTRODUCTION

Empty fruit bunch (EFB) and palm press fiber (PPF) are major cellulosic solid wastes of the palm oil industry. The availability of EFB and PPF in 1985 is estimated to be 2.09 and 2.17 million tones (dry weight basis) respectively, which is likely to increase in future (Husin et al 1985). These by-products are mainly discarded or under-utilized. EFB is normally incinerated to produce bunch ash which is used as potash fertilizer or distributed in the field as mulch, while PPF is extensively used as fuel for the production of steam in the mills. However, burning and incineration processes emit considerable amount of smokes and pollutants thus affecting surrounding areas. Upgrading of these fibers into useful end-products are expected not only to reduce pollution but also to increase their values.

Recently, it has been realized that there is a need to utilize these by-products effectively in order to improve the economic situation of the oil palm. Research on the conversion of EFB and PPF into useful end-products has been reported. Both EFB and PPF were shown to have considerable potential as animal feeds (Hutagalung and Mahyuddin, 1985). Being fibrous, they also provide possibilities for the production of pulp and paper (Kobayashi et al 1985), and with their high carbohydrate content, they could be viable substrates for the synthesis of furfural (Ariffin 1985). In spite of these studies, their utilization is still limited and mainly under experiment. More efforts are required to optimize the utilization of these oil palm wastes.

Gamma irradiation is presently being utilized for sterilization of medical products and preservation of foods in bulks by virtue of its high penetration ability. In relation to the similar application, we have been investigating the upgrading of cellulosic wastes by radiation and fermentation.

This paper describes the results on upgrading of oil palm wastes (mainly EFB) to animal feeds by radiation and fermentation treatment which were obtained under the research cooperation in the field of radiation processing between the Japan Atomic Energy Research Institute (JAERI) and the Malaysian Institute for Nuclear Technology Research (MINT) signed on 9 December 1987. Under the Annex 1 (1987 - 1992); 1) Identification and decontamination of micro-organisms by radiation, 2) Physico-chemical properties of irradiated wastes, 3) Survey of useful micro-organisms and fermentation conditions, 4) Evaluation of nutritional values of products, and 5) Techno-economical feasibility study were investigated. In the Annex 2 (1992 - 1997); 1) Feasibility study for animal feed production by using pilot plant, and 2) Production of bioactive materials were performed.

2. BACKGROUND FOR UPGRADING OF OIL PALM BY-PRODUCTS

2.1 Situation for Upgrading of Cellulosic Agro-wastes

A large quantity of cellulosic agro-wastes or by-products like sugar cane bagasse, rice straw, etc. are discarded or under utilized through out the world. It has been estimated that 2 billion ton of cereal straw, 500 million ton of leguminous crop residues, 200 million ton of sugar cane bagasse and 300 million cubic meter of forest residues are generated and a great deal of them becomes wastes (Chahal, 1991). If these wastes are upgraded into useful end-products, the technology may be a potential solution to the demands of the environmental pollution. One of the ideas is to change them into animal feeds. The point to be solved is the high content of lignin which animal cannot digest. The forest biomass contains 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). It is required to decrease the lignin content and increase the digestibility of cellulosic wastes to use as ruminant feeds. To dissolve these problems, radiation sterilization and fermentation is considered as a effective process for upgrading of cellulosic wastes to animal feeds.

2.2 Oil Palm Production in Malaysia

Oil palm is cultivated in the tropical regions within 20 degrees of north and south latitude, with 1500-2000 mm rain /year, temperature 22-30℃ and sunshine more than 5 hr. Southeast Asia, Africa and South America are including in these area. Two kinds of oil, palm oil and palm kernel oil, are obtained from the oil palm fruit. One fruit bunch consists of 1000-3000 fruits and the weight is 20-30 kg. It can be harvested during 3-20 years after plantation. As 10-12 fruit bunches are produced from one tree throughout the year, its yield is 12-18 times higher than that of soybean which is the biggest oil crops (Photo. 2-1 - 2-4). Palm oil is mainly used for food (90%) and the other residues are used in various fields such as detergent industry. In Japan, 20% of oil for food has been depended to oil palm.

Malaysia, the biggest palm oil producing country in the world, produces the palm oil from 250 million ton in 1980 to 600 million ton in 1989, which increases more than two times (Table 2-1). The production is increasing yearly and it will reach to 775 million ton in 2000.

2.3 By-products of oil palm

A brief description of palm oil processing is shown in Fig. 2-1 with many kinds of by-products generated. The by-products are empty fruit bunch (EFB) 24%, palm press fiber (PPF) 21%, kernel shell 8%, kernel cake 2.5% and palm oil sludge (POS) 10%. EFB and PPF are the major cellulosic wastes. PPF and kernel shell are used as fuel to produce steam (generator), but it causes the black smoke containing the burnt carbon fly ash. EFB is mainly incinerated to produce potassium ash for fertilizer, causing white smoke with high moisture (Photo. 2-5 - 2-7). The treatment of oil palm waste is becoming important because the palm oil industry is rapidly expanding in Asia. Then, there is also a national concern on the pollution of the oil palm waste. At present most of the waste is incinerated, which causes a serious pollution nearby areas. The burning of the cellullosic wastes in the field is prohibited and the new construction of incinerator is not permitted by the environmental pollution regulation in Malaysia. Recently, it has been realized the needs to utilize these by-products effectively in order to improve the economic situation of the oil palm industry as well as to reduce pollution problems. From these reasons, it is necessary to develop the process to utilize the cellulosic wastes especially EFB. The current production of EFB and PPF in Malaysia, 1989 is estimated as 3.2 million ton and 2.8 million ton, respectively.

2.4 Upgrading Process of EFB

The research has been carried out for utilizing this potentially useful cellulosic waste by upgrading with nuclear technology. As EFB is produced in palm oil mill, it is easily obtained without collection from distributed area as other cellulosic wastes. The process is considered for the upgrading of EFB to useful products such as animal feed and mushroom.

The process is as follows (Fig. 2-2):

1) to cut EFB sample to the length of 2-3 cm
2) to add inorganic nutrients or rice bran and adjust moisture content
3) to pasteurize or sterilize the fermentation media by irradiation
4) to inoculate useful microorganisms
5) to ferment the EFB into useful products

To perform the process, each step is investigated in detail under the project.

Table 2-1 Palm oil production and by-products generation in Malaysia

Year	Cultivated area (x 1000ha)	Crude oil (x 1000t)	PPF* (x 1000t)	EFB** (x 1000t)
1970	291	431		
1980	1043	2573	1210	1360
1986	1599	4544	2220	2400
1987	1672	1800	2220	2540
1988	1786	5030	2330	2660
1989	1951	6050	2800	3200

* palm press fiber

** empty fruit bunch calculated at 53% of crude oil

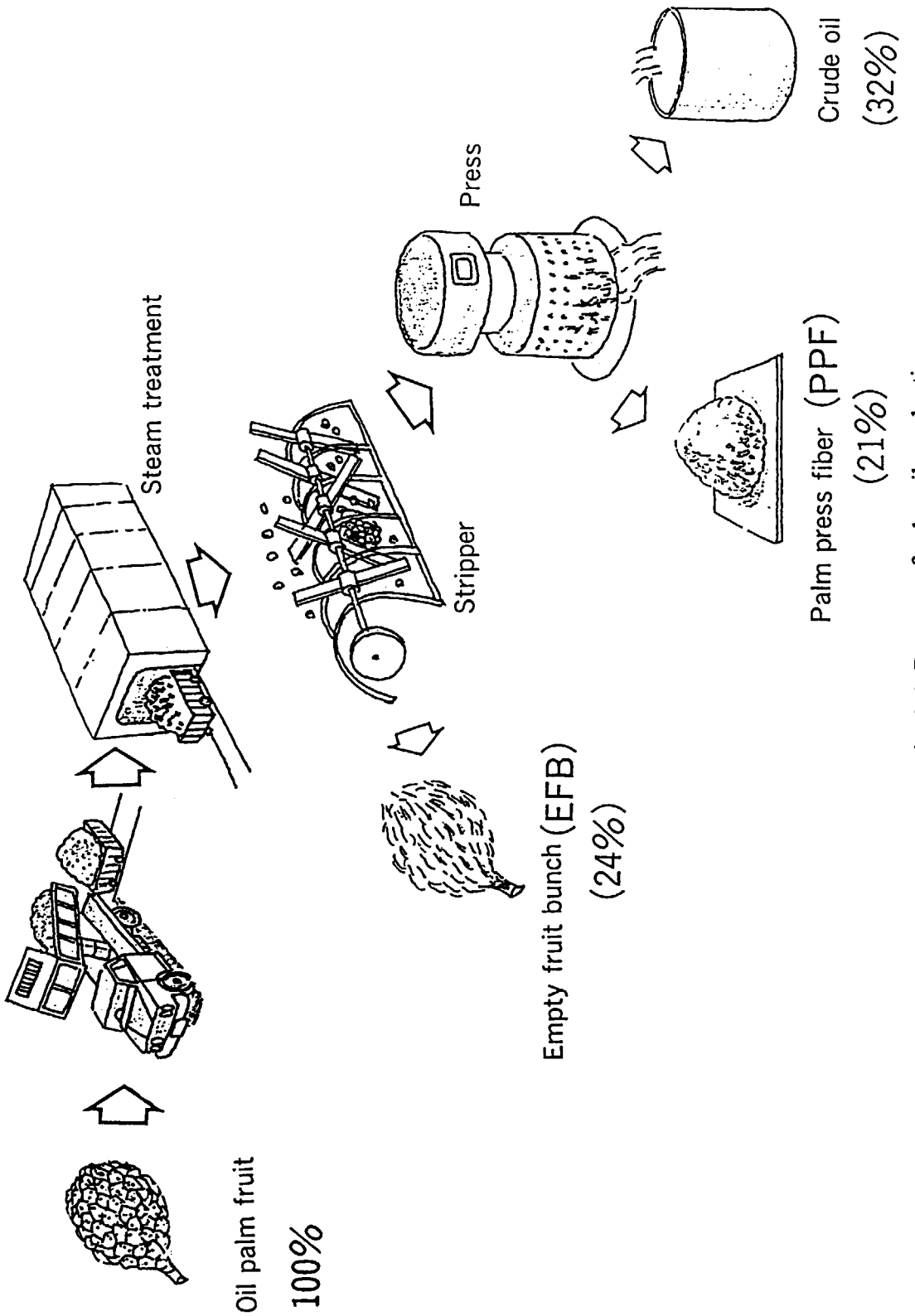

Fig. 2-1. Process of palm oil production

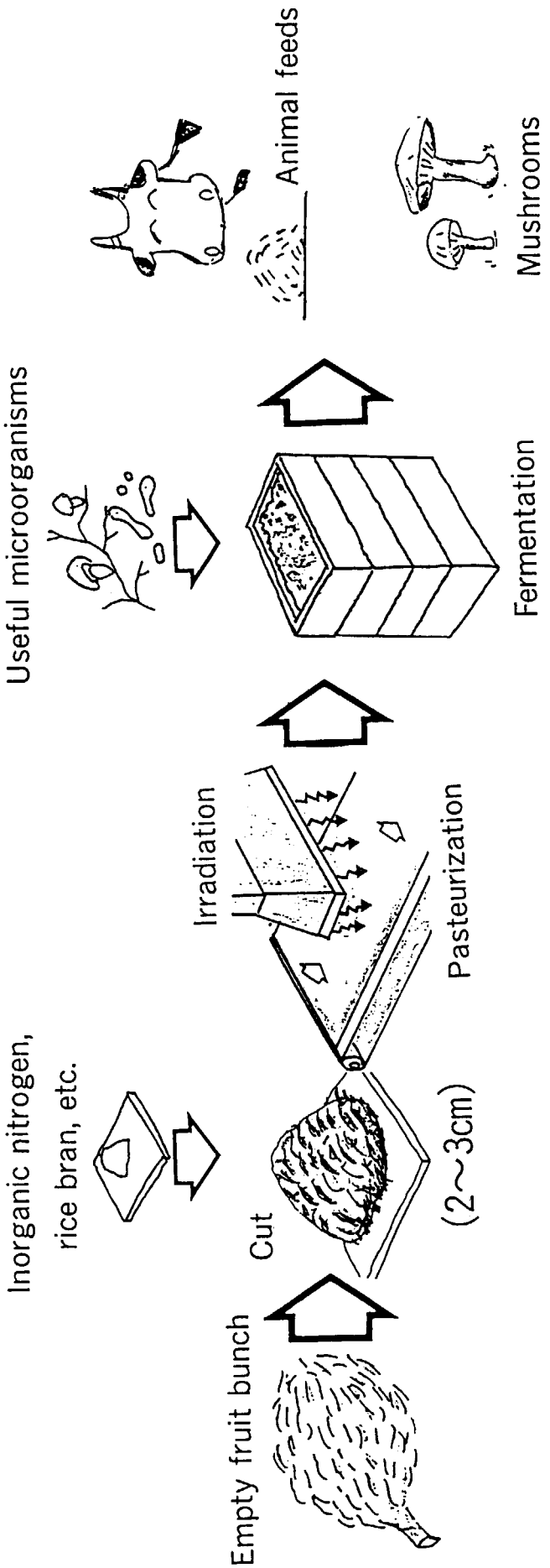

Fig. 2-2. Process for upgrading of palm oil wastes by radiation and fermentation treatment

Photo. 2-1. Oil palm tree and fruits

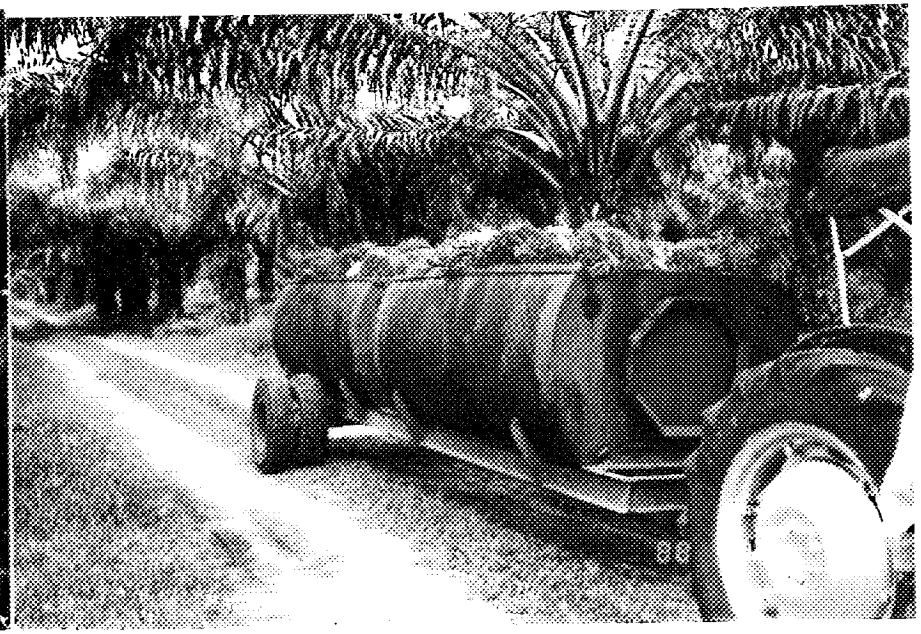

Photo. 2-2. Cultivation of oil palm fruits

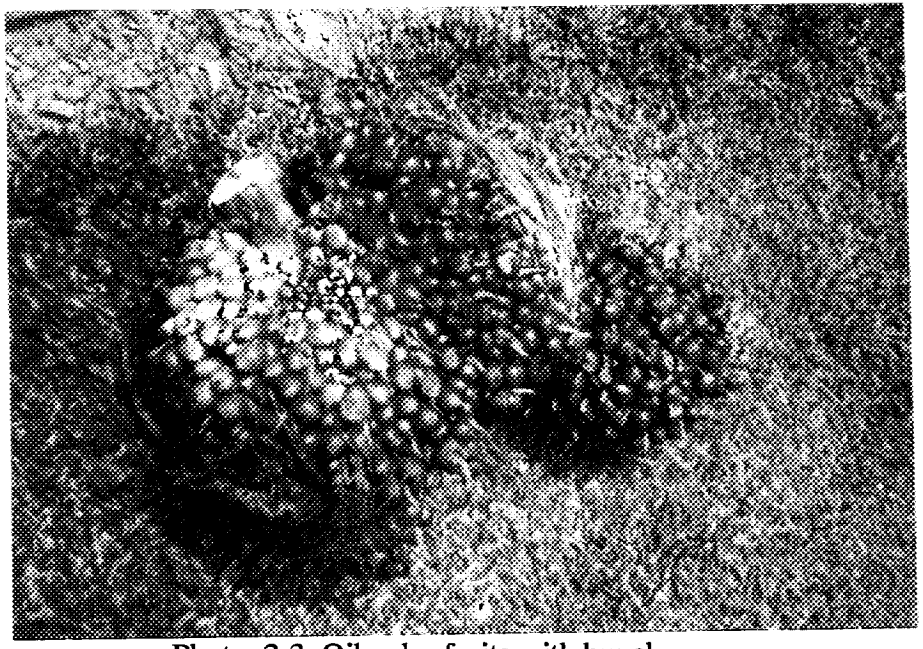

Photo. 2-3. Oil palm fruits with bunch

Photo. 2-4. Oil palm fruits

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Photo. 2-5. Palm press fiber (PPF)

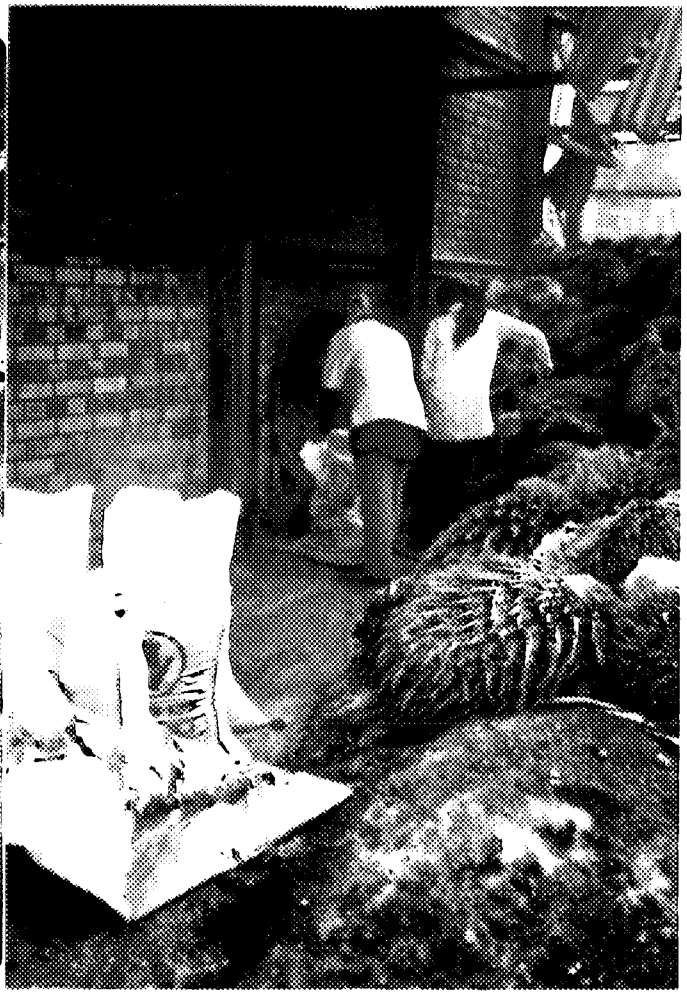

Photo. 2-6. Empty fuit bunch (EFB) and incinerator

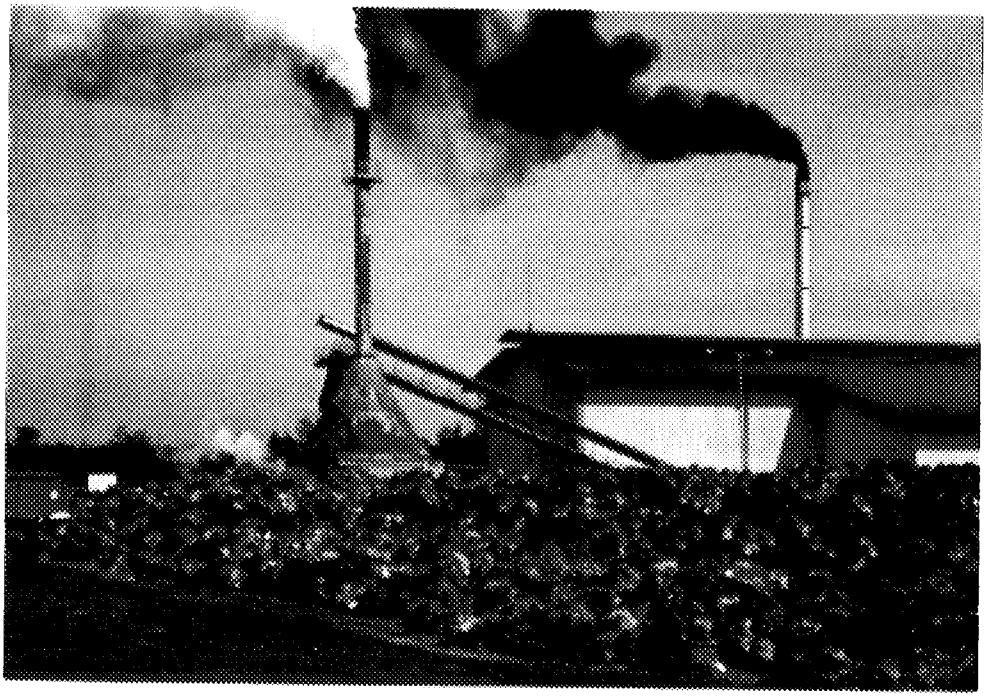

Photo. 2-7. Smoke from burning of PPF (black) and incinerator of EFB (white)

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3. BASIC STUDY ON CHEMICAL AND BIOLOGICAL PROPERTIES OF OIL PALM WASTES

3.1 Distribution and Radiation Decontamination of Microorganisms

3.1.1 Introduction

It is considered that the degree of microbial contamination of oil palm wastes is different with the sample conditions. Microbial fermentation of these wastes by desired microorganisms is useful to convert to useful products but it is greatly interfered by natural contamination. It is, therefore, required to study the number, identification and radiation sensitivity of contaminated microorganisms to determine the necessary dose for pasteurization or sterilization of wastes. In this section, the distribution of microorganism in EFB and PPF, identification of dominant species and the irradiation effect on microbiological load were studied as the preliminary study for the fermentation of oil palm wastes.

3.1.2 Materials and methods

(1) Materials

Samples of PPF and EFB were collected from 15 palm oil mills in the Peninsular Malaysia (Fig. 3.1-1). These samples were kept for 2 - 14 days after oil extraction process. In addition, fresh samples were also collected from Mill 2. Fresh samples referred to samples collected immediately after discharged from "screw press" in the case of PPF, and "stripper" in the case of EFB.

(2) Gamma Irradiation

The samples were cut into small pieces (*ca.* 2 cm length) and used for the assay of microorganisms. These samples (5 g) were packed in polyethylene pouches and irradiated at room temperature using cobalt-60 slab source (100 kCi) at JAERI or the gamma-cell 400A at MINT. The dose rates used were 1 - 10 kGy/h as determined by Fricke dosimetry.

(3) Assay of microorganisms

The numbers of microorganisms were determined by the surface plate agar method (Kume et al 1983). Total aerobic bacteria and fungi were enumerated separately on Difco-nutrient agar and MYG-chloramphenicol agar plates, respectively.

Identification to the levels of genus and species was performed mainly by referring to "Bergey's Manual of Determinative Bacteriology" (Buchanan & Gibbons 1974) and "The Genus Aspergillus" (Raper & Fennell 1965).

3.1.3 Results and discussion

(1) Distribution of microorganisms and moisture content

Samples from 15 mills were enumerated for total aerobic bacteria and fungi. Figure 3.1-2 shows the distribution of microorganisms and moisture content in various EFB samples. Total aerobic bacteria range from 10^8 to 10^{10} cells/g, and fungi range from 10^4 to 10^8 cells/g. All EFB samples collected from various mills were highly contaminated with bacteria and fungi. The contamination of the microorganisms stood in the same high level except the samples from Mill 7 in which the moisture content and the contamination of microorganisms were relatively low. The results presented in Fig. 3.1-3 show that total aerobic bacteria in PPF range from 10^7 to 10^{10} cells/g and fungi range from 10^4 to 10^8 cells/g.

EFB samples have generally higher moisture content (44 - 74% except Mill 7 of 24%) compared to PPF (10 - 38%) because the former is kept outside the mills while the later is stored under shade. The initial contamination of microorganisms in EFB is higher than that in PPF corresponding to the moisture content. However, it is considered that there is no significant difference in the numbers of total aerobic bacteria and fungi between samples collected from the various locations in the Peninsular Malaysia.

(2) Decontamination of microorganisms by irradiation

Figures 3.1-4 and 5 show the decrease in numbers of microorganisms in EFB and PPF after irradiation, respectively. Bacteria in both EFB and PPF samples were radioresistant and the dose required for elimination below the detectable level was more than 15 kGy. Fungi were eliminated below detectable level by the irradiation of 5 - 6 kGy. These results suggest that irradiation dose of 5 to 10 kGy is sufficient for pasteurization of EFB and PPF since the decontamination of fungi is important for the fermentation using useful fungi, the pH requirement of which is lower than the optimum pH for bacterial growth.

(3) Distribution of microorganisms in fresh PPF and EFB samples

Table 3.1-1 shows the distribution of microorganisms at various stages of post-

extraction of oil. In fresh EFB sample, total aerobic bacteria of 10^7 cells/g increased to 10^8 cells/g in 1-day old EFB. The sample of EFB (inside) has lower bacterial counts (10^2 cells/g) with negligible fungi count. The degree of contamination in PPF increased in the order of hottest, hot and 1 day old samples. Fungi was below the detectable level in hottest sample and detected in hot sample (8×10^2 cells/g) which increased to a level of 10^5 cells/g after 1 day of storage. These results indicate that fungi began to infect PPF a few hours after being ejected from the fiber cyclones and increased rapidly during storage under shade.

Figure 3.1-6 shows the decrease in numbers of total aerobic bacteria of fresh EFB and PPF samples by irradiation. Total aerobic bacteria in fresh samples decreased below the detectable level when subjected up to 6 kGy irradiation even in the most resistant samples (1 day old PPF). As the initial counts of fungi were low and the fungi was radiosensitive, dose less than 2 kGy is sufficient to decrease below the detectable level.

From the results, it is apparent that pasteurization treatment of PPF and EFB is required in order to reduce microorganisms to acceptable level for fermentation regardless of the stages of samples except the hottest PPF.

(4) Identification of contaminated microorganisms

The contamination of microorganisms in EFB was remarkable as shown in Fig. 3.1-1, and the total number of aerobic bacteria in EFB which had been discarded more than one week after the oil extraction process in the factory were 5.5×10^6 to 1.6×10^9 per gram as shown in Table 3.1-2. These number of contamination of either bacteria or molds were not decreased even after pH adjustment in water for 1 or 2 days or washing by water. Coliforms were not detected on MacConkey agar. The species of bacteria, such as *Bacillus subtilis*, *B. firmus*, *B. coagulans* and *B. shaericus*, and a little amount of *Actinomycetes* and *Arthrobactor*. Molds were found to be 2.8×10^5 to 4.8×10^6 per gram (Table 3.1-3), which consist of mainly *Aspergillus flavus*, *A. flavus var columnaris*, *A. phoenicis*, *A. alliaceus*, *Penicillium* and *Rhizopus*. In some of these samples, *A. versicolor* and *A. fumigatus* were also isolated but less frequently found than *A. flavus* group. *A. flavus* was the most dominant among of these molds, and many isolates had the ability to produce aflatoxin B_1, B_2 and others when examined by the conventional plate agar method as reported previously (Muhamad et al 1986). Many isolates of molds, including *A. flavus*, had the ability to grow on cellulosic substrates. This evidence has been confirmed by incubation of mold on a defatted cotton supplemented within organic nutrient solution. The isolated microorganisms contaminated in EFB

was measured in Table 3.1-4.

In the case of a fresh EFB which had been discarded 1 or 2 days after the oil extraction process, the main contaminants were *Aureobasidium* sp. and *Candida* sp. with count of 1.1×10^9 per gram. The molds consist of mainly the yellow fungi, *Neurospora* sp. and the count was 8×10^6 per gram. *Aureobasidum* was resistant to radiation, as similar to spore-forming bacteria, whereas *Neurospora* can be eliminated below 5 kGy of irradiation (Fig. 3.1-7).

3.1.4 Conclusion

The distribution of microorganisms in EFB and PPF was enumerated using the samples collected from 15 palm oil mills in peninsular Malaysia. All samples were highly contaminated with bacteria (10^8 - 10^{10} cells/g) and fungi (10^4 - 10^8 cells/g). The dominant spices in EFB and PPF were identified and it was found that some strains of fungi produce mycotoxin. From the results of the radiosensitivity obtained by survival curves of contaminating microorganisms, it was concluded that the necessary doses were 10 kGy for pasteurization and 30 kGy for sterilization.

Table 3.1-1. Distribution of microorganisms in fresh EFB and PPF

Samples		Total aerobic bacteria (cells / g)	Fungi (cells / g)
EFB	fresh[a]	1.6×10^7	ND
	fresh(inside)[b]	6.0×10^2	ND
	1 day old	1.1×10^8	1.9×10^2
PPF	hottest[c]	1.3×10^3	ND
	hot[d]	3.4×10^3	8.0×10^2
	1 day old	2.6×10^8	1.2×10^5

a: Samples collected on conveyor belt which was immediately ejected from the "stripper".
b: Samples collected asceptically from the base of fresh EFB.
c: samples ejected immediately at the exit of "screw press".
d: Samples collected a few hours after exposure to the environment and still within the vicinity of "screw press".
Nd: Below the detectable level.

Table 3.1-2. Distribution of bacteriain EFB and PPF

Sample	Total bacteria (counts/g)	Main species
EFB, No. 1	1.6×10^9	*Bacillus firmus, B. subtilis*
EFB, No.2	3.6×10^8	*B. coagulans, B. subtilis*
EFB, No. 3	1.2×10^9	*B. subtilis, B. firmus*
EFB, heated (100℃, 15min)	4.4×10^7	*B. coagulans, B. firmus*
EFB, alkali-treated (1% NaOH, 24hr)	1.0×10^6	*B. subtilis, B. firmus*
PPF, No. 1	4.6×10^7	*B. shaericus, B. subtilis*
PPF, No. 2	5.5×10^6	
EFB, fresh	negligible	

Actinomycetes and *Arthrobacter* were also isolated.

Table 3.1-3. Distribution of fungi in EFB and PPF

Sample	Fungi (counts/g)	Main species
EFB, No. 1	6.0×10^5	*Aspergillus ochraceus group;A. allisceus,*
EFB, No.2	2.8×10^5	*A. niger group; A. phoenicis,*
EFB, No. 3	6.2×10^5	*A. flavus group; A. flavus,* *A. flavus* var *columnaris,*
PPF, No. 1	2.1×10^6	*Penicillium,* *Rhizopus*
PPF, No. 2	4.8×10^6	
EFB, fresh		
moulds	7.5×10^6	*Neurospora*
yeasts	1.1×10^9	*Pullularia, Candida*

Table 3.1-4. Isolation of contaminated microorganisms in EFB

	Species
Bacteria	*Micrococcus, Bacillus, Actinomycetes,* *Pseudomonas, Alcaligenes, Coliform*
Molds	*Aspergillus flavus, A. flavus* var *columnaris,* *A. fumigatus, A. tamarri* *A. allisceus, Penicillium, Cunninghamella,* *Helminthosporium, Oidiodendron* sp.

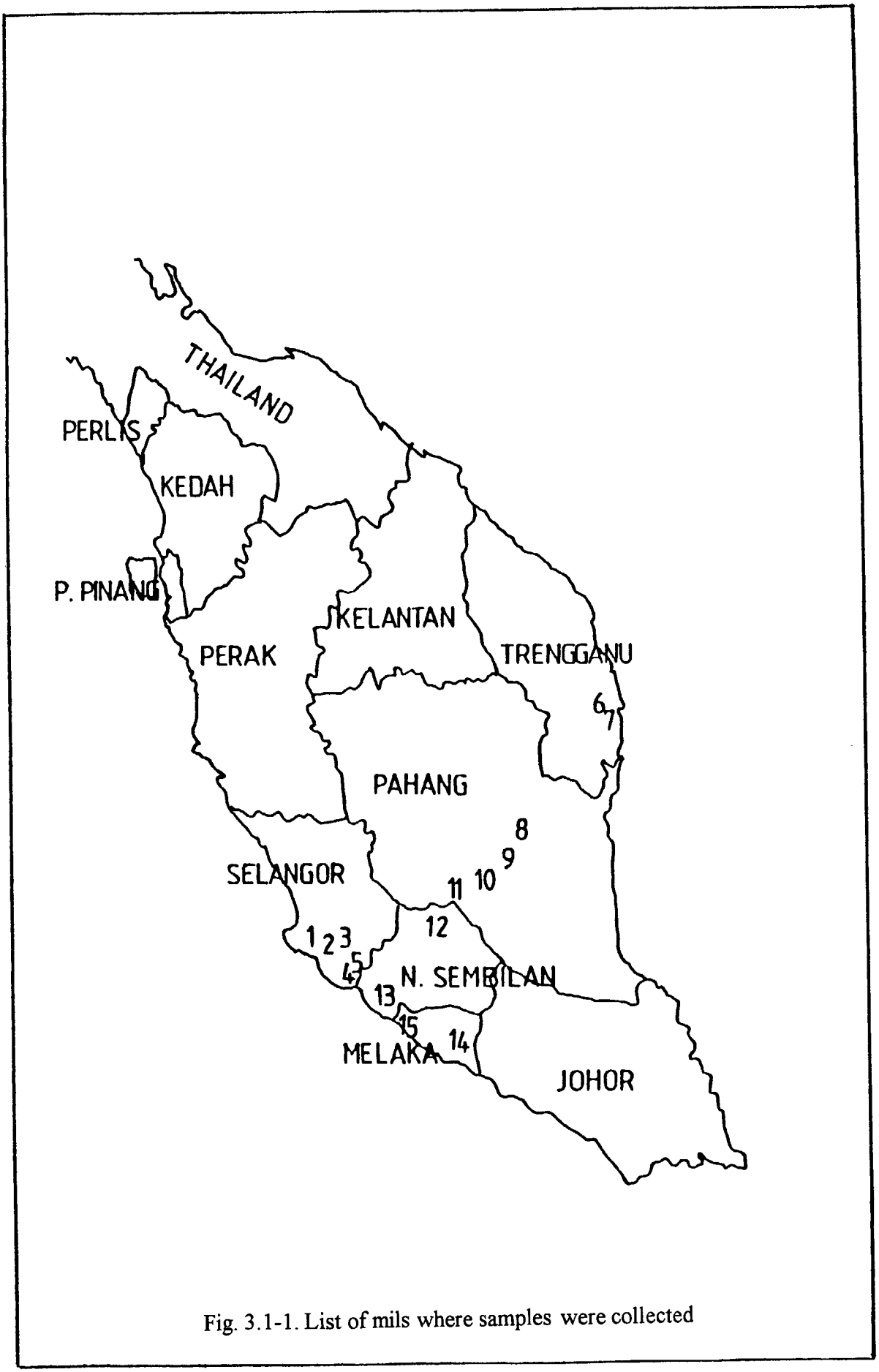

Fig. 3.1-1. List of mils where samples were collected

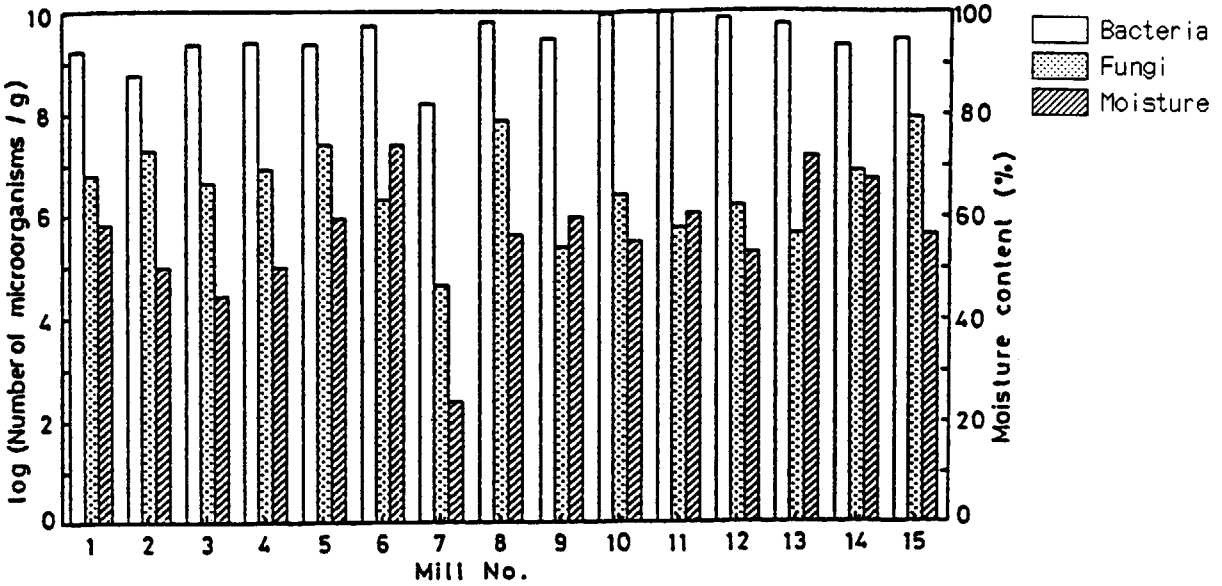

Fig. 3.1-2. Distribution of microorganisms and moisture content in EFB collected from 15 mills (Kume et al., 1990)

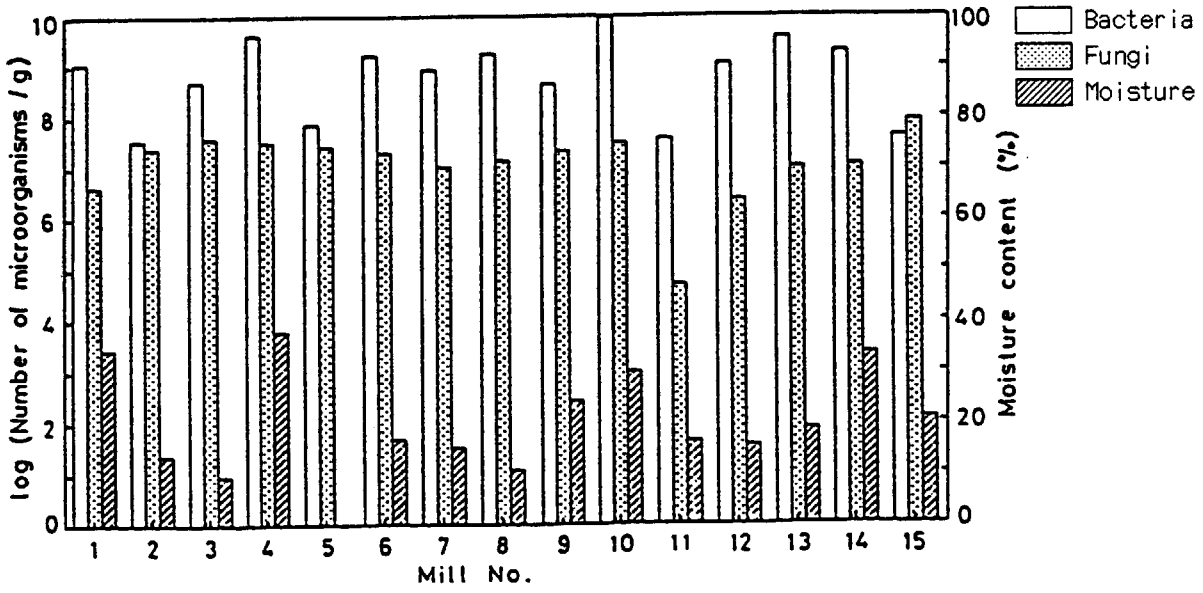

Fig. 3.1-3. Distribution of microorganisms and moisture content in PPF collected from 15 mills (Kume et al., 1990)

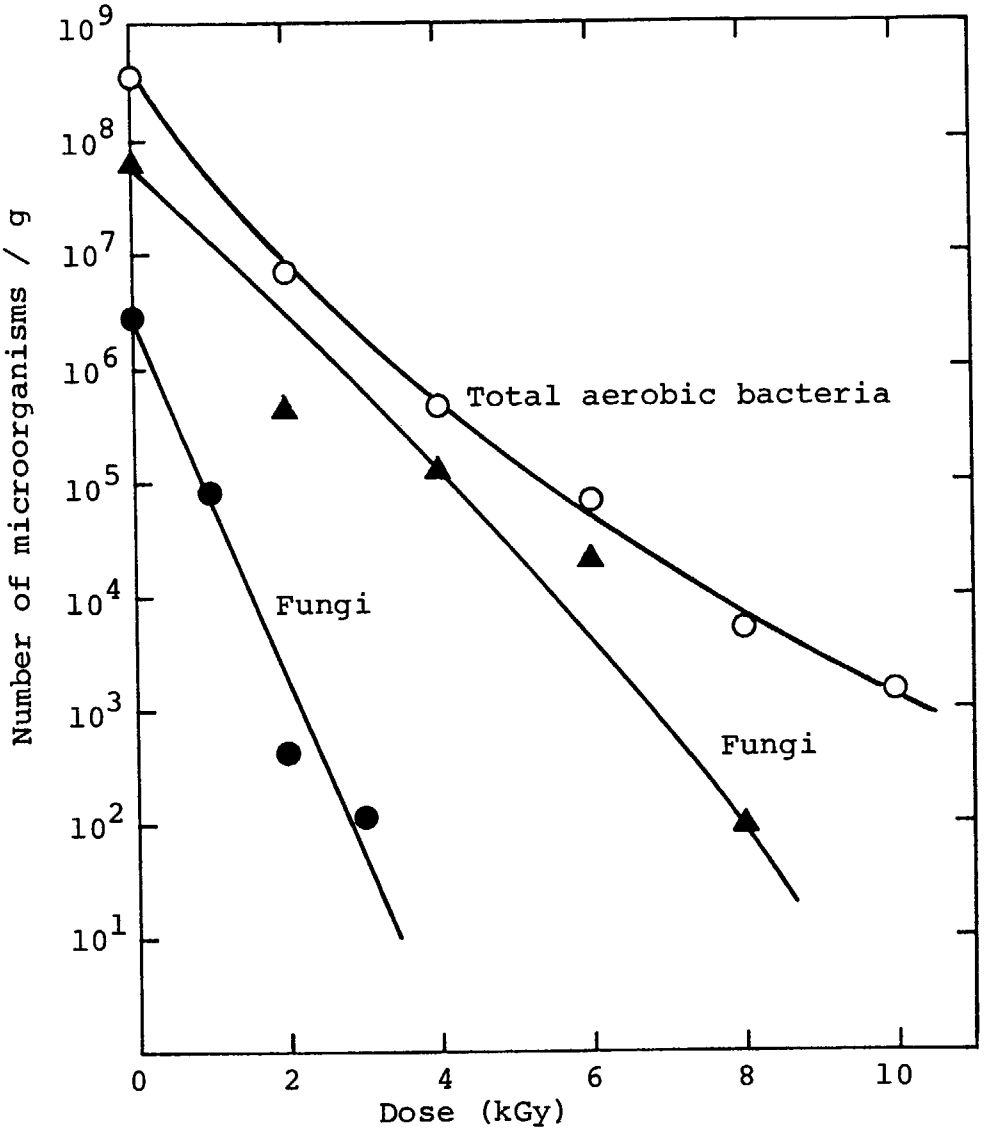

Fig. 3.1-4. Decrease in number of microorganisms contaminating in EFB by γ-irradiation

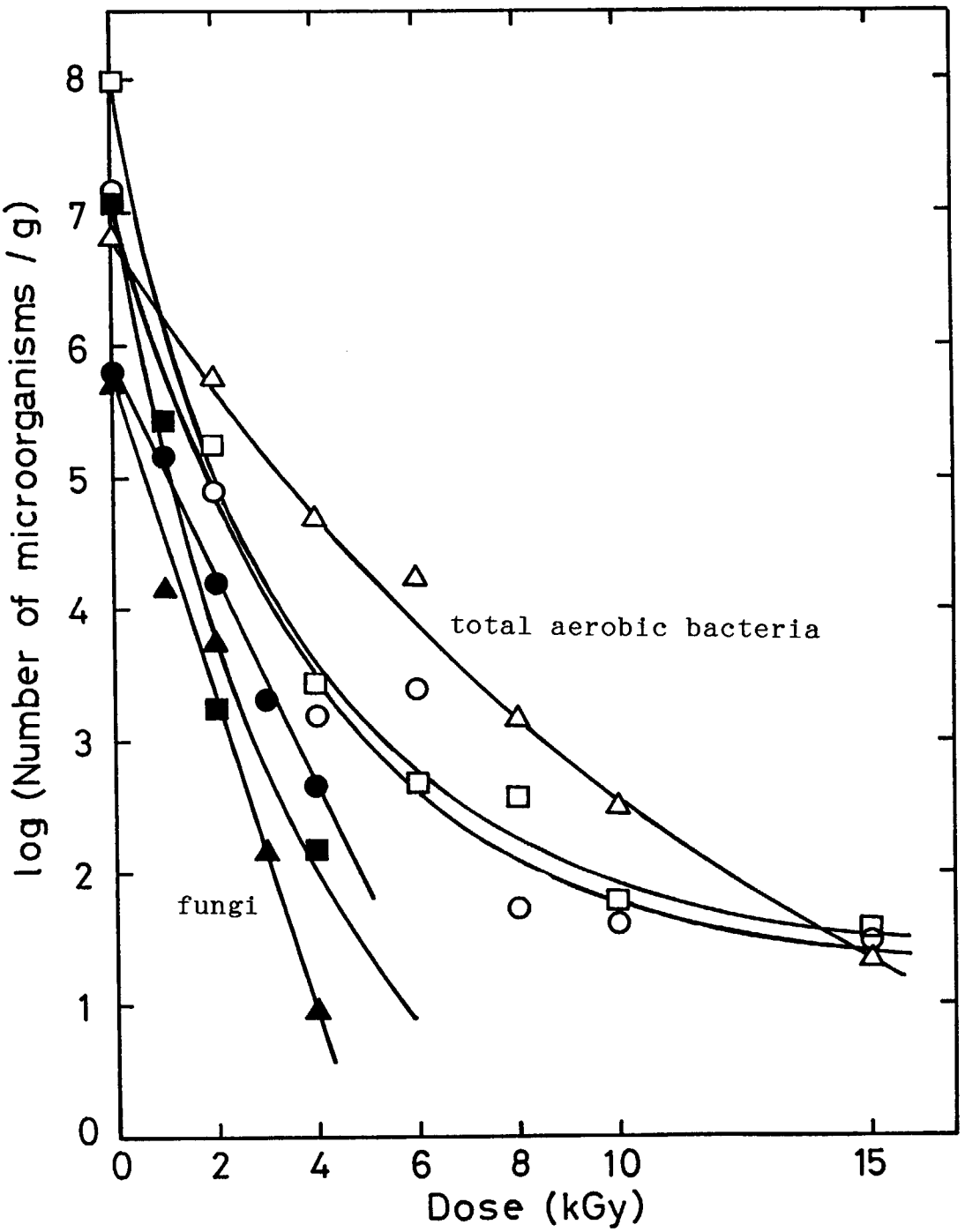

Fig. 3.1-5. Decrease in number of microorganisms contaminating in PPF by γ-irradiation (Kume et al., 1990)

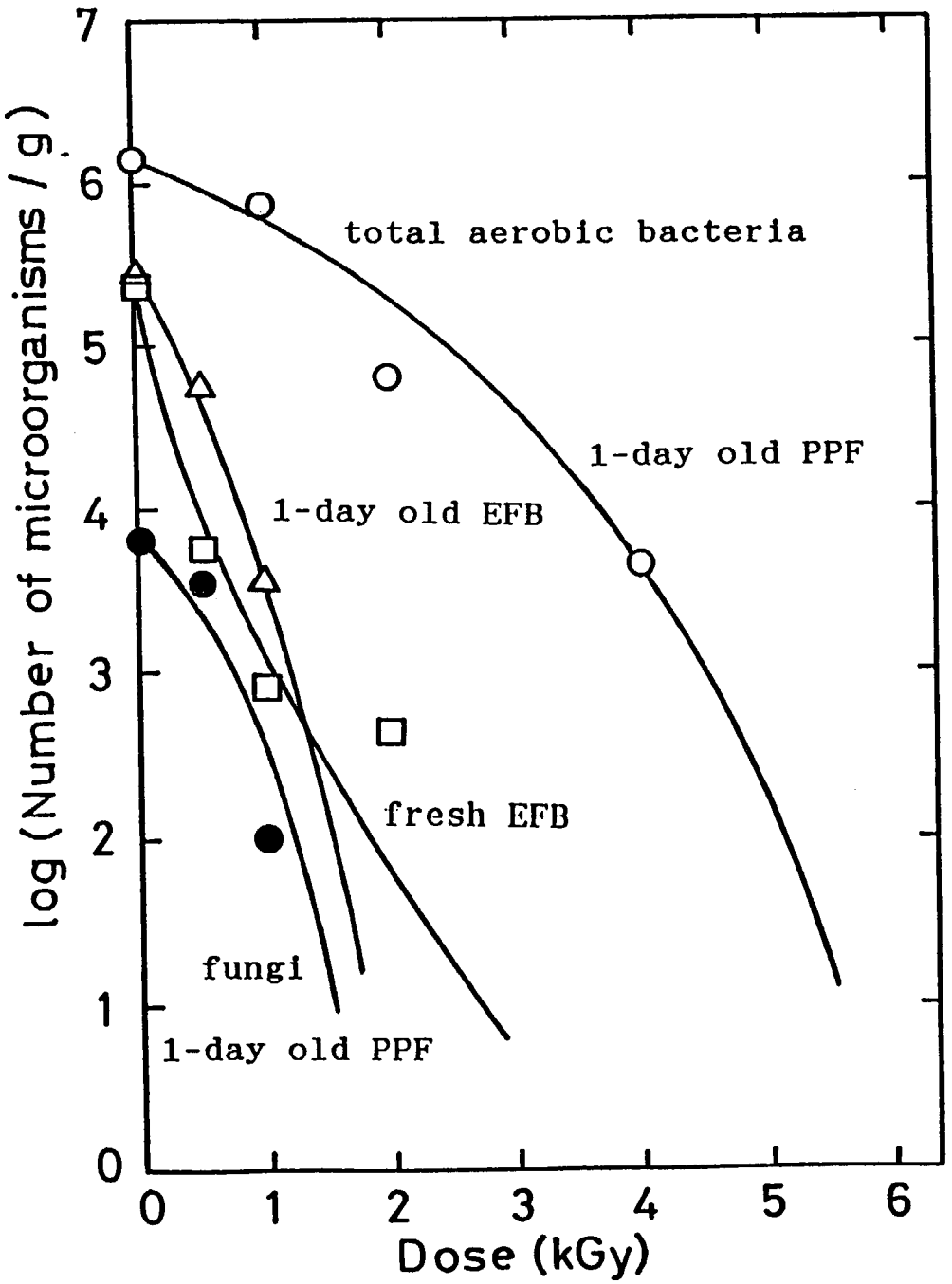

Fig. 3.1-6. Decrease in number of microorganisms contaminating in fresh EFB and PPF by γ-irradiation (Kume et al., 1990)

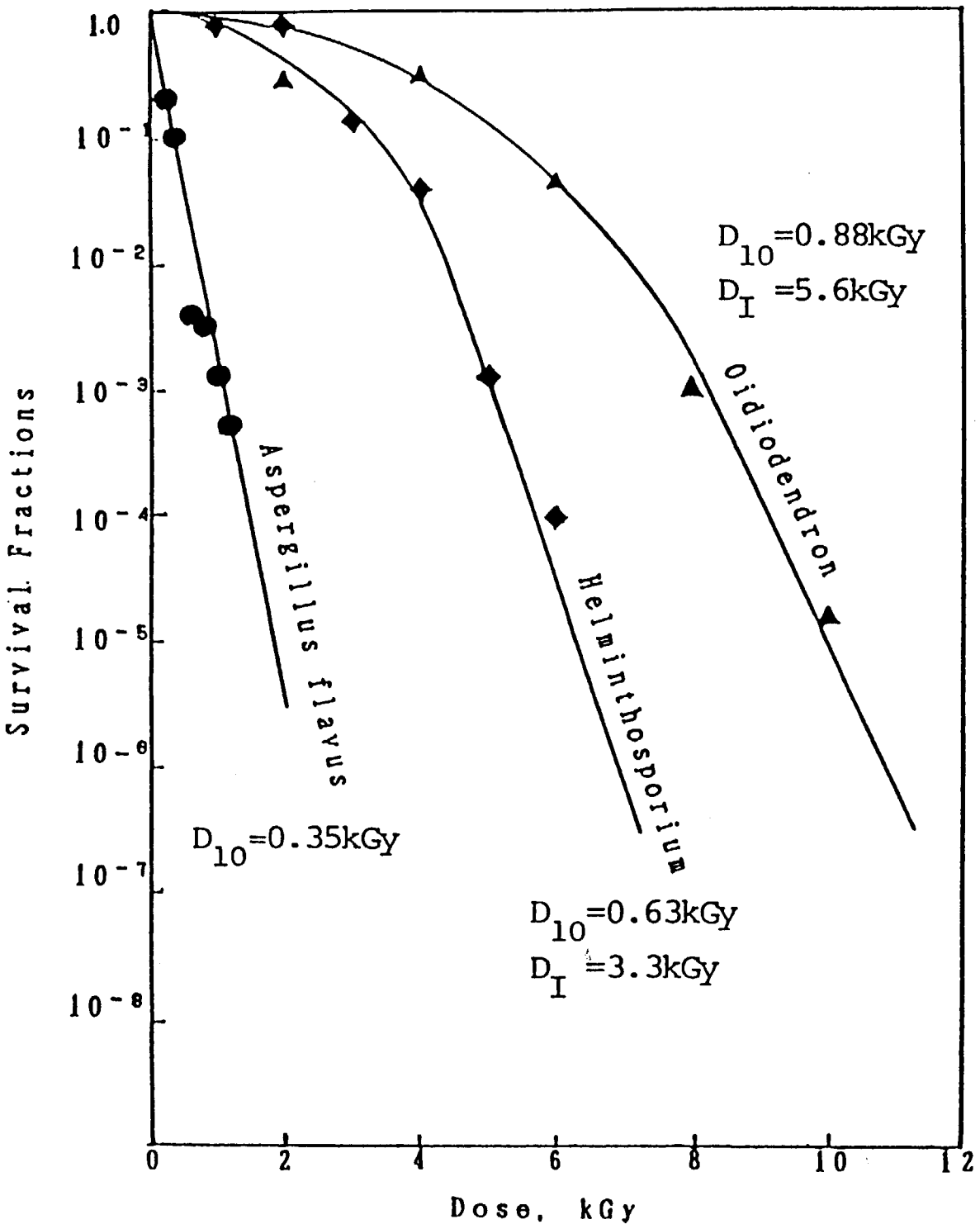

Fig. 3.1-7. Survival curves of imperfect fungi isolated at high dose

3.2 Analysis of Chemical Components

3.2.1 Introduction

Cellulosic wastes such as EFB and PPF contain high amount of cellulose, hemicellulose and lignin, and the digestibility of these lignocellulose is generally very low. One of the ideas is to change them into animal feeds by fermentation but the point to be solved is the high content of lignin which animal cannot digest. It is, therefore, required to decrease the lignin content and increase the digestibility of cellulosic wastes to use as the ruminant feeds. Prior to the fermentation treatment, it is necessary to analyze the components of EFB and PPF.

In this section, chemical components and the physico-chemical properties of EFB and PPF are analyzed and the effect of irradiation on these components are studied for the base of upgrading of oil palm wastes to useful products.

3.2.2 Materials and methods

(1) Moisture content

The moisture contents of the samples were determined using Mettler system (Mettler Inst., Switzerland) at 135℃ for 4 hr.

(2) Gamma irradiation

The samples were cut into small pieces (*ca.* 2 cm length) and ground to particle size of 180 - 250 μ m. These samples (5 g) were packed in polyethylene pouches and irradiated at room temperature using cobalt-60 slab source at JAERI or the gamma-cell 400A at MINT. The dose rates used were 1 - 10 kGy/hr as determined by Fricke dosimetry.

(3) Analysis of chemical components

The chemical components were determined in accordance with the modified TAPPI standards method (1974). The outline of procedure for chemical components analysis was shown in Fig. 3.2-1. All measurements were done in duplicates and the values were expressed as weight percentage on moisture-free basis.

(4) Water holding capacity (WHC)

The water holding capacities of EFB and PPF were determined (McConnell et al

1974) as follows; Samples (0.45 g), weighed in centrifuge tubes, were stirred in 25 ml distilled water for 16 hr. After centrifugation at 14,000g for 1 hr the supernatant was discarded and the tubes were weighed, and the dry weight of pellet was measured. Results were expressed as grams of water per gram of dry sample.

(5) Water extracts

Ground samples of EFB and PPF (3 g in 30 ml distilled water) were shaken overnight and the extracts were collected as supernatant upon filtration. Water extractives were determined by measuring the dry weight of solid matters in the extract.

3.2.3 Results and discussion

(1) Chemical components of EFB and PPF

Table 3.2-1 shows the chemical components of EFB collected from 3 mills. Some differences in alcohol-benzene extracts, holocellulose and lignin contents were observed between the samples while hot-water solubles and alpha-cellulose content show no differences. The values obtained were in agreement to that reported by Husin et al (1985). PPF samples from 3 different mills also showed some variations in their chemical components (Table 3.2-2). These variations may be due to several reasons such as difference in maturity (age) of samples and the differences in processing in each mills.

Some differences in the chemical components were observed between EFB and PPF. Hot-water soluble contents of EFB were almost 4 times higher than that of PPF while alcohol-benzene extract was slightly lower. Cellulose content appeared slightly higher in EFB whereas lignin content was slightly lower. It can be generally concluded that the chemical components of EFB and PPF showed little variations among different mills and hence the fermentation conditions are not likely to be affected by these little variations.

The results show that both EFB and PPF have high content of cellulosic components and lignin. It is, therefore, suggested that the lignin digestible fungi is necessary to use for their fermentation. Physical and/or chemical pretreatment for delignification may also be effective to facilitate the fermentation process.

(2) Effect of irradiation on chemical components

Tables 3.2-3 and 3.2-4 show the change in components of EFB and PPF by

irradiation. Alcohol-benzene extracts and hot-water solubles of EFB were slightly increased by irradiation up to 50 kGy. Whereas, holocellulose and alpha-cellulose were slightly decreased at a high dose of 50 kGy and lignin content did not change. In the case of PPF, the hot-water solubles were slightly increased but holocellulose and alpha-cellulose contents seemed to decrease slightly at 50 kGy. There is very little effect of irradiation on the amount of alcohol-benzene extract and lignin. From these results, it can be concluded that some slight degree of degradation in the chemical components of EFB (Fig. 3.2-2) and PPF have occurred by irradiation up to 50 kGy but the overall effects were not significant.

(3) Effect of irradiation on water holding capacity of various samples

Water holding capacity is an important factor for the fermentation substrate. Table 3.2-5 shows the change in water holding capacity of EFB and PPF by irradiation. The results of the commonly used fermentation media such as sawdust and rice bran samples were also shown for comparison. Both EFB and PPF have a high water holding capacity and these values were almost the same to those of rice bran and sawdust. These results suggest that they are suitable to use as fermentation substrate. Irradiation dose up to 50 kGy has no effect on the water holding capacities of all the samples examined.

(4) Components in fresh PPF and EFB samples

The chemical components in fresh samples were analyzed immediately after collection. The results presented in Table 3.2-6 show that the chemical components of fresh samples were apparently different from stored samples. The alcohol-benzene solubles were significantly higher in fresh EFB and PPF while hot-water solubles were much lower than old samples shown in Tables 3.2-1 and 3.2-2. Fresh samples were very oily, which account for obtaining higher quantity of alcohol-benzene solubles. It is considered that the oil components were consumed in a short time by fungi such as *Neurospore* which grow rapidly on fresh samples.

(5) Change in reducing sugar contents in water extracts by irradiation

Total and free reducing sugars were determined in water extracts of EFB and PPF. Ground samples (3 g in 30 ml distilled water) were irradiated at doses of 5, 10, 25 and 50 kGy. A set of sample was autoclaved (121℃, 15 min) for comparison. The samples were shaken overnight and the water extracts were collected as supernatant upon filtration. Water extractives were determined by measuring the dry weight of solid

matters in the extract. The water extractives of EFB were much higher than PPF, and little increase was observed by irradiation in both samples (Fig. 3.2-3). The contents of reducing sugar in water-extracts of EFB and PPF increased with increase in dose, while autoclaving treatment increased water extractives but the contents of reducing sugar decreased significantly (Fig. 3.2-4). These results suggest that the irradiation treatment up to 50 kGy was mild and the remaining reducing sugar was higher than the autoclaving treatment.

3.2.4 Conclusion

EFB are composed of 54 - 60% holocellulose (36 - 40% of cellulose), 22 - 27% lignin and other materials. Soluble fraction of EFB was slightly increased by irradiation up to 50 kGy, whereas holocellulose and α-cellulose were slightly decreased at a high dose of 50 kGy and lignin content did not change. Similar results were obtained in the case of PPF. From these results, it can be concluded that slight degree of degradation in the chemical components of EFB and PPF occurred by irradiation up to 50 kGy but these changes were not significant.

Table 3.2.-1. Chemical components of EFB collected from different palm oil mills (Kume et al., 1990)

Chemical component	Palm Oil Mill No.		
	Mill 1	Mill 6	Mill 14
Alcohol-benzene extracts	1.8	2.6	3.8
Hot water solubles	16.4	16.6	15.6
Holocellulose	60.3	54.2	58.9
(Alpha-cellulose	40.0	36.0	36.5)
Lignin	21.5	26.6	21.7

Values are means of duplicates and expressed as weight percentage based on moisture-free sample.

Table 3.2.-2. Chemical components of PPF collected from different palm oil mills (Kume et al., 1990)

Chemical component	Palm Oil Mill No.		
	Mill 1	Mill 5	Mill 6
Alcohol-benzene extracts	4.0	5.5	5.5
Hot water solubles	4.0	5.0	5.6
Holocellulose	67.7	63.1	60.3
(Alpha-cellulose	32.2	29.0	27.8)
Lignin	24.3	26.4	28.6

Values are means of duplicates and expressed as weight percentage based on moisture-free sample.

Table 3.2.-3. Change in chemical components of EFB by irradiation (Kume et al., 1990)

Chemical component	Dose (kGy)			
	Unirradiated	10	25	50
Alcohol-benzene extracts	1.8	1.6	2.5	2.2
Hot water solubles	16.4	18.5	17.5	19.6
Holocellulose	60.3	58.1	59.0	56.4
(Alpha-cellulose	40.0	37.4	33.8	31.3)
Lignin	21.5	21.8	21.0	21.8

Values are means of duplicates and expressed as weight percentage based on moisture-free sample.

Table 3.2.-4. Change in chemical components of PPF by irradiation (Kume et al., 1990)

Chemical component	Dose (kGy)			
	Unirradiated	10	25	50
Alcohol-benzene extracts	4.0	4.2	3.7	4.3
Hot water solubles	4.0	4.1	6.4	5.3
Holocellulose	67.7	66.0	65.1	64.4
(Alpha-cellulose	32.2	35.5	32.4	28.5)
Lignin	24.3	25.7	24.8	26.0

Values are means of duplicates and expressed as weight percentage based on moisture-free sample.

Table 3.2.-5. Effect of irradiation on water holding capacities (WHC) (Kume et al., 1990)

Sample	WHC (g water/g dry sample)			
	Unirradiated	10 kGy	25 kGy	50 kGy
EFB	4.9	5.0	4.8	5.2
PPF	4.2	4.5	4.1	4.4
Sawdust	4.8	5.0	4.9	5.2
Sawdust (fine)	3.6	3.7	3.4	3.6
Rice bran	4.1	4.0	4.0	4.0
Rice bran (fine)	3.5	3.4	3.6	3.4

Values are means of duplicates.

Table 3.2.-6. Chemical components of fresh EFB and PPF (Kume et al., 1990)

Chemical component	EFB		PPF
	outer	inner	
Alcohol-benzene extracts	13.4	8.0	10.8
Hot-water solubles	1.2	0.9	1.2
Holocellulose	67.2	64.4	67.7
(Alpha-cellulose	39.9	33.8	36.3)
Lignin	18.2	26.7	20.3

Values are means of duplicates and expressed as weight percentage based on moisture-free sample.

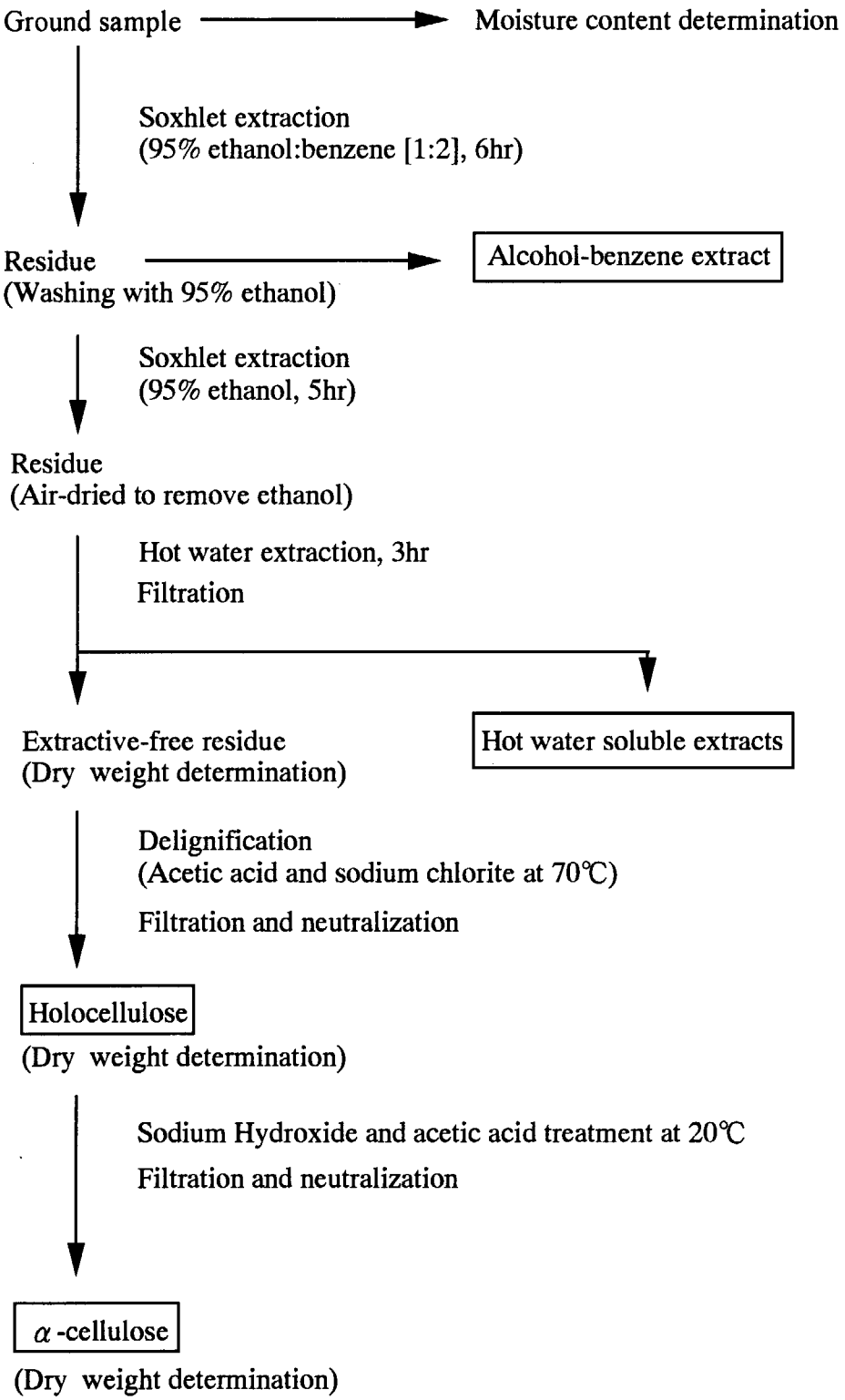

Fig. 3.2-1 Outline of procedure for chemical components analysis

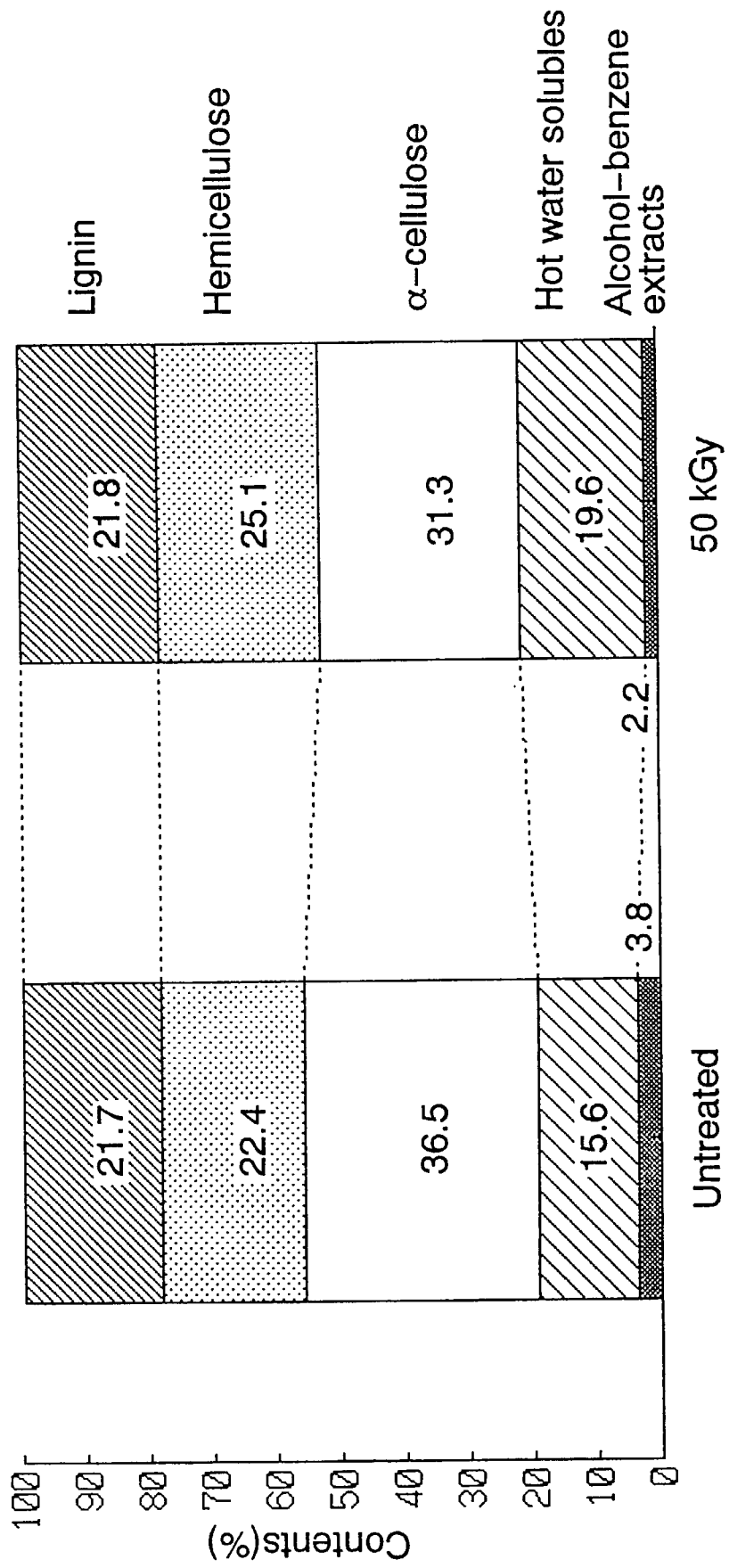

Fig. 3.2-2. Change in chemical components of EFB by irradiation

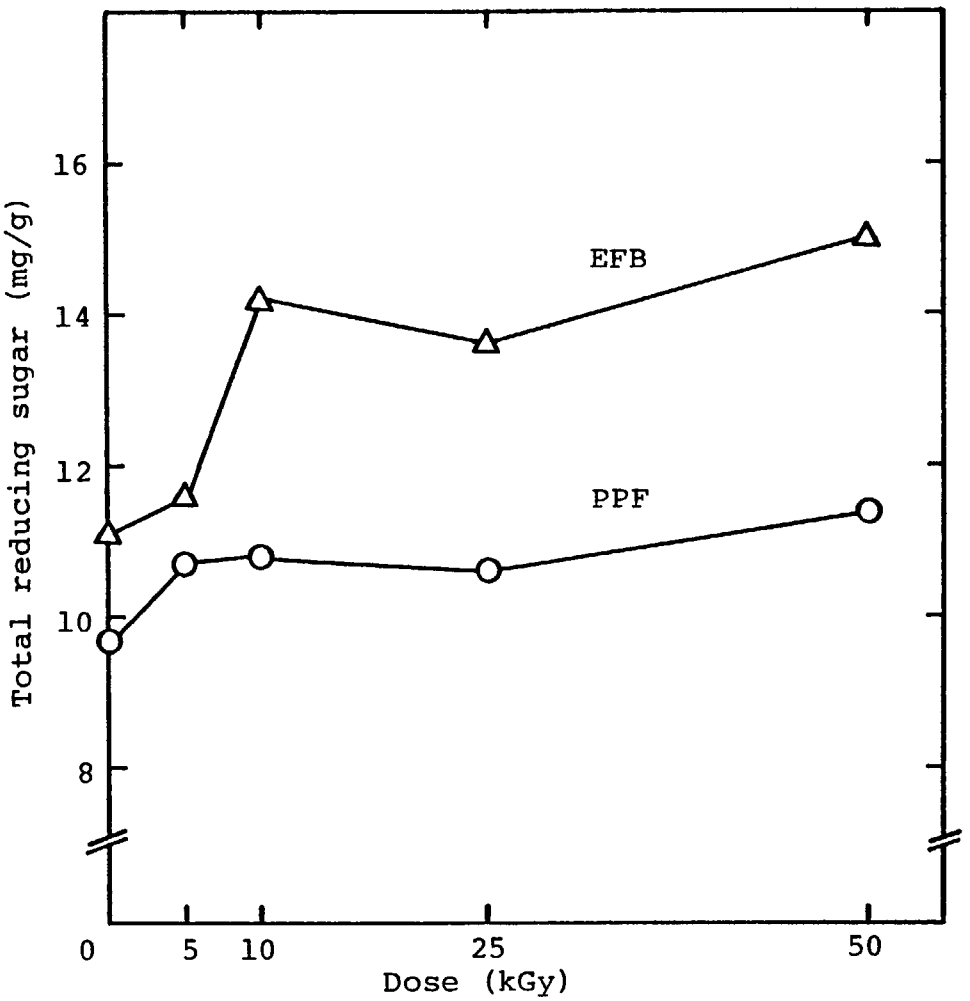

Fig. 3.2-3. Change in total reducing sugar in water extracts of EFB and PPF by irradiation

3g samples were suspended in 30ml of distilled water and shaken for 16 hr at room temperature.

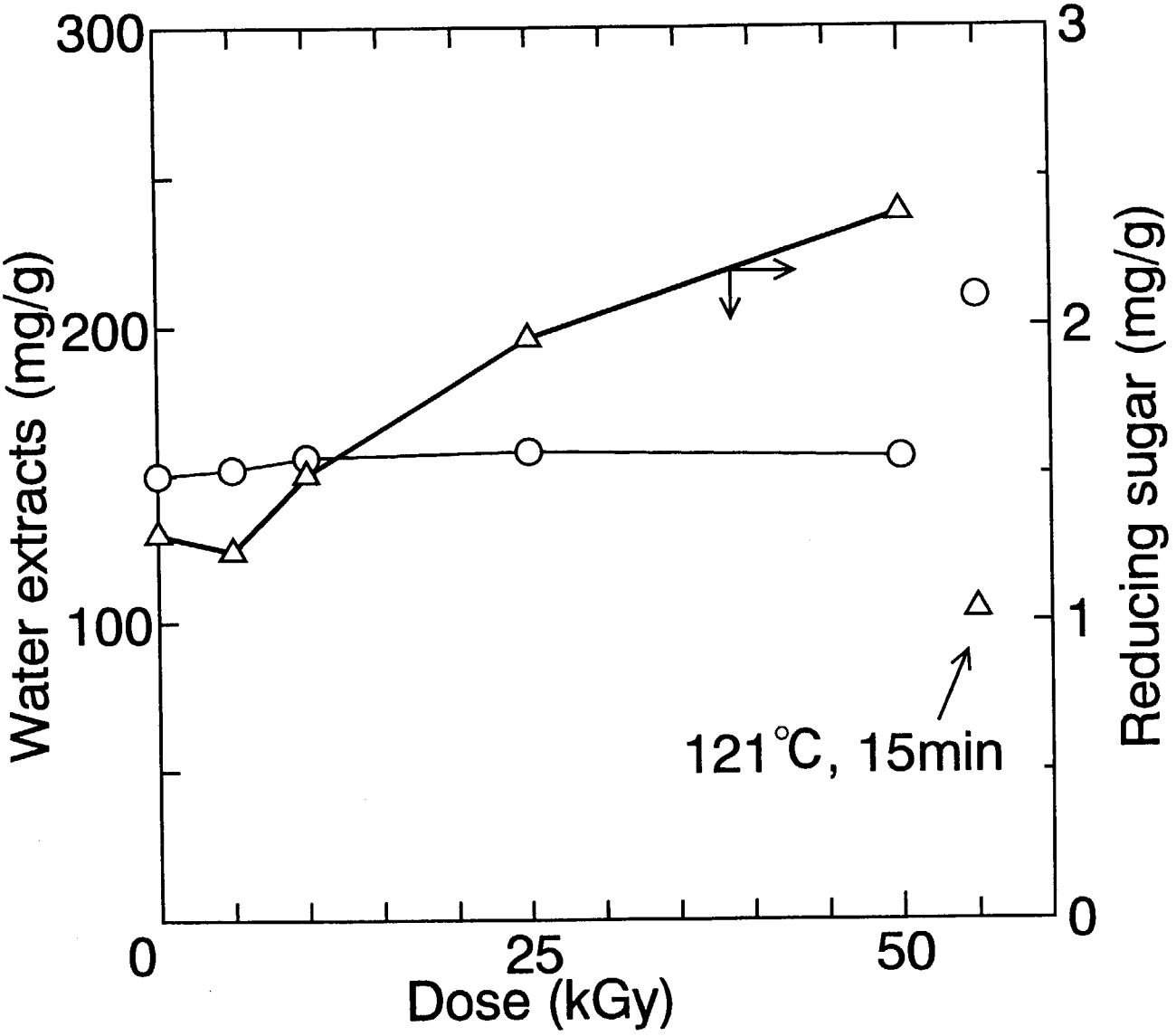

Fig. 3.2-4. Change in water extracts and reducing sugar in EFB by irradiation

3.3. Analysis of Mycotoxin

3.3.1 Introduction

Oil palm wastes were heavily contaminated with fungi including *Aspergillus flavus* (Table 3.1-3), a potential aflatoxin producers. The importance of aflatoxin in oil palm wastes hence animal feed production is by virtue of aflatoxin stability and harmful when consumed. Aflatoxin has been reported as potent hepatocarcinogens. It also can induce acute liver necrosis, growth depression and may lead to death in animal (Ostrowski, 1984). The maximum acceptable concentration of aflatoxin in animal feed has been established by FAO as 50 μ g/kg (Allcroft and Carnagh, 1963).

This chapter describes the results of aflatoxin production by 6 strains of *Asspergillus flavus* isolated from EFB and animal feeds. The contents of aflatoxins in fermented EFB were also analyzed.

3.3.2 Materials and methods

(1) Materials

Four strains of *Aspergillus flavus* isolated from EFB were tested the ability of aflatoxin production and 2 strains isolated from animal feeds were used as a comparison.

(2) Production of aflatoxin

Rice (30 g) was mixed with 15 ml of distilled water and autoclaved for 15 min at 121℃. Conidia of *A. flavus* was inoculated on rice substrate and incubated at 26 - 30℃ with 85% RH for 10 days.

(3) Extraction and purification

Sample in the flask was mixed with 200 ml methanol-1% NaCl (55:45). All methanol layer was collected, added with 100 ml chloroform, and shaken again for 5 min. All chloroform layer was collected and evaporated. Dried sample was dissolved in 10 ml chloroform-hexane (3:7), and eluted through silica-gel column in the following order: 10 ml hexane, 10 ml benzene-acetic acid(95.5:4.5), 10 ml diethyl ether-hexane (6:4) and finally with 15 ml dichloromethane-acetone (9:1). The solution was evaporated, re-dissolved in 5 ml methyl sulfoxide.

(4) HPLC analysis

An HPLC analysis of aflatoxins was performed on a JEOL liquid chromatography.

The column used was ODS-1201-P(2) (6 ϕ x 200 mm) and the eluent was 1% acetic acid-acetonitrile (70:30). The flow rate was 1.5 ml/min and aflatoxins were detected by UV spectrophotometor at 350 nm. Four components of aflatoxin B_1, B_2, G_1 and G_2 could be separated well under this HPLC condition.

3.3.3 Results and discussion

(1) Destruction of aflatoxins in solution

Pure aflatoxins (Sigma) B_1, B_2, G_1 and G_2 were irradiated in a solution (10 μ g/ml 1/15M phosphate buffer, pH 7.0). Figure 3.3-1 shows the destruction of aflatoxins by irradiation. All aflatoxins decreased exponentially and the sensitivities of B_1 and G_1 were higher than those of B_2 and G_2. The destruction of aflatoxins were also tested by autoclaving at 121℃ for 15 min and steaming at 100℃ for 25 min. Autoclaving and steaming did not cause any destruction of aflatoxins as shown in Table 3.3 -1.

(2) Potential for aflatoxins production by *A. flavus* isolated from EFB

Table 3.3-2 shows the potential for aflatoxins production of various strains isolated from EFB. *A. flavus* strains of E11, E14, E15 except E3 produced aflatoxins. Strains E11 and E15 produced aflatoxins of B_1 and B_2, and E14 produced B_2 and G_2. Other 2 strains 56915 and M13 isolated from animal feeds did not produce aflatoxins in this experimental condition.

(3) Aflatoxins in fermented EFB

Aflatoxin contents in EFB sample and the effect of irradiation and fermentation on aflatoxins in EFB were tested to evaluate the final products as animal feed. Table 3.3-3 shows the content of aflatoxins in various EFB. The content of most toxic component B_1 was lower than the FAO standard of 50 μ g/kg in the stored EFB sample. The level of other two components of B_2 and G_1 were the same but only G_1 was high. The contents were not changed by radiation and fermentation.

Aflatoxins in the dry conditions (even in the high moisture sample in cooked rice) are quite stable against irradiation (Kume et al., 1989). Figure 3.3-2 shows the radio-sensitivities of *A. flavus* and aflatoxin B_1 which has the highest radiation sensitivity among 4 aflatoxins. The toxicity of aflatoxins could be decreased by irradiation even in dry state but quite high dose is required. Consequently, it is concluded that the decontamination of fungi by 10 kGy irradiation prior to the production of aflatoxins is effective for the prevention of mycotoxin production.

3.3.4 Conclusion

EFB is highly contaminated with fungi including *A. flavus* and 3 of 4 strains have the potential to produce aflatoxins. Aflatoxins are quite radio-resistant and it is very difficult to destroy by irradiation. It is, therefore, recommended that the decontamination of fungi by 10 kGy irradiation prior to the production of aflatoxins is essential for the prevention of mycotoxin production. The aflatoxins level in fermented EFB after irradiation is low and therefore it could be used as feed supplement.

Table 3.3-1. Effect of autoclaving and steaming on denaturation of aflatoxins

	Concentration of aflatoxins (μ g / ml)			
	B_1	B_2	G_1	G_2
Control	15	15	15	15
Autoclaving (121℃, 15min)	15	15	15	15
Steaming (100℃, 25min)	15	15	15	8.2

Table 3.3-2. Aflatoxin production by various strains of A. flavus in sterile rice

Strains		Aflatoxins (μ g / g)			
		B_1	B_2	G_1	G_2
E3	a	—	—	—	—
	b	—	—	—	—
E11	a	—	—	0.32	6.56
	b	—	—	2.18	—
E14	a	0.48	—	—	—
	b	0.77	—	0.90	—
E15	a	—	—	—	7.60
	b	—	—	—	66.5
56915	a	—	—	—	—
	b	—	—	—	—
M13	a	—	—	—	—
	b	—	—	—	—

a and b represent the duplicate samples.

Table 3.3-3. Aflatoxins content in EFB samples (Awang et al., 1993)

	Concentration of aflatoxins (μ g / kg)			
	B_1	B_2	G_1	G_2
Stored EFB	2.3	1.9	13.6	74.5
Irradiated EFB	0.9	1.9	26.0	69.1
Fermented EFB	2.2	1.8	17.8	57.9

Values are means of 4 replicates.

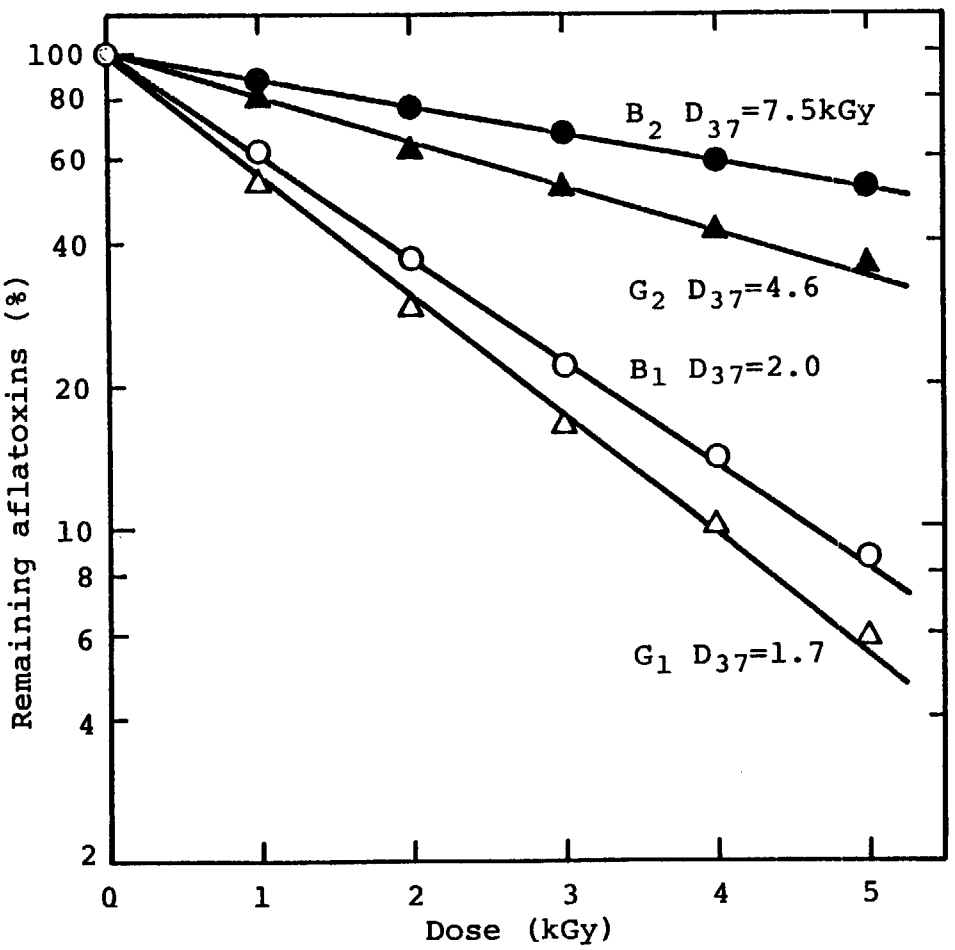

Fig. 3.3-1. Destruction of aflatoxins in solution by irradiation
Aflatoxins were dissolved in 1/15M phosphate buffer, pH 7.0 (10 μg/ml) and irradiated at room temperature.

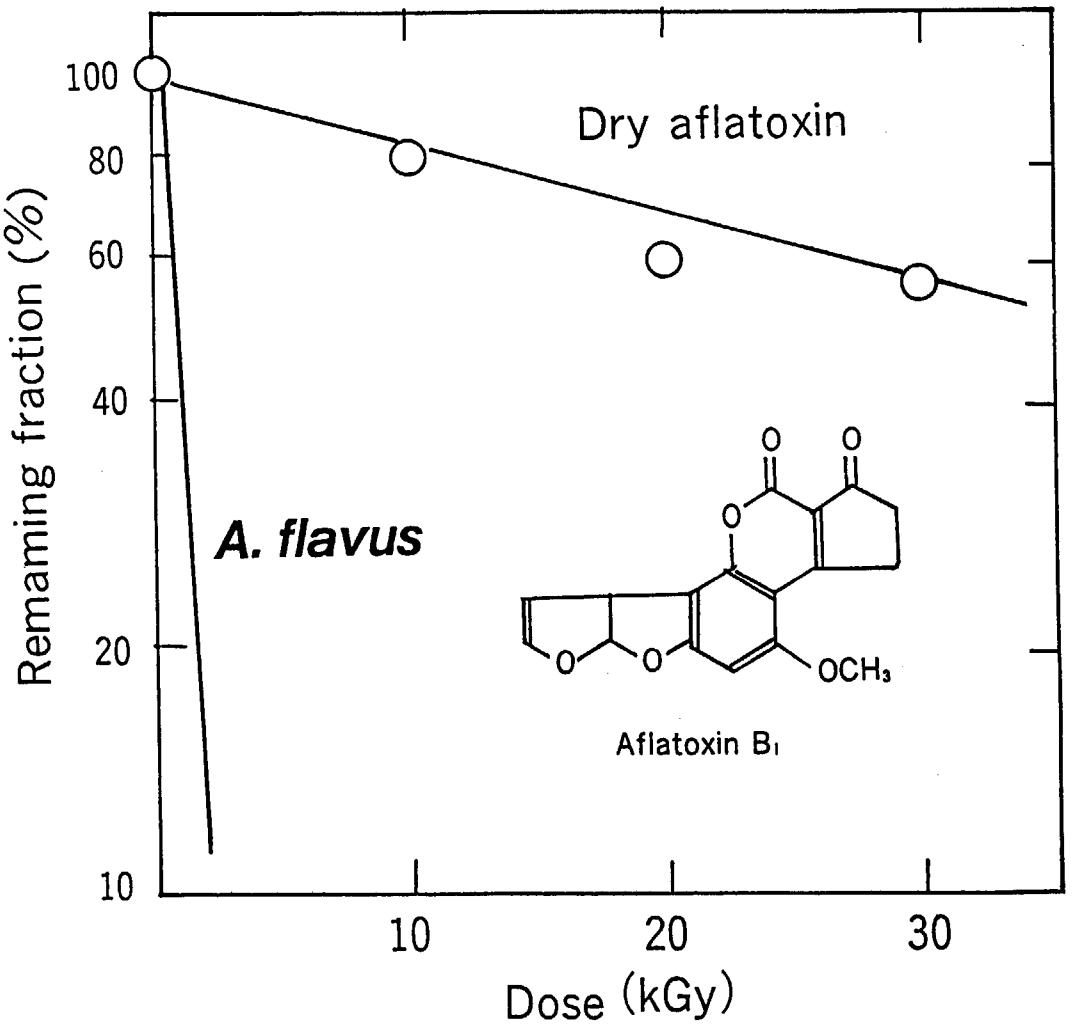

Fig. 3.3-2. Radiation destruction of aflatoxin B_1 in dry condition and radio-sensitivity of *A. flavus*

3.4 Enzymatic Digestion of EFB

3.4.1 Introduction

Wood-rotting fungi, some of them are mushrooms, can digest lignocellulosic substrates by enzymes such as cellulase, hemicellulase and pectinase (Kerem et al 1992, Nicolini et al 1993, Buswell et al 1994). On the other hand, biological degradation of lignocellulose takes long period. It needs an effective pre-treatment to substrate for shortening the period of biological digestion. The results in chapter 3.2 showed that more than 15 kGy irradiation was necessary to sterilize bacteria in EFB and each component of EFB did not change markedly up to 50 kGy. While it is not clear the effect of irradiation at sterilization dose after alkali treatment on enzymatic digestibility of cellulosic waste. In this chapter, the effect of irradiation and combination treatment with alkali was studied to increase the efficiency of enzymatic digestibility of EFB as substrate for fungal fermentation.

3.4.2 Materials and methods

(1) Preparation of EFB sample

EFB collected in Malaysia was dried and cut twice with a straw cutter VL-56 (Marumasu Co Ltd, Tokyo, Japan) and then chopped by Willey Mills WSX-200 (Kiya Ltd, Tokyo, Japan). Length of the sample was less than 5 mm after treatment. For the alkali treatment, the sample was dipped in 1% NaOH solution for 1 h, then neutralized and dried at room temperature.

(2) γ -irradiation

EFB sample was irradiated at room temperature using gamma-rays of cobalt-60 (4.66 PBq), dose rate of 5.0 kGy/h.

(3) Enzymatic digestion of EFB and PPF

Samples (0.2 g in 10 ml 0.5M acetate buffer, pH4.5) were irradiated at dose of 25 kGy and incubated with enzyme cellulase Onozuka 3S (50 mg) at 40℃ in shaking water bath. The free-reducing sugar content of the samples were measured by Somogyi-Nelson method.

(4) Degradation of EFB by Driselase

Commercial mushroom enzyme, Driselase (from *Polyporus tulipiferae*, Kyowa

Hakko Co Ltd, Tokyo, Japan), was dissolved to 50 mM acetate buffer, pH 5.0 (20 g/500 ml). The reaction mixtures with total volume of 5.0 ml containing 0.1 g of EFB, 0.5 ml of 1% glycerol (internal standard), 1.0 ml of Driselase solution, and 3.5 ml of 50 mM acetate buffer (pH 5.0), were incubated for various periods at 30℃. After each incubation period, EFB fiber, enzymes and high molecular weight materials remaining in the reaction mixture were removed with Millipore Molcut II GC (1×10^4 Da exclusion limit for globular protein).

(5) HPLC analysis of reducing and neutral sugars

Reducing sugar and neutral sugar were separated by Shodex SUGAR SH1821 column (ϕ 8×300 mm) that separation mechanism is ion elimination and gel filtration. Analytical condition of HPLC was: mobile phase; 0.001 N sulfuric acid, flow rate; 1.0 ml/min, column temperature; 40℃, detector; JASCO 830-RI reflect index detector (1.6×10^{-5} RIU at 40℃). The amount of galacturonic acid was calculated from the peak area ratio to glycerol added in the reaction mixture as internal standard.

(6) Identification of neutral sugars

Tandem columns of Shodex SUGAR SP0810 (ϕ 8×300 mm) were used for separation of neutral monosaccharides. Same condition of HPLC described above was used except the mobile phase of distilled water.

3.4.3 Results and discussion

(1) Effect of irradiation on the enzyme digestion of EFB and PPF

Four different samples, namely, ground-raw form, extractive-free form, holocellulose and alpha-cellulose were prepared and the digestion of EFB and PPF by various commercial enzymes was investigated (Table 3.4-1). The treatment of NaOH increased the digestion of EFB but the effect of alkaline treatment was not clear in PPF except some enzymes. Cellulase Onozuka 3S (*Trichoderma* enzyme) was the most effective for EFB while pectinase SS was effective for PPF. As Cellulase Onozuka 3S was also effective for PPF, this enzyme was used for the following study of enzyme digestion

Change in the digestion of ground samples and extractive-free samples are shown in Fig. 3.4-1 and 3.4-2, respectively. Only small differences were observed between digestion of unirradiated and irradiated ground, raw samples of EFB and PPF while irradiation appeared to have no effect on digestion of extractive-free samples. The results

obtained for holocellulose and alpha-cellulose prepared from EFB and PPF are shown in Fig. 3.4-3 and 3.4-4, respectively. The reducing sugar content of holocellulose and alpha-cellulose samples were much higher than ground and extractive-free samples, indicating that delignification enhanced the digestion of EFB and PPF by the enzyme. Irradiated holocellulose of PPF appeared to be slightly less digested while that of irradiated EFB appeared to be slightly more digested than unirradiated samples upon 2 days incubation. Irradiation have no effect on enzyme digestion of alpha-cellulose of EFB but digestion of PPF samples was slightly decreased. These results show that the irradiation of 25 kGy has almost no effect on enzyme digestion of any sample conditions of EFB and PPF. The higher digestion of holocellulose by the enzyme cellulase Onozuka 3S than that of alpha-cellulose suggests that the enzyme is effective for the digestion of hemi-cellulose.

(2) Monosaccharides released by Driselase digestion

Woody fiber generally contains neutral and reducing sugars. Figure 3.4-5 shows the HPLC chromatograms of Driselase digestion products from unirradiated and 50 kGy irradiated EFB. The peak of glycerol added prior to the digestion test as internal standard for HPLC and other peaks were observed in both chromatograms. The peaks 1 and 2 are presumed galacturonic acid and neutral sugar from the retention time, respectively (Matsuhashi et al 1992). Unirradiated EFB released small amount of galacturonic acid and neutral sugar, and the peak size of the neutral sugar was a little larger than that of galacturonic acid. Both peaks of galacturonic acid and neutral sugar were increased in 50 kGy irradiated EFB.

Figure 3.4-6 shows relationship between dose and the amount of galacturonic acid released from EFB. The amount of galacturonic acid was slightly increased from 150 ppm to 200 ppm by 25 kGy irradiation. Furthermore, galacturonic acid was remarkably increased at above 25 kGy and twice amount of that (ca. 400 ppm) was obtained at 50 kGy. This result shows that 25 kGy was not enough to increase the enzymatic digestibility of EFB and 50 kGy was required to improve the digestibility. The released amount of galacturonic acid during the incubation period was shown in Fig. 3.4-7. Without alkali treatment, the initial rates of galacturonic acid production were 20 ppm/day for unirradiated and 60 ppm/day for 50 kGy. The curve of unirradiated EFB was leveled off after 5 days. It means that the enzyme digestible galacturonan was consumed completely during 5 days incubation. Compare with this, the digestion speed of 50 kGy was a little decreased after 5 days, and the released amount of galacturonic acid was increased through 15 days incubation. After 15 days incubation,

the amounts of galacturonic acid were 155 ppm for unirradiated and 385 ppm for 50 kGy. The enzymatic digestibility of galacturonan was increased 2.5 times by irradiation. From this result, irradiation is effective not only to increase digestion products but also to increase digestion speed of galacturonan fraction in EFB. With alkali treatment, the slope of unirradiated EFB was 175 ppm galacturonic acid/day and leveled off after 5 days. The released galacturonic acid was 550 ppm after 5-15 days. The initial rate for 50 kGy was almost the same for unirradiated, but after 2 days incubation, the amount of galacturonic acid was remarkably decreased. It is presumed that released galacturonic acid was adsorbed to EFB again or made complexes with another materials released from EFB. Further work is required to clarify the mechanism. The initial rate of the enzymatic digestion of galacturonan was increased 3 times by 50 kGy irradiation and 9 times by alkali treatment. However, the combination treatment of alkali and irradiation did not enhance the initial rate comparing with alkali treatment. The result show that the alkali treatment is quite effective to increase the enzymatic digestibility of galacturonan fraction of EFB and initial rate of digestion.

(3) Neutral sugar released by Driselase digestion

The amount of neutral sugar was also calculated as the peak area ratio of neutral sugar to glycerol from HPLC chromatograms. Released neutral sugar from irradiated EFB by Driselase was shown in Fig. 3.4-8. The amount of released neutral sugar was not increased up to 25 kGy while it was slightly increased by 50 kGy. Figure 3.4-9 shows the released amount of neutral sugar from EFB with and without alkali treatment during 15 days incubation. The released amount of galacturonic acid from unirradiated EFB was leveled off after 5 days incubation (Fig. 3.4-7), whereas the released amount of neutral sugar was still increased even after 15 days incubation. The enzymatic digestion speed was decreased at the longer incubation time. Without alkali treatment released amounts of neutral sugar after 15 days incubation were 850 ppm for unirradiated and 1155 ppm for 50 kGy. Irradiation on EFB did not increase the enzymatic digestibility of neutral sugar fraction comparing with the galacturonan. The digestibility of neutral sugar fraction increased only 1.4 times, while the enzymatic digestibility of galacturonan was 2.5 times increased by irradiation (Fig. 3.4-7). With alkali treatment, enzymatic digestibility of neutral sugar fraction was further increased and released amount of neutral sugar after 15 days incubation were 1900 ppm for unirradiated and 1750 ppm for 50 kGy. These results show that, enzymatic digestibility of EFB was slightly increased by irradiation. While the alkali treatment

increased the enzymatic digestibility of EFB advantageously. However, it seems that the combination treatment of alkali and irradiation on EFB does not enhance the released amount of neutral sugar comparing with alkali treated EFB.

(4) Components of neutral sugar

Neutral sugars from digested EFB were separated by Shodex SUGAR SP0810 column (Fig. 3.4-10). Neutral monosaccharides of glucose, xylose, arabinose and mannose were able to separate each other, but galactose and rhamnose were difficult to separate because of same retention time. Without alkali treatment, glucose, xylose and trace of galactose and/or rhamnose, arabinose and mannose were released from unirradiated EFB. The main components of neutral sugars were glucose and xylose. Figure 3.4-11 shows the released amount of glucose and xylose from EFB digested by Driselase at various conditions. Without alkali treatment, released amount of glucose (765 ppm) was increased 1.2 times and 878 ppm glucose was obtained at 50 kGy. While the amount of xylose (85 ppm) was significantly increased to 277 ppm by 50 kGy irradiation. In xylose, the increase was 2.7 times. Xylose from unirradiated EFB comprises only 10% of total neutral sugar. After irradiation, xylose comprises 24% of total neutral sugar. This result shows that irradiation is mainly effective to increase the enzymatic digestibility of xylan rather than glucose. With alkali treatment, the amount of glucose (1159 ppm) was decreased to almost half (587 ppm). However, the amount of xylose (741 ppm) increased 1.6 times and 1173 ppm was obtained. Furthermore xylose from irradiated EFB after alkali treatment was 14 times that of unirradiated EFB without alkali treatment. Xylose from alkali treated EFB (unirradiated) comprises 39% of released total neutral sugar. Comparing with this, xylose from irradiated EFB after alkali treatment comprises 67% of the total neutral sugar released. Irradiation or alkali treatment on EFB enhanced the release of xylose, and the amount of xylose from irradiated EFB after alkali treatment was close to the sum of their values for irradiated EFB and alkali treated EFB separately. These results show that the combination treatment of alkali and irradiation did not increase the amount of neutral sugar but enhance the release of xylose. Morrison (1988) and Barlet al (1991) show that alkali treatment on cellulosic material is effective to remove lignin. The results obtained in this experiment show that the combination treatment of alkali and 50 kGy irradiation on EFB is effective not only remove lignin but also increase the enzymatic digestibility of xylan. It is, therefore, expected that the combination treatment is able to enhance enzymatic digestibility of EFB.

3.4.4 Conclusion

Effect of γ -irradiation and combination treatment with alkali on empty fruit bunch (EFB) was studied to enhance the efficiency of enzymatic digestibility. γ -Irradiation was effective to enhance the enzymatic digestibility, and the amount of released galacturonic acid and neutral sugars from EFB by Driselase were increased with the increase in irradiation dose. The main components of neutral sugars released from EFB were glucose and xylose. EFB treated with alkali and 50 kGy irradiation released 1200 ppm of xylose that was twice amount of glucose showing that the combination treatment was effective especially to increase the digestibility of xylan.

Table 3.4-1. Digestion of EFB and PPF by Various Commercial Enzymes

	Glucose (mg/g)			
	EFB		PPF	
	(-)	NaOH*	(-)	NaOH*
Macerozyme 2S	27	34	37	25
Pectinase SS	60	116	112	101
Pectinase 3S	20	108	24	97
Pectinase HL	5	78	89	75
Cellulase Onozuka 3S	85	195	81	121
Cellulase Y-NC	25	117	98	99

* Treated with 10% NaOH for 1 day.
0.5g samples were incubated with 50mg enzyme in 10 ml of 0.05M acetate buffer (pH 4.5) at 40°C for 2 days.

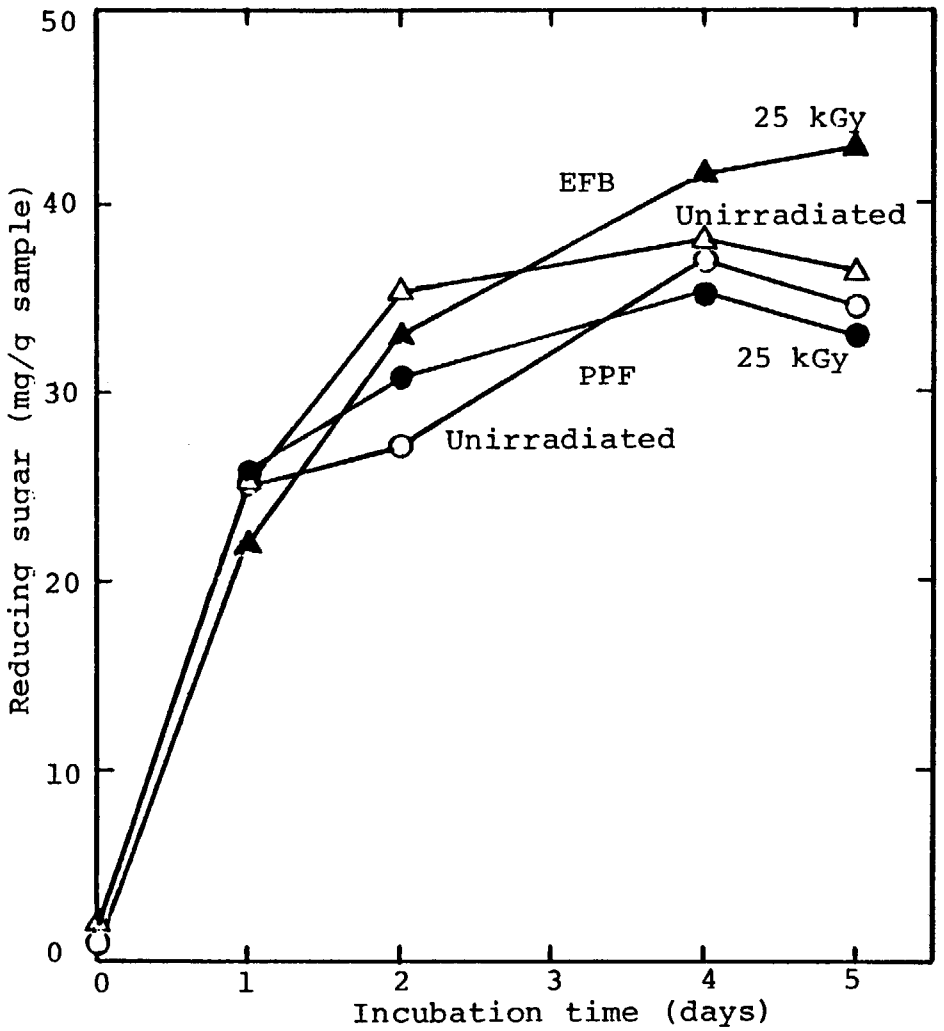

Fig. 3.4-1. Effect of irradiation on digestion of ground samples of EFB and PPF by cellulase

Samples (0.2g in 10ml acetate buffer, pH 4.5) were incubated with cellulase Onozuka 3S (50mg) at 40℃ in shaking water-bath.

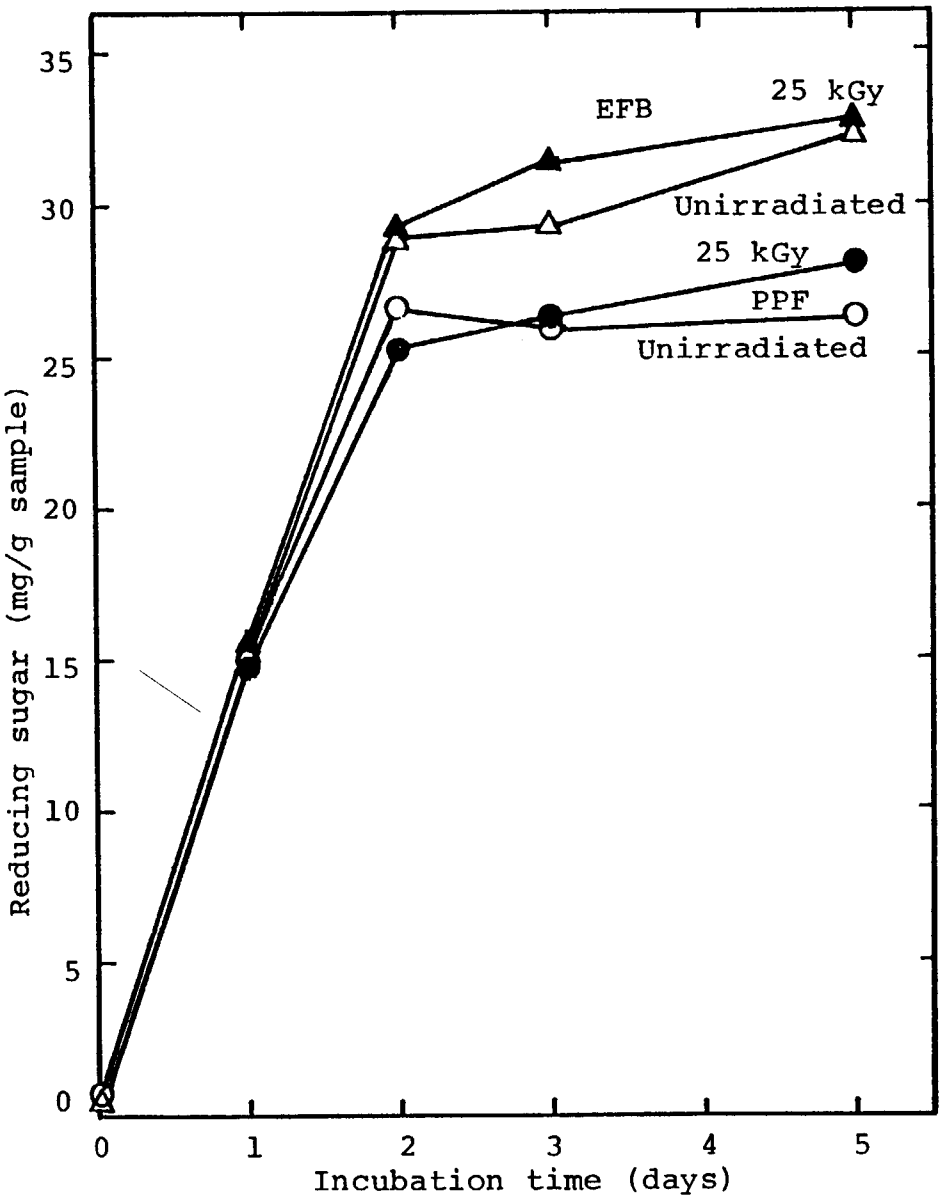

Fig. 3.4-2. Effect of irradiation on digestion of extractive-free samples of EFB and PPF by cellulase

Samples (0.2g in 10ml 0.5M Acetate buffer, pH4.5) were incubated with cellulase Onozuka 3S (50mg) at 40°C in shaking water-bath

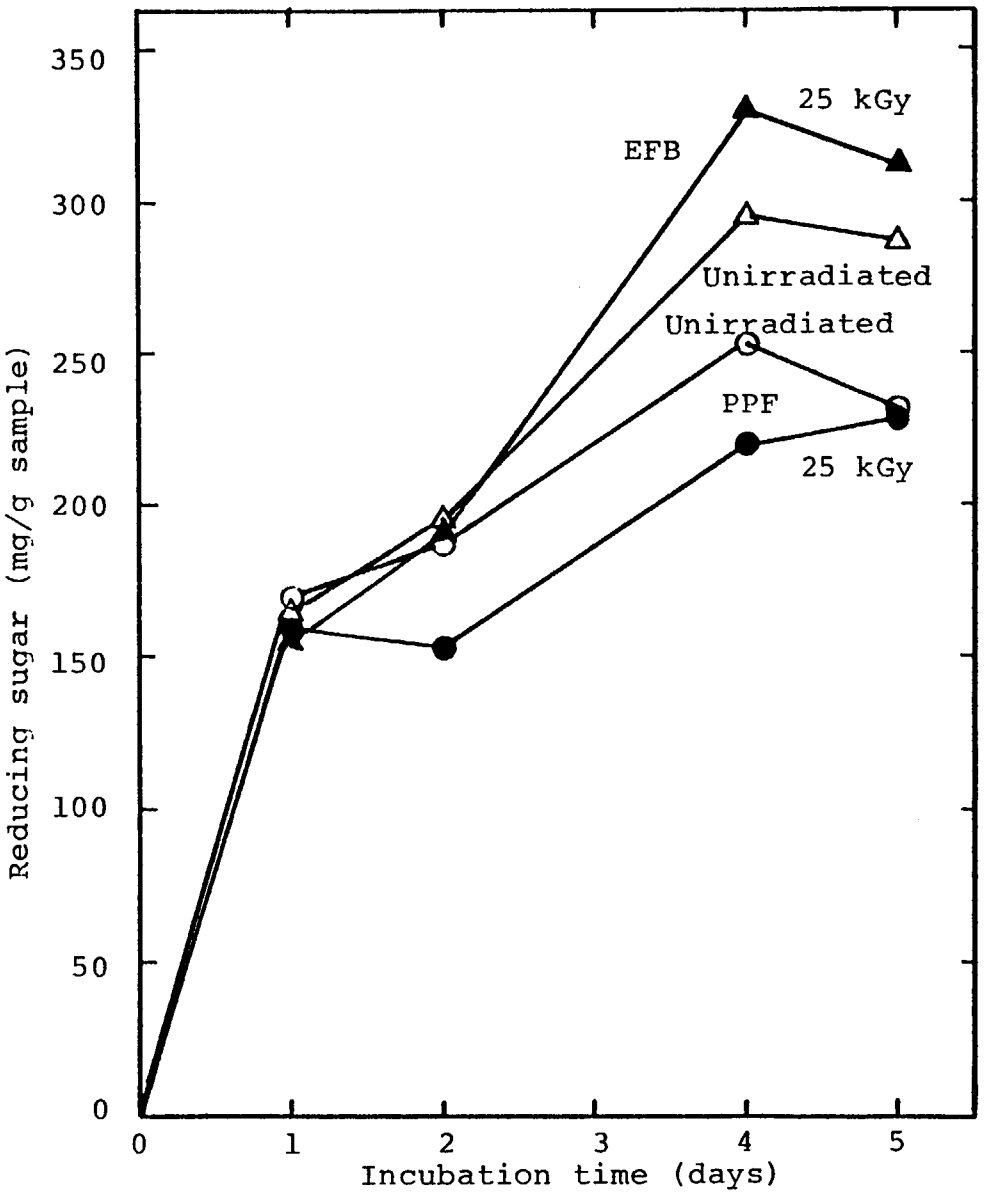

Fig. 3.4-3. Effect of irradiation on digestion of holocellulose of EFB and PPF by cellulase

Samples (0.2g in 10ml 0.5M Acetate buffer, pH4.5) were incubated with cellulase Onozuka 3S (50mg) at 40°C in shaking water-bath.

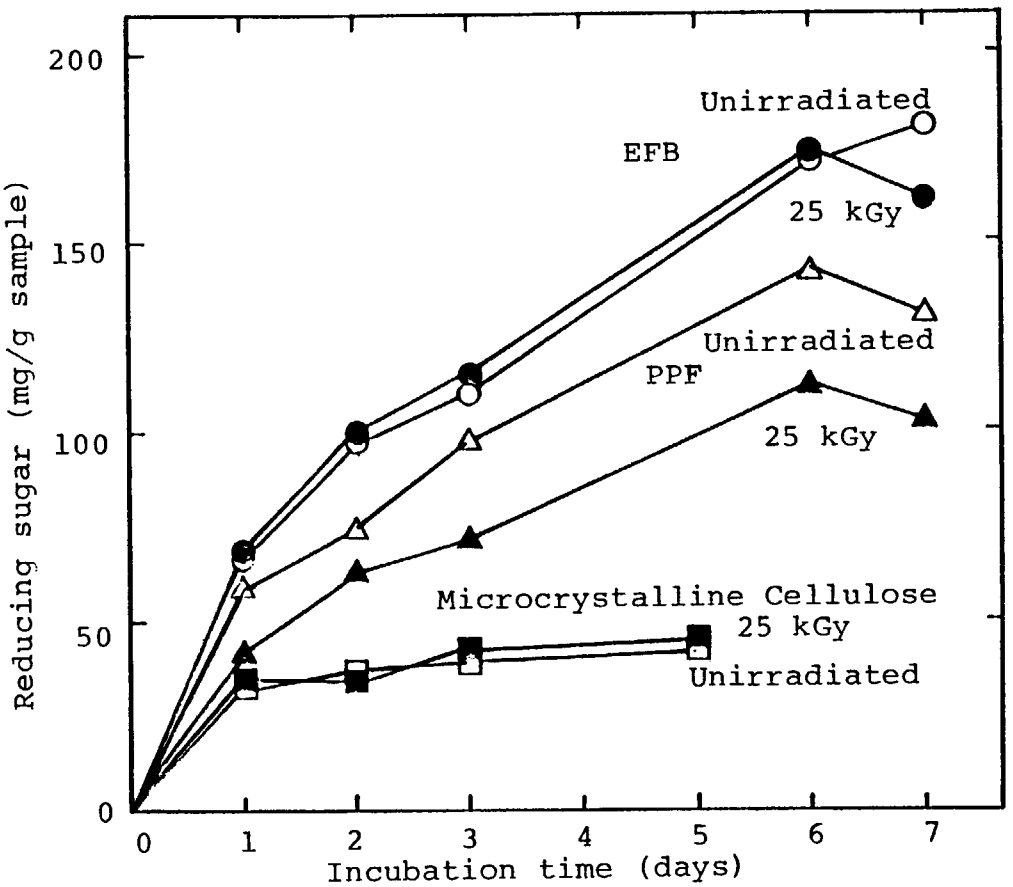

Fig. 3.4-4. Effect of irradiation on digestion of α-cellulose of EFB and PPF by cellulase

Samples (0.2g in 10ml 0.5M Acetate buffer, pH4.5) were incubated with cellulose Onozuka 3S (50mg) at 40°C in shaking water-bath.

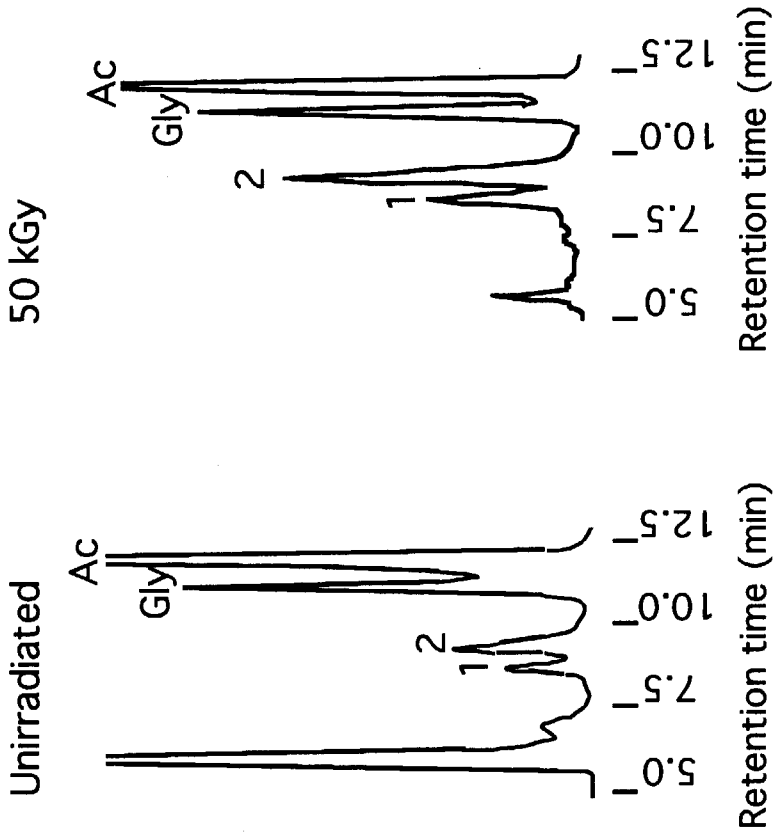

Fig. 3.4-5. HPLC chromatograms of degradation products released from EFB by Driselase

Reaction mixture was incubated at 30° C for 15 days. Peak identify: 1, galacturonic acid; 2, neutral sugar; Gly, glycerol; Ac, acetate.

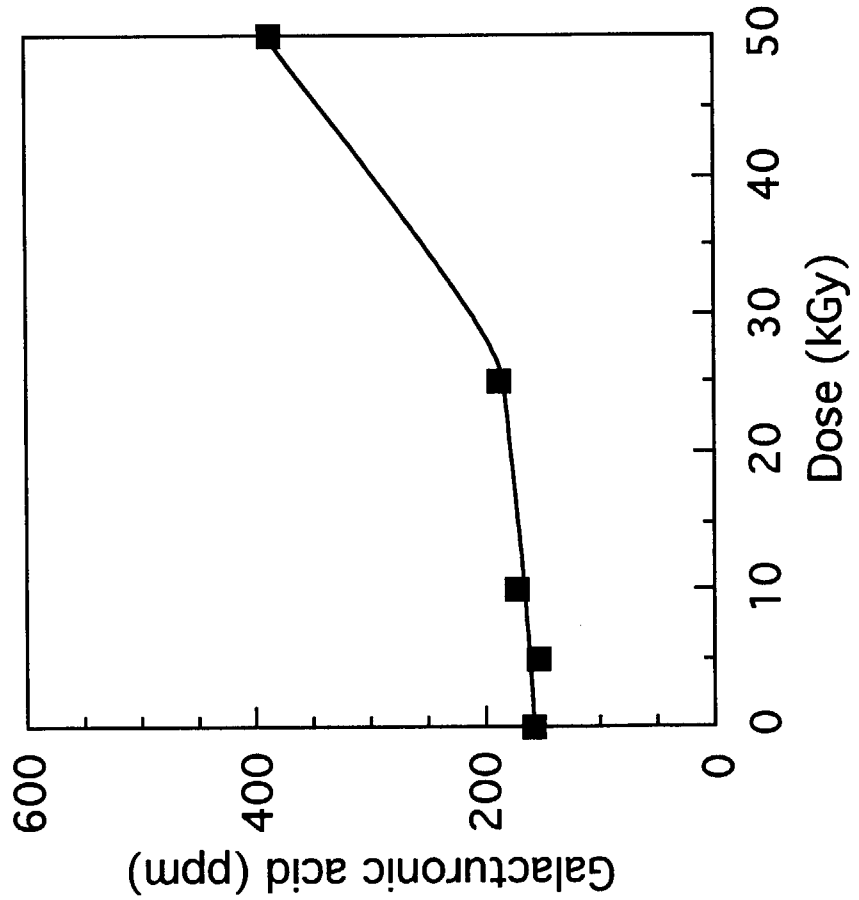

Fig. 3.4-6. Release of galacturonic acid from irradiated EFB by Driselase
The amount of galacturonic acid was calculated from peak area of HPLC chromatograms (incubation period: 15 days).

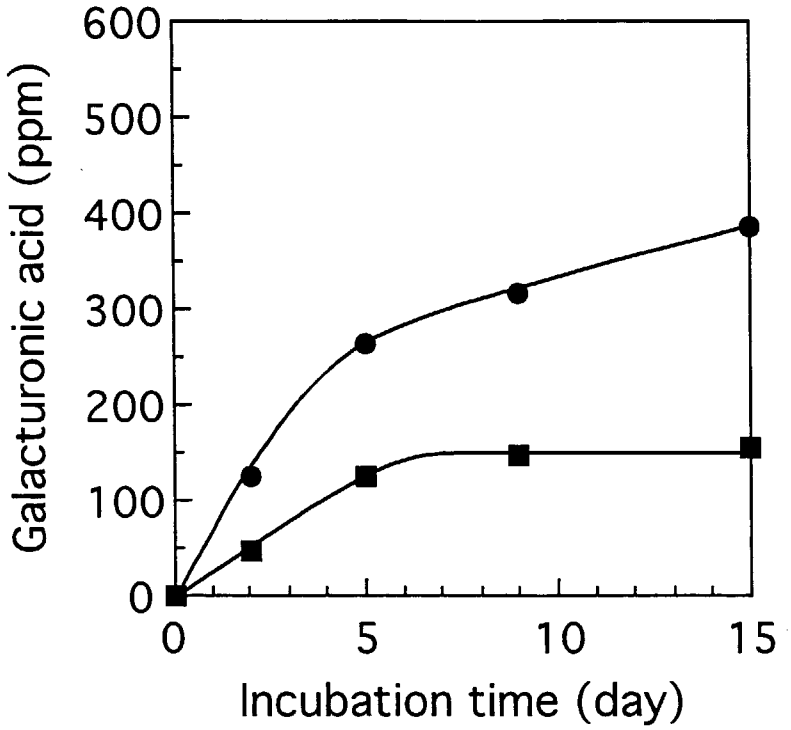

Fig. 3.4-7. Galacturonic acid released from EFB
Symbols: ■, unirradiated; ●, 50 kGy (Matsuhashi et al., 1995).

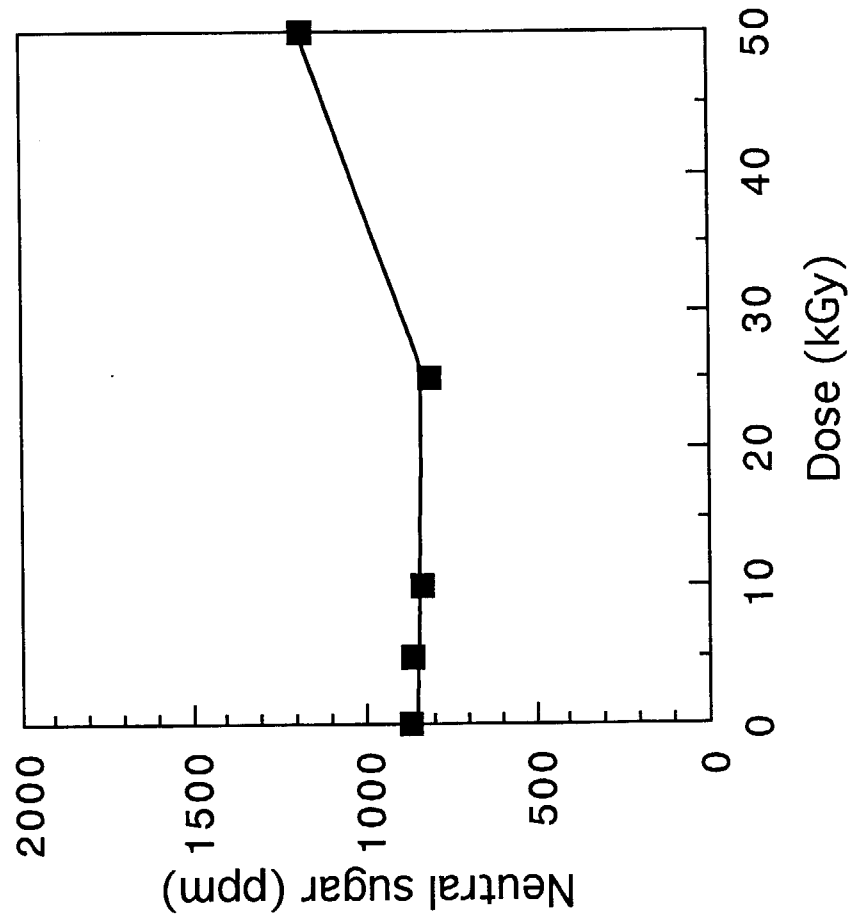

Fig. 3.4-8. Dose effect on enzymatic digestibility of neutral sugar fraction of EFB
The amount of released neutral sugar was calculated from peak area of HPLC chromatograms (incubation period: 15 days).

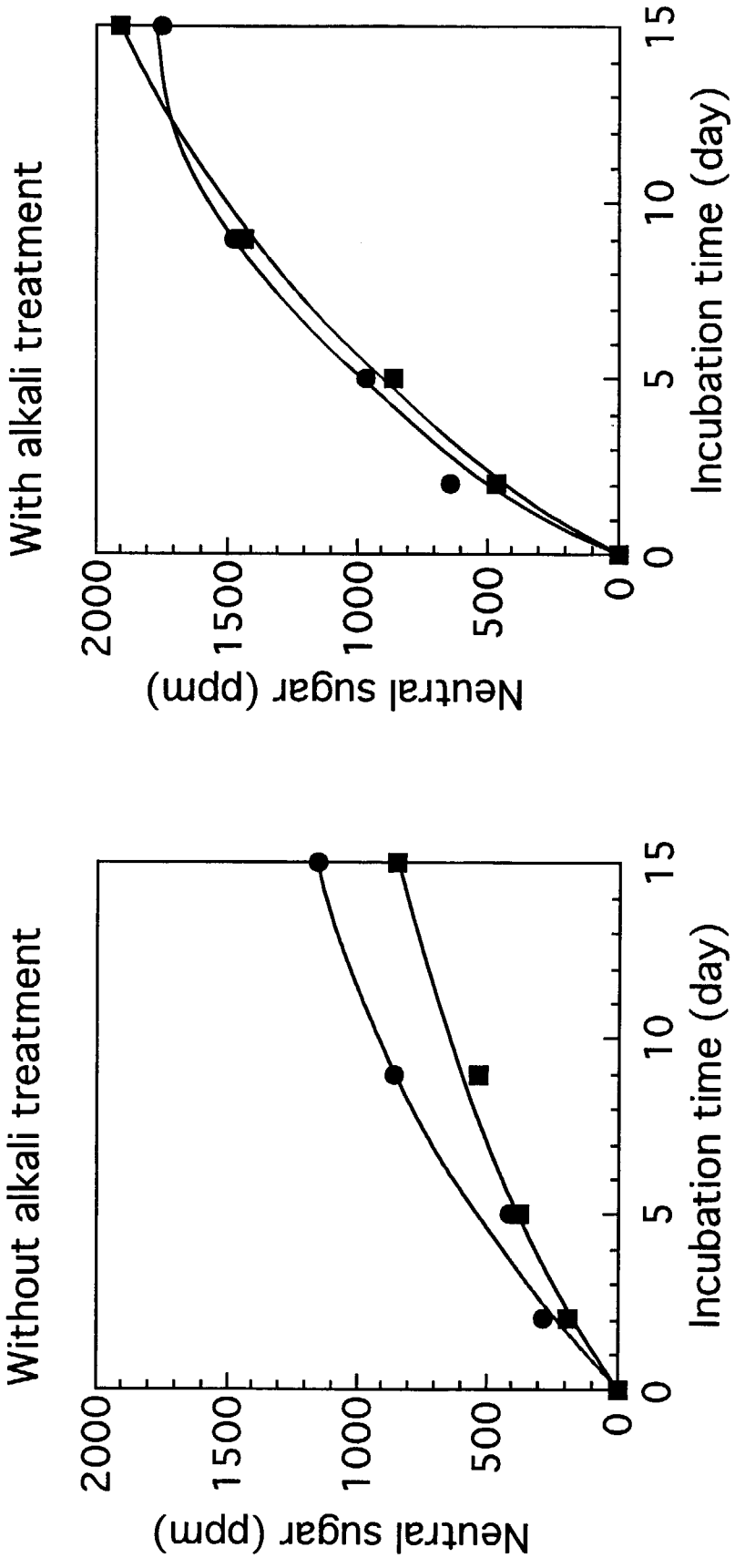

Fig. 3.4-9. Neutral sugar released from EFB with and without alkali treatment
Symbols: ■, unirradiated; ●, 50 kGy (Matsuhashi et al., 1995).

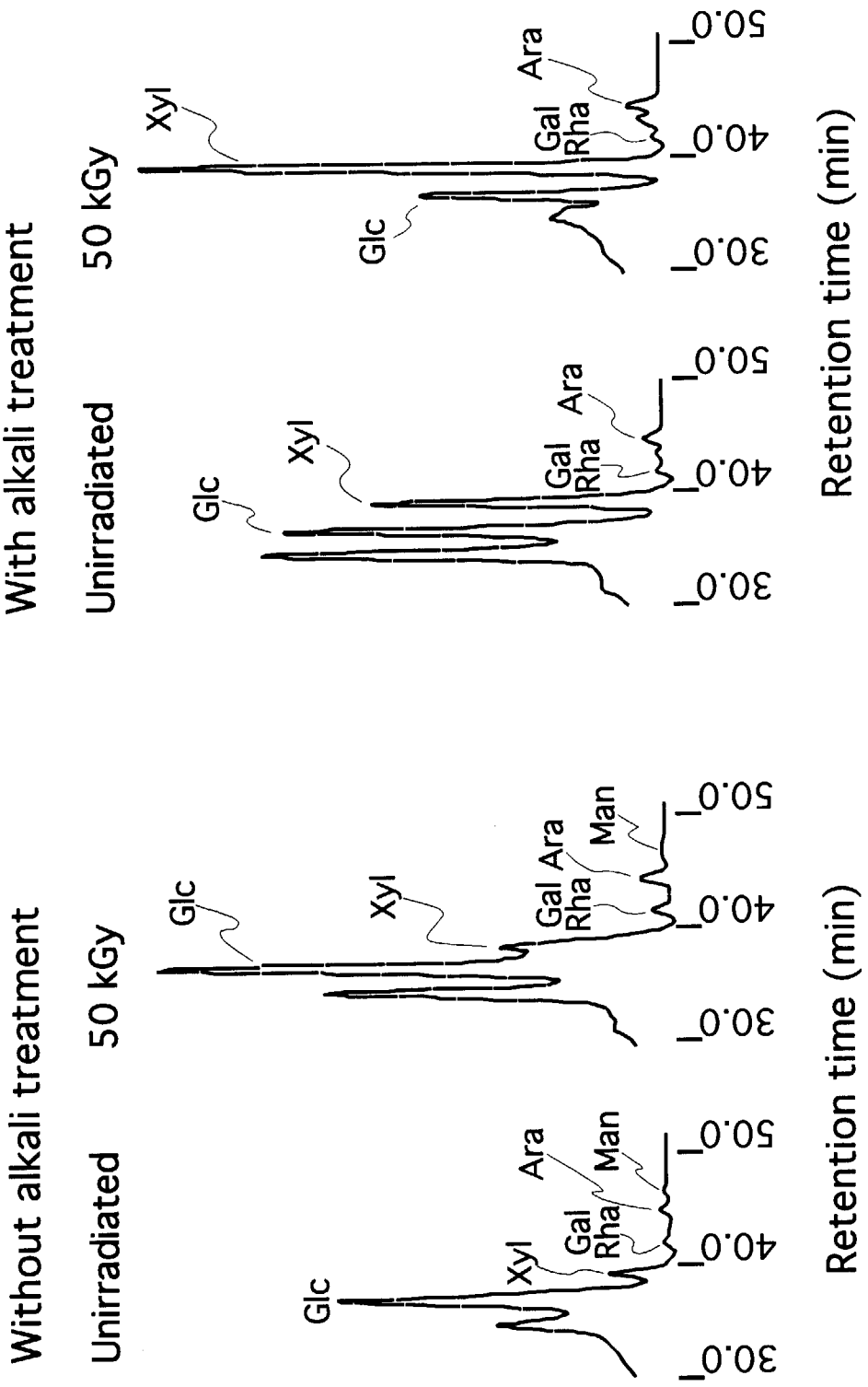

Fig. 3.4-10. HPLC chromatograms of neutral sugar released from EFB by Driselase Reaction mixture was incubated at 30° C for 15 days. Peak identify: Glc, glucose; Xyl, xylose; Gal, galactose; Rha, rhamnose; Ara, arabinose; Man, mannose.

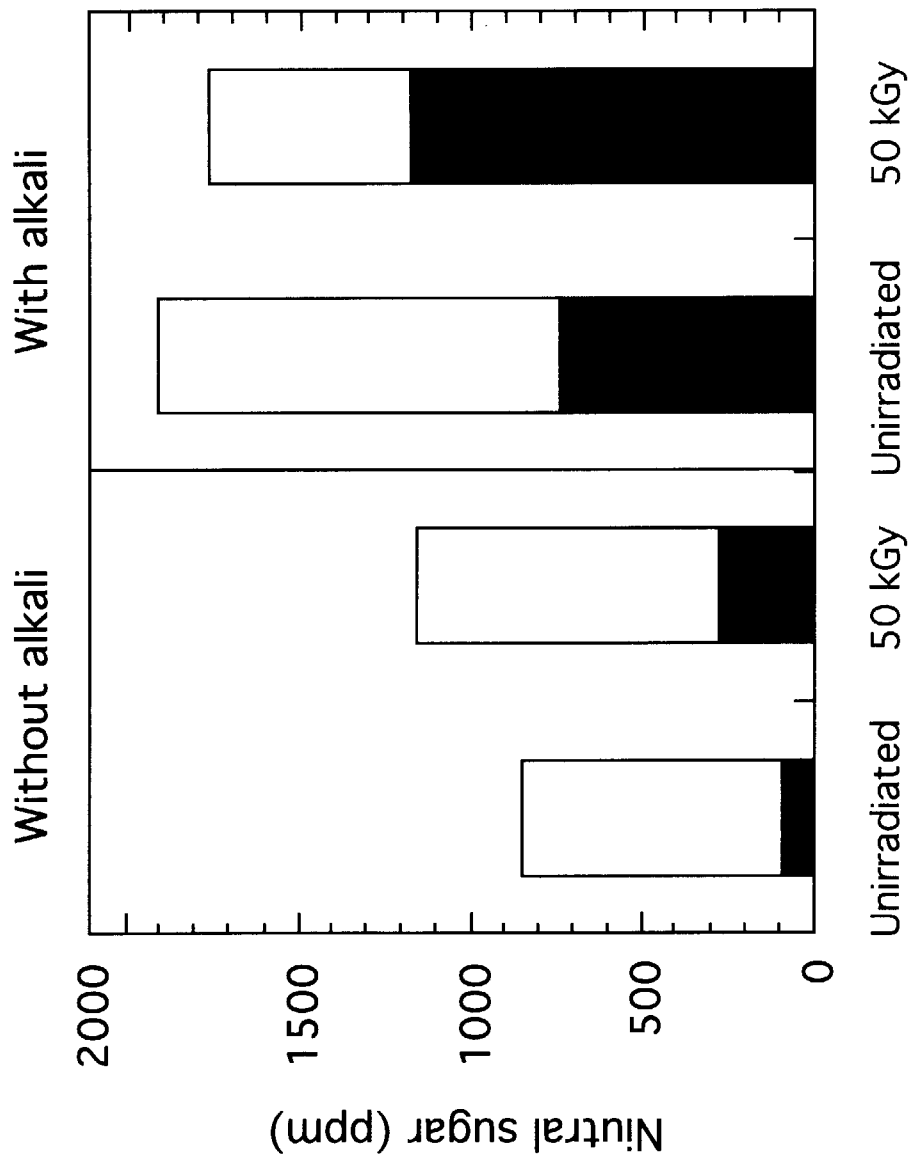

Fig. 3.4-11. Xylose and glucose released from EFB

Symbols: □, glucose; ■, xylose (Matsuhashi et al., 1995).

4. ESTABLISHMENT OF FERMENTATION CONDITIONS FOR EFB

4.1 Selection of Useful Microorganisms

4.1.1 Introduction

Oil palm empty fruit bunch contains 22 - 27% of lignin and therefore it cannot be used for animal feed directly. To decrease the lignin content and increase the digestibility, the selection of useful microorganisms suitable for the fermentation of EFB to the ruminant feed is important. In this chapter, various kinds of mushroom fungi and other microorganisms were tested for the fermentation of EFB to select the effective fungi for the production of animal feeds from EFB by radiation and fermentation treatment.

4.1.2 Materials and methods

(1) Organisms

Pleurotus djamor (Fr. : Fr.) Boedijin *(=Pleurotus flavellatus), Pleurotus sajor-caju* (Fr. : Fr.) Fr., *Pleurotus cystidiosas* and *Trichoderma koningi* were obtained from Agricultural University of Malaysia. *Coprinus cinereus* (Schaeff. Fr.) S. F. Gray was obtained from Prof. A. Suzuki, Chiba University in Japan. *Verticillium* sp. S1 was isolated from rotted sawdust sample (Ito et al 1986). Strains of *T. viride* IFO 31137, *Aspergillus niger* IAM 202 were also used in this study.

(2) Culture media

EFB samples were collected from Sri Ulu Langat Palm Oil Mill, Kajang, Malaysia. EFB was shredded fibrous form and soaked overnight in tap water and adjusted the pH by HCl solution to 4.0. The solid-state culture media were prepared with addition of nutrient solution containing KH_2PO_4, $(NH_4)_2SO_4$, $NaNO_3$, urea and other salts. Dry EFB of 3 - 6 g was put into 200 ml conical flasks with addition of 40 - 100 ml nutrient solution, and pH adjusted to 5.0 before radiation pasteurization. Various fungi were inoculated onto the solid state culture media in the flask with cotton plug and incubated under 85% relative humidity at 30℃ for 18 to 30 days.

In case of the fermentation in a plastic bag, inorganic nutrients or rice bran was added to the EFB substrate and adjusted the moisture content to *ca.* 60%.

(3) Analysis of crude fiber and protein

The product was freeze-dried before analysis. Crude fiber was determined by AOAC method (Horwitz 1980) by subjecting the sample in boiling 1.25% H_2SO_4 and 1.25% NaOH. Crude protein content was determined by Kjeldahl sulfuric acid digestion method of AOAC using Buchi 425 digester and Buchi 320 Nitrogen distillation unit.

4.1.3 Results and discussion

(1) Change in pH of EFB and PPF suspensions

One of the important factor for fermentation conditions is pH of the substrates. It is reported that EFB ash is extremely basic and contains 41% K_2, 4% P_2O_5, 6% MgO and 5% CaO on dry weight (Muhamad et al 1985). Therefore, the pH change in the media prepared with EFB and PPF was tested (Table 4.1-1). The pH of EFB was increased significantly during storage while the pH of PPF was increased a little. The pH of EFB samples were slightly increased by irradiation or autoclave treatment. Figure 4.1-1 shows the pH change in culture media sterilized by radiation with 25kGy or autoclave (121℃, 15min). These results suggest that the extraction of minerals in EFB increased by the treatments but the increase in pH of irradiated samples were still continued to increase. It is, therefore, considered that the pH of EFB media should be adjusted to 4 - 5 before sterilization treatment and wait for 1 day for inoculation. One procedure to avoid this pH increase in media is to wash the samples with HCl solution prior to preparation of media but the problem of this procedure is the production of other wastes.

(2) Fermentation of EFB by various fungi

EFB media in conical flask irradiated at 10 kGy were inoculated with various fungi such as *C. cinereus*, *Vertisillium* sp., *A. niger*, *T. viride*, *T. koningi*, *Sporotricum* sp, *P. flavellatus*, *P. cystidiosus* and *P. sajor-caju*. The changes in crude fiber and protein contents after fermentation by these fungi are shown in Table 4.1-2.

EFB media irradiated at 10 kGy were inoculated with various fungi such as *C. cinereus*, *Vertisillium* sp., *A. niger*, *T. viride*, *T. koningi*, *P. cystidiosus* and *P. sajor-caju*. Figure 4.1-2 shows the changes in crude fiber and protein contents after fermentation by these fungi. Many fungi used in this experiment digested the fiber and increased the protein content. Among the edible mushroom strains showing good results of fermentation, *C. cinereus* and *P. sajor-caju* were selected as the most suitable

microorganism for the fermentation of EFB. The protein content increased to 13% and the crude fiber content decreased to 20% after 30 days of incubation with *C. cinereus* at 30℃ in solid state fermentation. The amino acid compositions of fermented products was equivalent to that of usual animal feeds except the low content of methionine (Table 4.1-3). It is expected that these fermented products were suitable as feeds for the ruminant animals.

4.1.4 Conclusion

Various kinds of fungi can and decrease the crude fiber contents and increase the protein content of EFB. Among these fungi degraded EFB effectively, *C. cinereus* and *P. sajor-caju* were selected as the most useful fungi for the fermentation of EFB because they are edible mushroom. Usually to use as animal feeds, it is required to test the safety of fungi but the edible mushroom can be used for animal feeds without such kind of safety tests.

Table 4.1-1. Change in pH of Media Prepared with EFB or PPF

		pH			
		Before treatment	After treatment		
			6hr	1day	7days
EFB	Control	3.61	4.59	5.00	6.12
	5 kGy	3.52	4.56	5.02	5.59
	10 kGy	3.90	5.19	5.65	6.24
	25 kGy	4.24	5.31	5.71	6.08
	50 kGy	3.13	5.04	5.51	5.86
	121˚C, 15min	3.82	6.30	6.34	6.73
PPF	Control	3.45	3.62	3.64	-
	5 kGy	3.57	3.77	3.79	3.98
	10 kGy	3.58	3.87	3.92	4.01
	25 kGy	3.53	4.02	4.18	4.20
	50 kGy	3.48	4.27	4.49	4.50
	121˚C, 15min	3.45	3.62	3.64	-

1.5g sample was suspended in 20ml media and kept at room temperature.

Table 4.1-2(1). Fermentation of EFB with various fungi

Fungi	Exp. 1*	Exp. 2*	
	Crude fiber (%)	Crude fiber (%)	Crude protein (%)
Contents in EFB	49.7	50.4	2.6
Coprinus cinereus	37.7	20.1	13.1
Verticillium sp. S1	38.8		
Aspergillus niger	42.9	44.8	7.8
Trichoderma koningi	40.8		
T. viride	37.1	36.4	10.9
Pleurotus cystidiosas	42.8		
Pleurotus sajor-caju	37.5		
Pleurotus flavellatus		45.0	4.3

Table 4.1-2(2). Fermentation of EFB in plastic bag (400g EFB)with various fungi

Fungi	Crude fiber (%)	Crude protein (%)
Coprinus cinereus		
Fresh EFB	31.6	11.1
Stored EFB	33.6	13.9
Pleurotus sajor-caju		
Fresh EFB	41.9	12.3
Stored EFB	34.6	5.6
Pleurotus cystidiosas		
Stored EFB	26.6	16.1
Auricularia politrica		
Stored EFB	26.0	16.1

Table 4.1-2(3). Chemical components of EFB fermented with various fungi

Fungi	Chemical components (%)				
	Crude fiber	Crude protein	Crude lipid	Ash	Lignin
Coprinus cinereus	20.7	8.8	1.0	3.1	21.3
Pleurotus flavellatus	51.4	2.9	0.6	0.5	17.7
Sporotricum sp.	34.9	5.8	0.5	1.3	19.9

Table 4.1-3. Amino Acid Composition of EFB Fermented Product using Various Fungi

Amino acids	Contents (g/100g EFB fermented product)		
	C. cinereous	P. flavellatus	Sporotrichum sp.
Aspartic acid	1.25	0.61	0.87
Threonine	0.70	0.29	0.54
Serine	0.61	0.32	0.50
Glutamic acid	1.38	0.81	1.26
Glycine	0.76	0.32	0.52
Alanine	0.71	0.37	0.53
Valine	0.62	0.28	0.38
Methionine	0.12	0.04	0.08
Isoleucine	0.66	0.30	0.43
Leucine	1.43	0.61	1.02
Tyrosine	0.32	0.14	0.16
Phenylalanine	0.59	0.32	0.31
Lysine	0.61	0.35	0.44
Histidine	0.35	0.13	0.19
Arginine	0.57	0.45	0.39
Proline	0.94	0.28	0.48

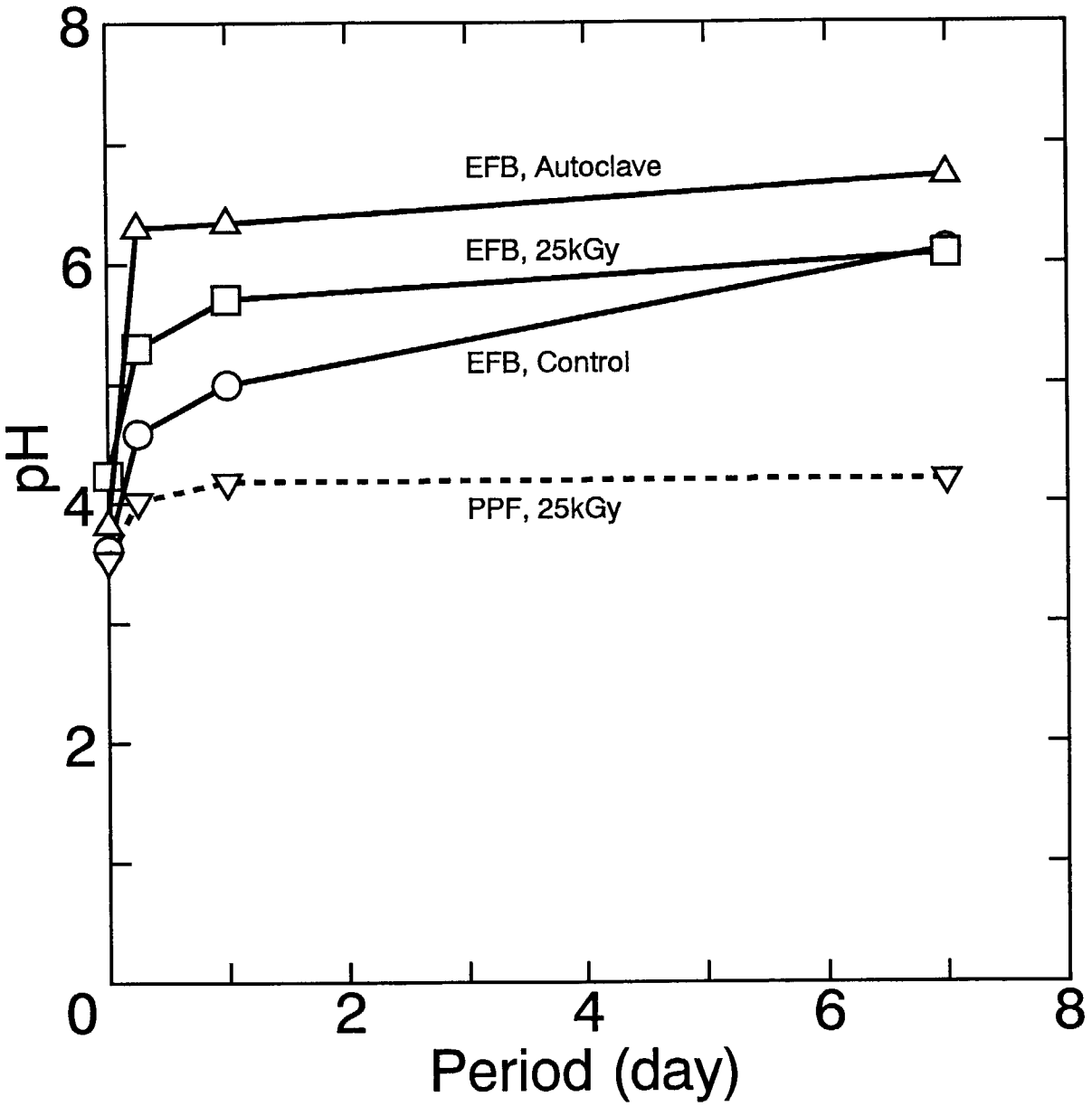

Fig. 4.1-1. Change in pH of media with EFB and PPF

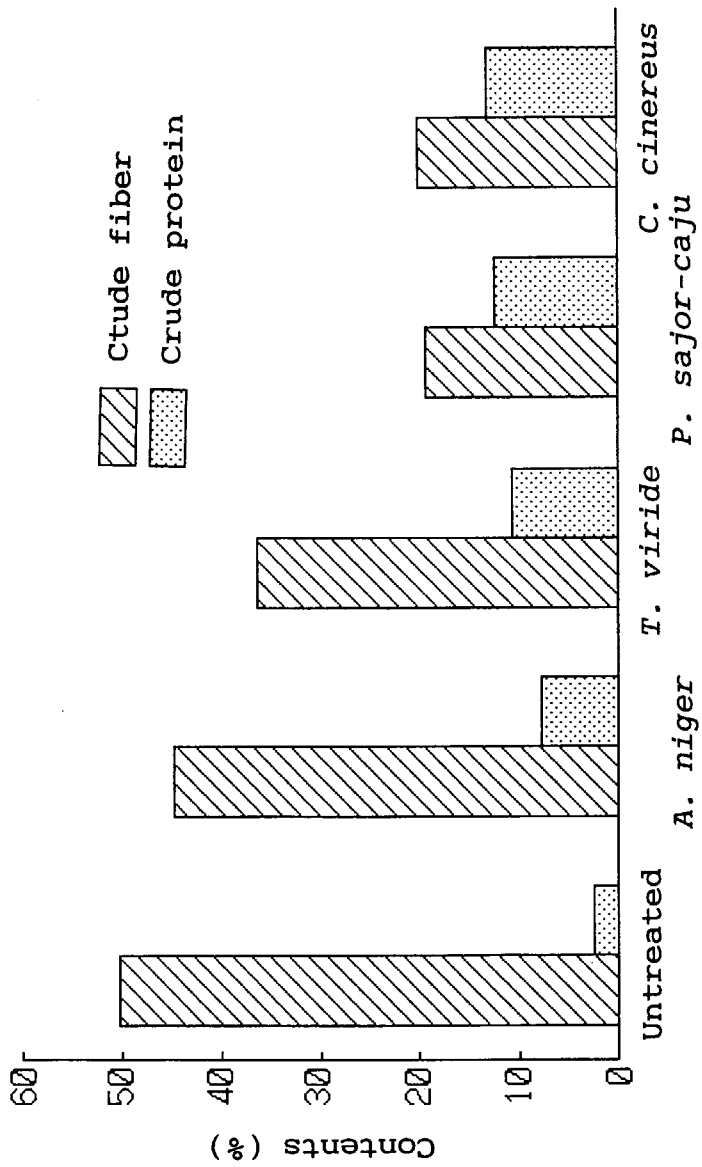

Fig. 4.1-2. Components of EFB after fermentation by various fungi (Kume et al., 1993)

4.2 Optimum Condition for Useful Fungi

4.2.1 Introduction

Coprinus cinereus and *P. sajor-caju* have been selected as the most suitable strains for the fermentation of EFB. The easier and effective procedures for fermentation are required for the production of a large amount of animal feeds. Therefore, the optimum growth condition for *C. cinereus* and *P. sajor-caju* was investigated for the base of scale-up fermentation.

4.2.2 Materials and methods

(1) Growth rate

About 20g of EFB substrate (moisture content: ca. 60%) with or without rice bran was packed into a test tube (ϕ 30 x 250mm). The substrate was sterilized by 30 kGy irradiation or autoclaving. Various fungi were inoculated and the growth was measured during incubation period at 30℃.

(2) Determination of optimum temperature and pH for fermentation

EFB (2-3 cm) was soaked in water for 2 days, drained the water and dried. The dried EFB was mixed with 3% rice bran and the moisture content was adjusted to 60%. The EFB substrate was irradiated at 30 kGy and fermented using solid state mini-fermentor (Photo. 4.2-1) at various temperature. The aeration rate was kept at 100 ml/min and the concentration of carbon dioxide was monitored during fermentation. The CO_2 concentration in exhaust gas from fermentors was measured during fermentation by CO_2 analyzer.

(3) Analysis of components in EFB fermented in bag

The fermented products after 1 month incubation were freeze-dried before analysis. The crude protein was determined by Kjeldahl method and various fiber components were determined by using Fibertec system 1010.

4.2.3 Results and discussion

(1) Optimum Conditions for fermentation of EFB by *Coprinus cinereus*

In the case of growth analysis using test tube, *C. cinereus* did not grow at temperature of 20, 25, and 45℃, however it grew well at 30, 35 and 40℃. Figures 4.2-

1 and 4.2-2 show the CO_2 evolution (g/ CO_2 /hr /kg dry matter) and carbon evolution (g carbon /kg dry solid) during fermentation at various temperature. The results show that *C. cinereus* grew well at 35 - 40℃. From these results it can be concluded that the optimum temperature for the fermentation by *C. cinereus* is 35 - 40℃.

It was observed by the fermentation in test tubes at 35℃ for 7 days that *C. cinereus* grew well at pH 6 - 9 and without pH adjustment (pH 8.5). However, no growth was observed at pH 4. A little growth of mycelium was observed at pH 5 and 10. The CO_2 evolution and carbon evolution during fermentation at various pH (Fig. 4.2-3 and 4.2-4) show the same result of the growth obtained in test tubes. From these results it is concluded that the optimum pH for the fermentation by *C. cinereus* is 7 - 9.

Previous works suggest that the pH of EFB substrate should be decreased to 4.5 before the fermentation. However, from these results, pH 4.5 is not favorable for *C. cinereus*. The substrate adjusted to pH 4.5 might gradually increased to pH 7 or 8 due to the dissolution of potassium contained in the EFB.

(2) Optimum condition for *P. sajor-caju*

Effects of irradiation and washing of EFB on growth rate of *P. sajor-caju* were studied using large test tubes. Figures 4.2-5 and 4.2-6 show the growth rates on EFB without or with washing, respectively. In case of without washing (Fig. 4.2-5), similar results were obtained on EFB with or without rice bran and 30 kGy irradiation or autoclaving. In case of washed EFB (Fig. 4.1-6), the growth rate on irradiated substrate was lower than that on autoclaved substrate. The results suggest that the low content of free sugars in washed EFB substrate affect the slow growth rate at early stage especially in the case of irradiation. However, the reason of the delay of growth on irradiated substrate is not clear and further study is necessary to clarify whether the irradiation causes the delay of growth or not.

The carbon dioxide evolution during fermentation at various temperature by *P. sajor-caju* were analyzed. Figure 4.2-7 shows that the optimum temperature for the EFB fermentation by *P. sajor-caju* is 30 - 35℃. In the previous section, *C. cinereus* also can grow well under the same conditions at pH 7 - 9 and 30 - 40℃. Therefore, the strains of *P. sajor-caju* and *C. cinereus* were selected as the most suitable fungi for EFB fermentation because these conditions are appropriate for the fermentation in Malaysia without pH adjustment and incubation at room temperature.

4.2.4 Conclusion

The optimum pH and temperature for the fermentation of EFB by *C. cinereus* is 7 -

9 and 30 - 40℃, respectively. Suspension of EFB in water showed the alkaline pH of 8 - 9 because it has a high potassium content. It is, therefore, considered that the fermentation of EFB using *C. cinereus* is appropriate for the fermentation in Malaysia without pH adjustment and incubation at room temperature. *P. sajor-caju* also can grow well under the same conditions.

Table 4.2-1. Cellulosic components of EFB, PPF and bagasse

Samples	Crude fiber (%)	NDF* (%)	ADF** (%)	Hemicellulose (%)	Lignin (%)
EFB, control	45.7	79.9	61.1	(18.8)	(15.4)
EFB, 10 kGy	44.7	80.2	60.4	(19.8)	(15.7)
PPF, control	41.9	85.2	64.5	(20.4)	(22.9)
PPF, 10 kGy	43.1	85.2	63.6	(21.6)	(20.5)
Bagasse		78.8	56.3	(22.5)	
Fermented EFB[1]	36.9	70.7	52.4	(18.3)	(15.5)
Fermented EFB[2]	27.4				
Fermented EFB[3]	9.3				

* Neutral detergent fiber, ** Acid detergent fiber,
1:Spray-tower type for 24days, 2:Erlenmyer flask for 1 month,
3:Horizontal screw type for several months.

Table 4.2-2. Components of fermented EFB by *P. sajor-caju*

	Crude fiber (%)	NDF (%)	ADF (%)	Hemicellulose (%)	Lignin (%)	Crude protein (%)
EFB	46.7	82.7	64.2	18.5	17.5	2.6
Fermented EFB	33.8	55.5	45.2	10.3	11.4	10.6

EFB was fermented by *P. sajor-caju* at 30°C for 1 month.

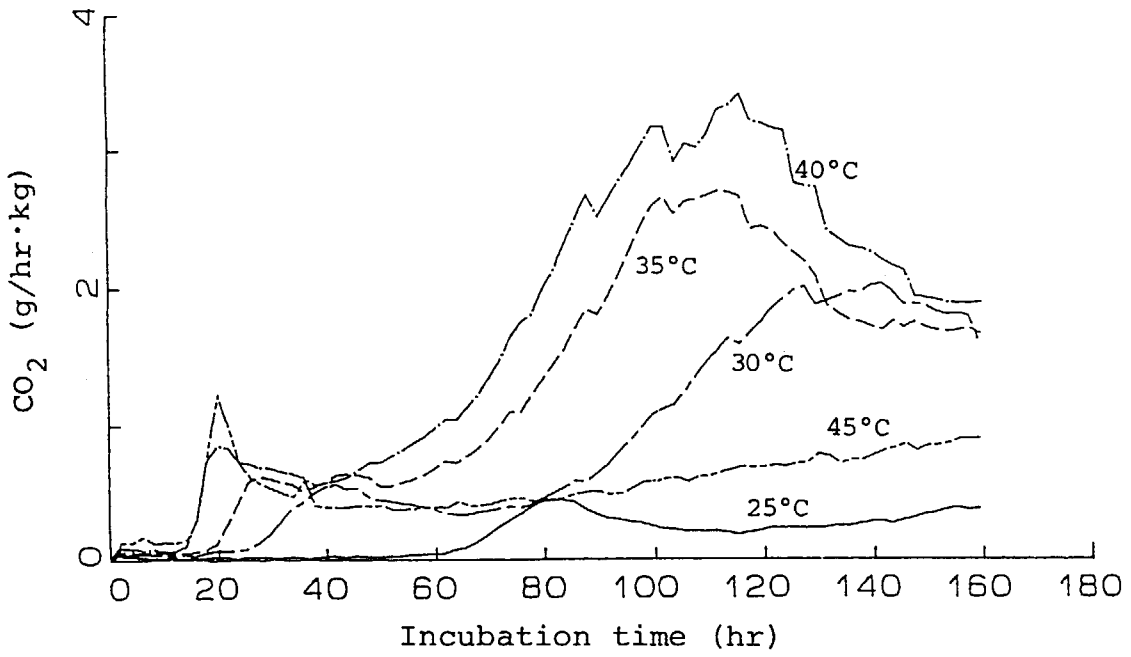

Fig. 4.2-1. Effect of temperature on CO_2 evolution during fermentation of EFB by *C. cinereus*

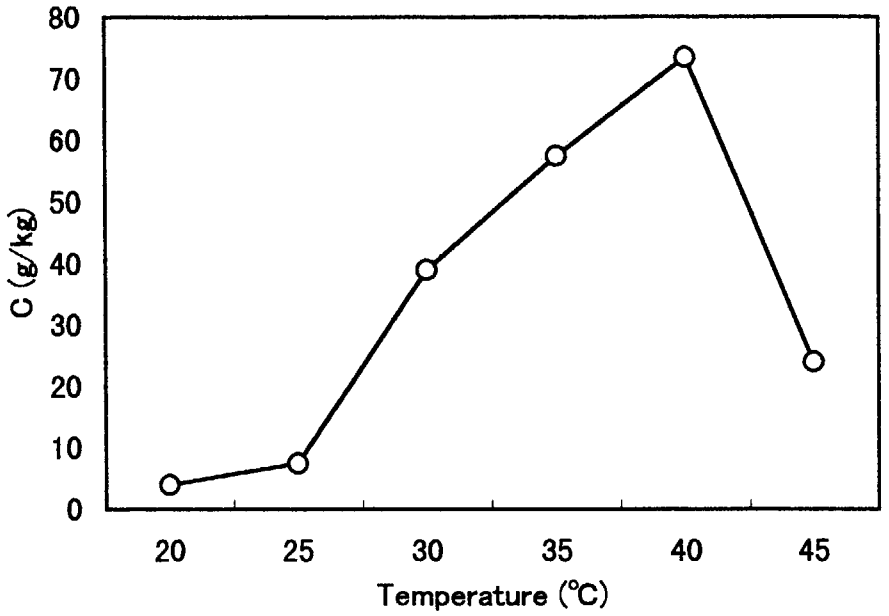

Fig. 4.2-2. Effect of temperature on carbon evolution during fermentation of EFB by *C. cinereus*

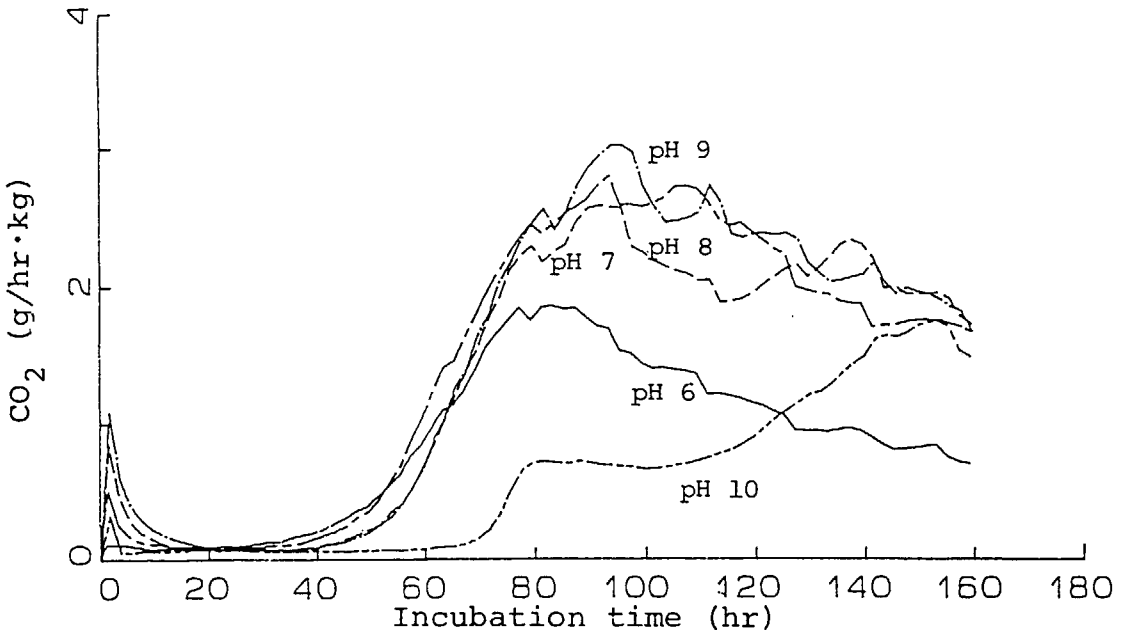

Fig. 4.2-3. Effect of pH on CO_2 evolution during fermentation of EFB by *C. cinereus*

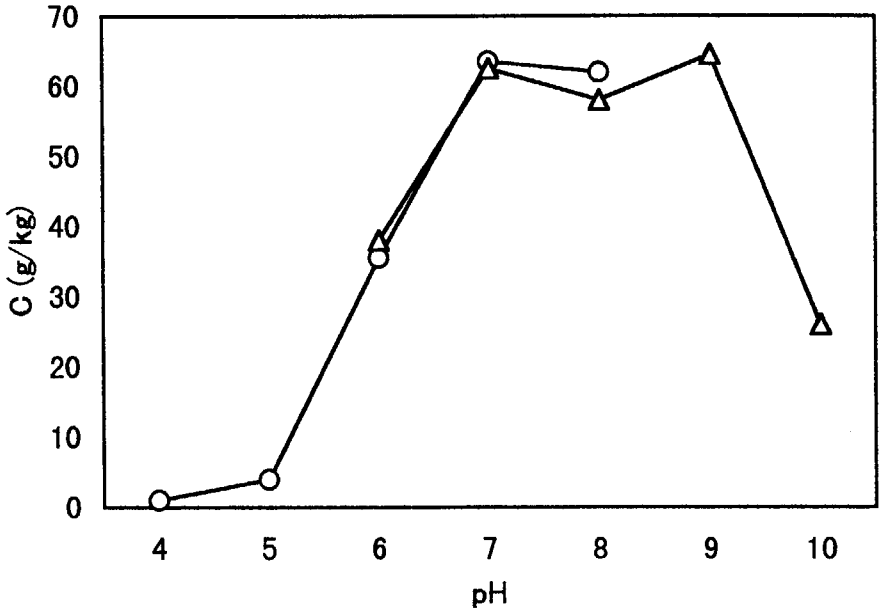

Fig. 4.2-4. Effect of pH on carbon evolution during fermentation of EFB by *C. cinereus*

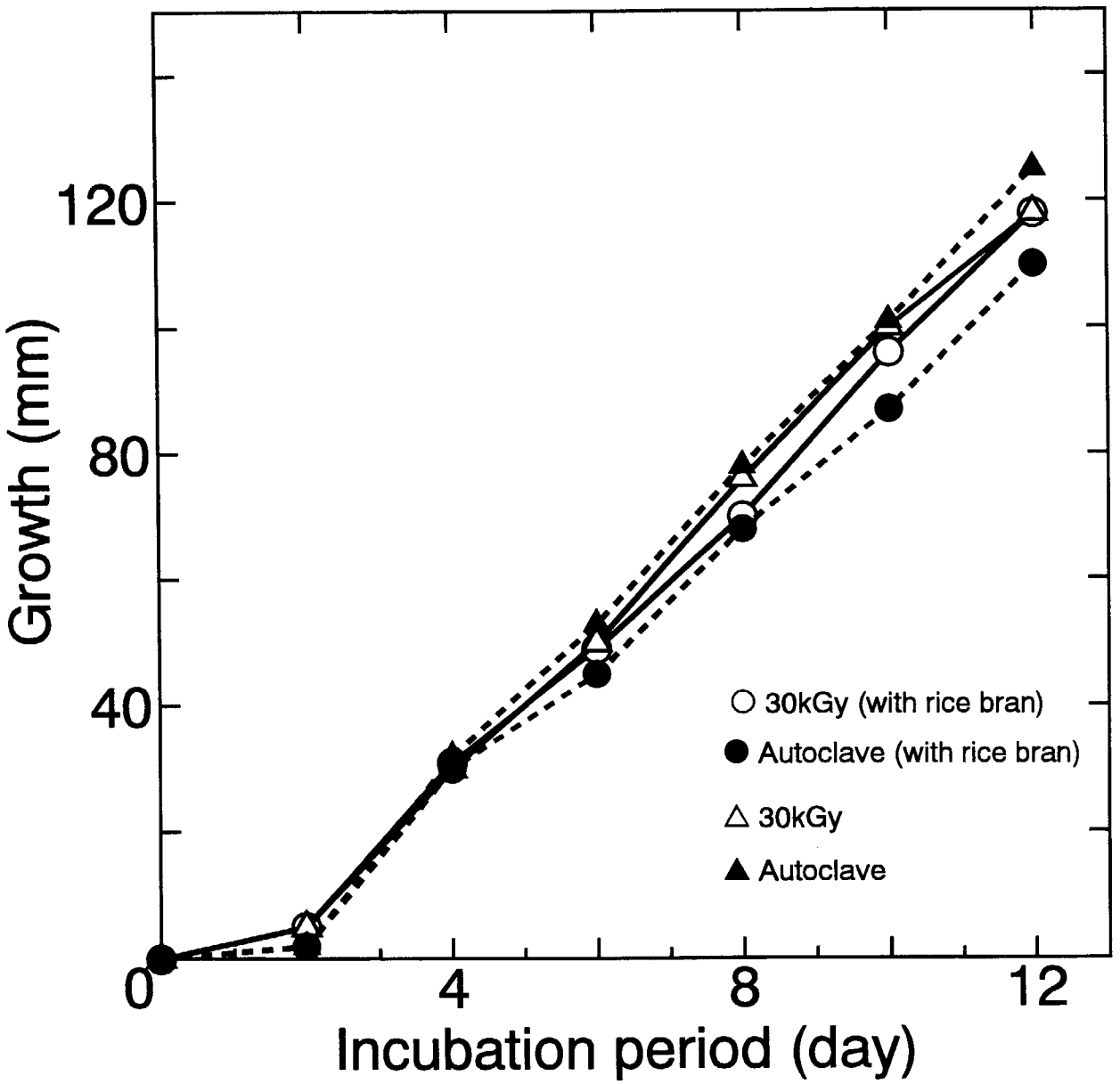

Fig. 4.2-5. Growth rate of *P. sajor-caju* on EFB media

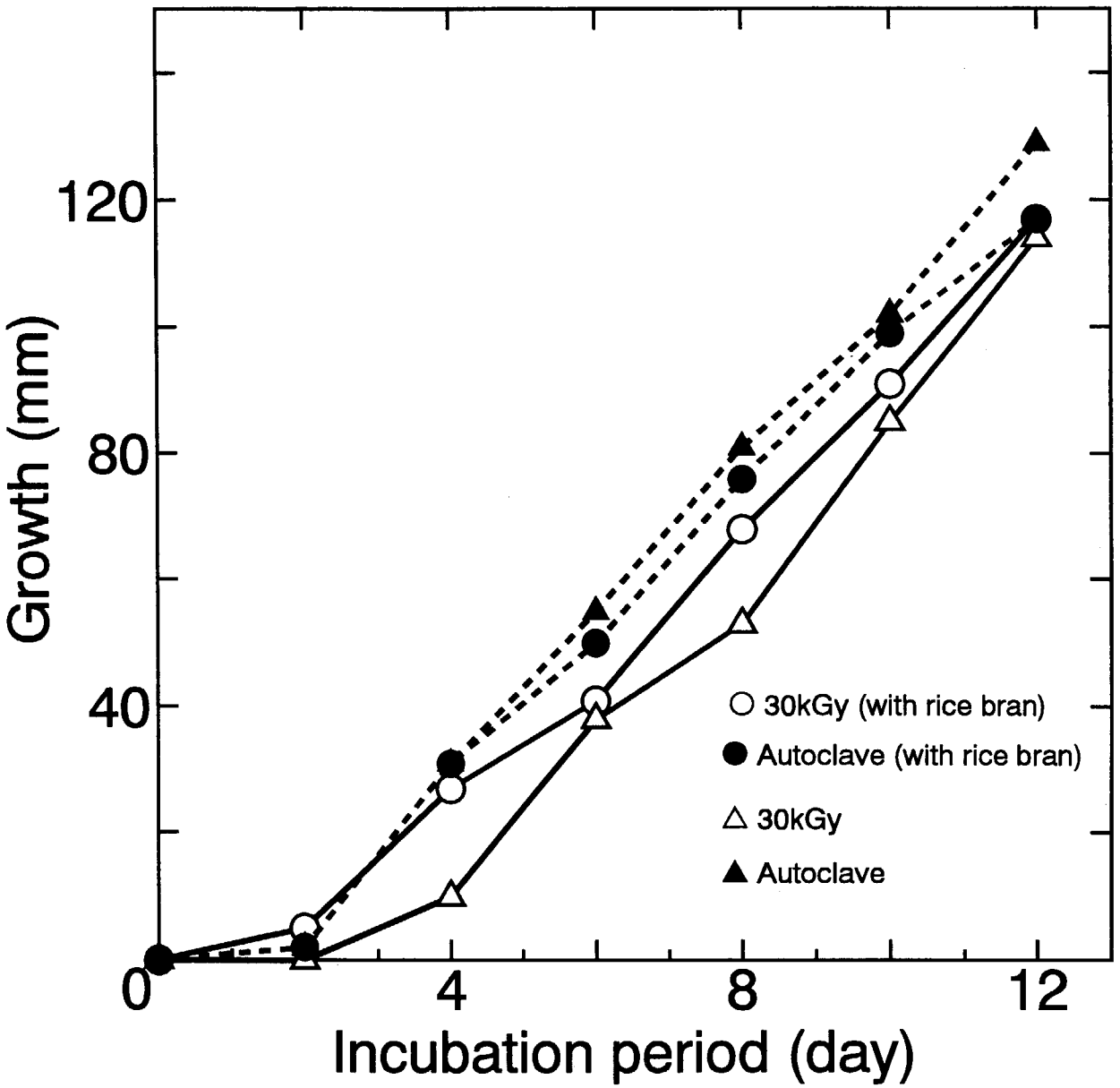

Fig. 4.2-6. Growth rate of *P. sajor-caju* on washed EFB media

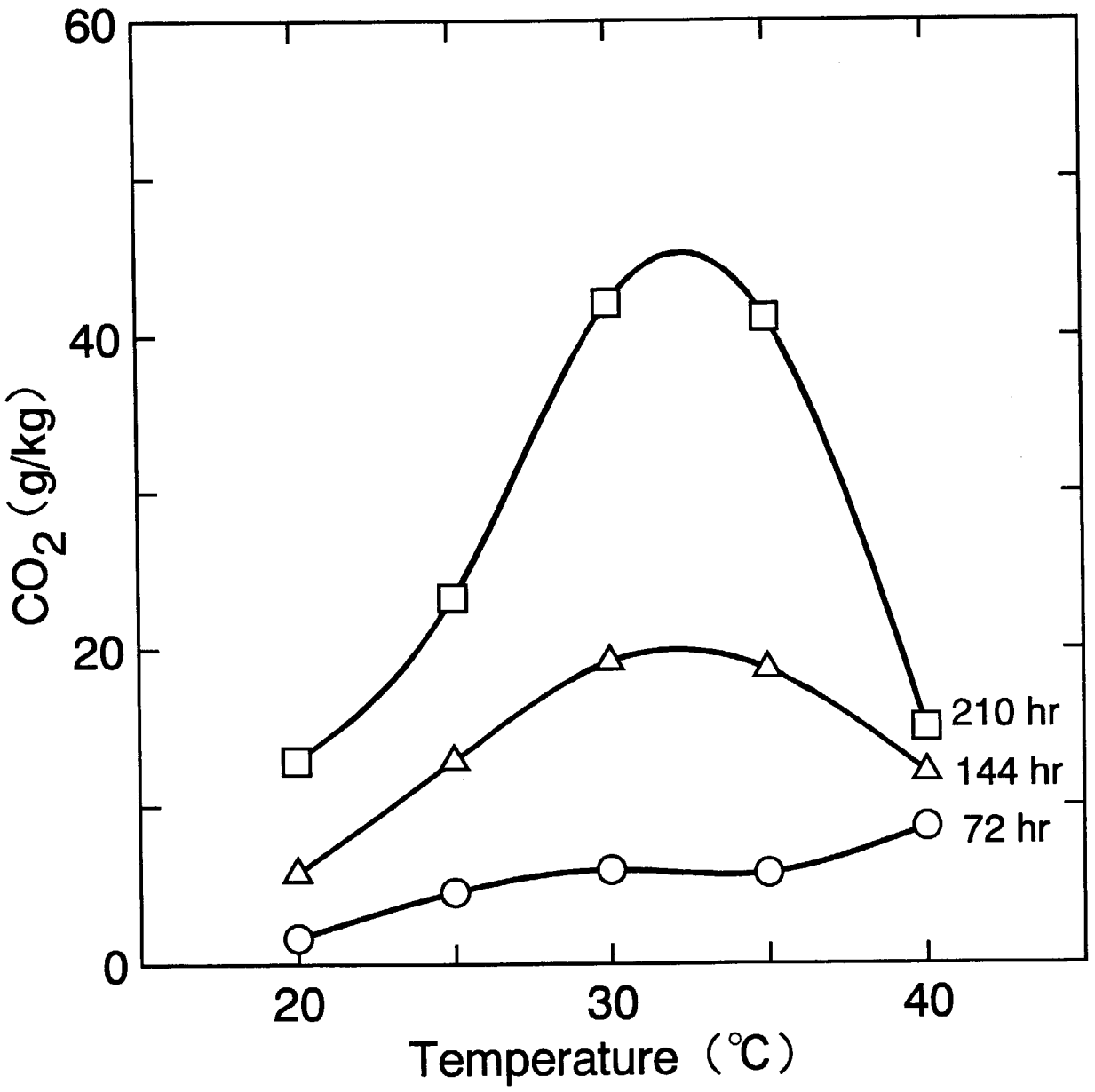

Fig. 4.2-7. Effect of temperature on EFB fermentation of EFB by *P. sajor-caju*

4.3 Design of Fermentors

4.3.1 Introduction

The plastic bags for mushroom cultivation containing ca. 40 g of EFB have been used for the fermentation of EFB. However, a large amount of fermented products from EFB are necessary for animal feeding test. The following methods are considered as the mass production of animal feeds;

1) increase the number of plastic bags for fermentation,

2) basic study using jar fermentor suitable for the solid state fermentation.

This chapter describes the design of fermentors tested for the basic study of scale-up fermentation.

4.3.2 Design of horizontal screw type fermentor

A small stainless steel fermentor was designed (Photo. 4.3-1). The capacity of the fermentor is 1000 ml and it has four screws shown in upper scheme (Fig. 4.3-1). The screw is rolling very slowly to prevent the damage of the fungus mycelia. However, EFB samples could not be stirred well by these screws. Therefore, the shape of the screws was changed (lower scheme) resulting the good stirring. When the EFB was fermented using this apparatus, it took for a long fermentation time during winter season as the equipment had no thermo-heater. It is a weak point of this equipment that the inside fermentation condition can not be seen.

4.3.3 Design of spray-tower type fermentor

A little bigger fermentor was designed for the basic study to get the scaling up factors (Fig. 4.3-2). This equipment is named as the spray-tower type fermentation system and consists the following parts:

1) tower type jar with spray system for nutrient solution,

2) control system for temperature, air flow rate and pH,

3) detector for CO_2, pH and temperature,

This system has two jars and the capacity of one jar is 2000 ml, and can ferment ca. 200 g of EFB per batch. The jar is see- through as it made by glass (Photo. 4.3-2).

4.3.4 Conclusion

Liquid seed preparation and inoculation system were developed for large-scale fermentation. The system consists the liquid fermentor and injector. The seed was cultivated in liquid media in 10 ℓ bottle with air bubbling and stirring for 3 - 4 days.

The seed suspension can be injected semi-automatically into the EFB substrate through needle.

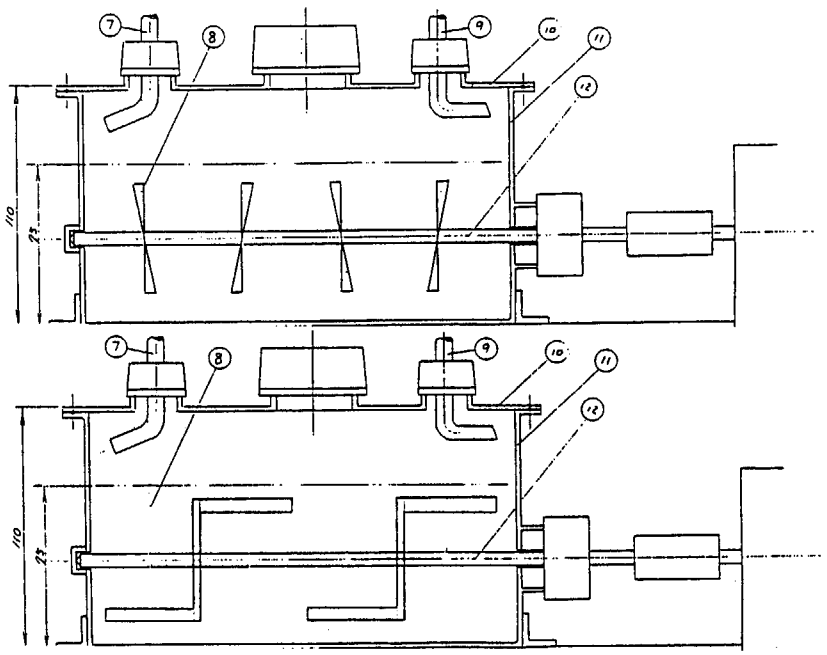

Fig. 4.3-1. Schematic figure of horizontal screw type fermentor

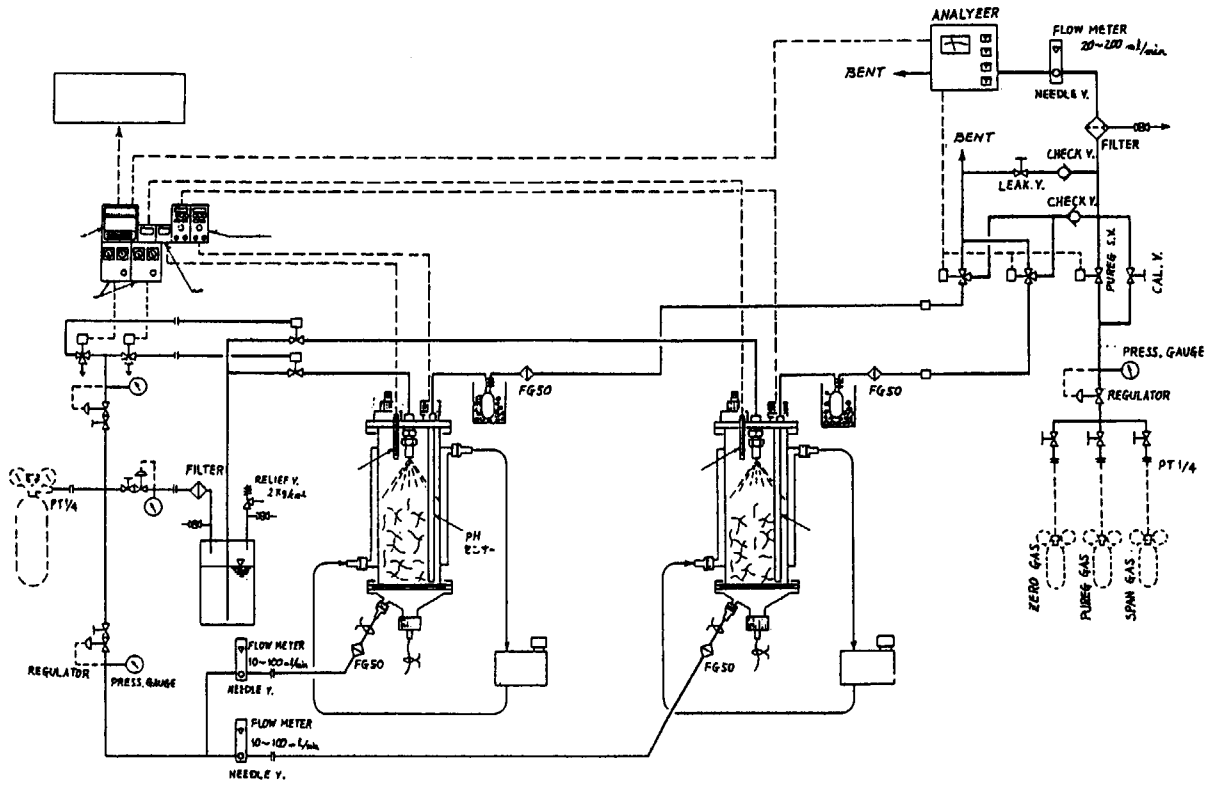

Fig. 4.3-2. Flow diagram of spray type fermentor

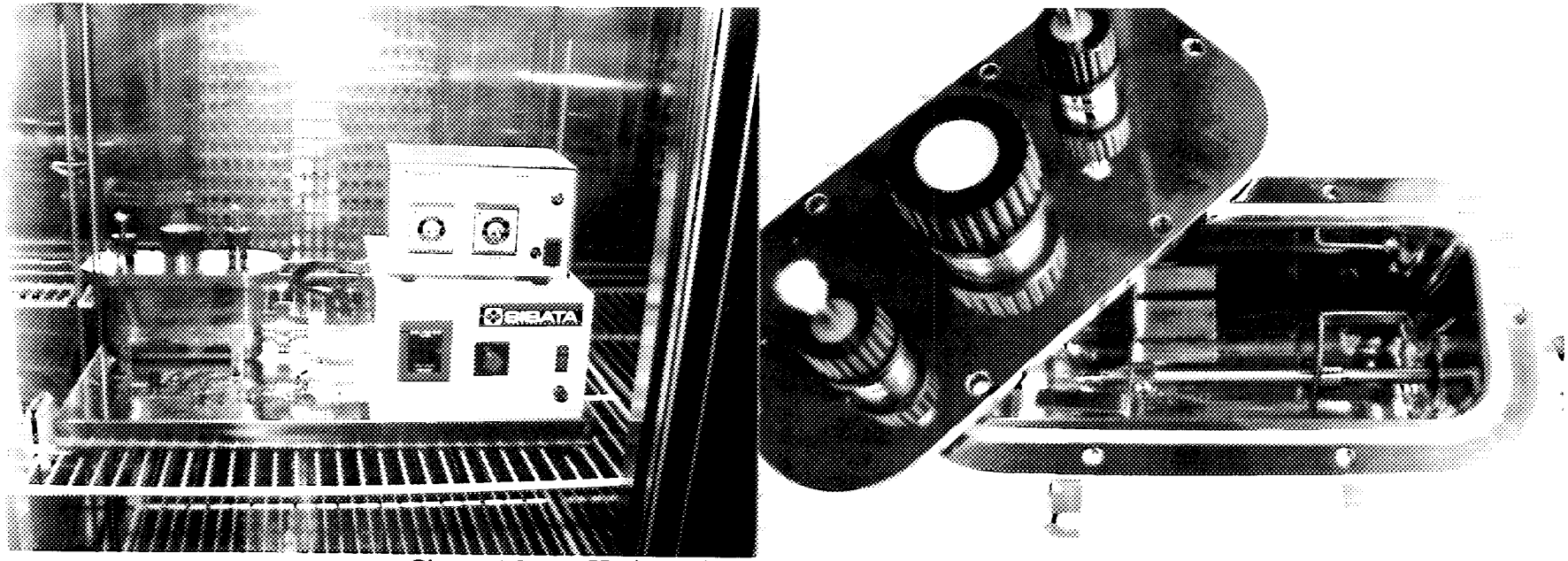

Photo. 4.3-1. Horizontal screw type fermentor

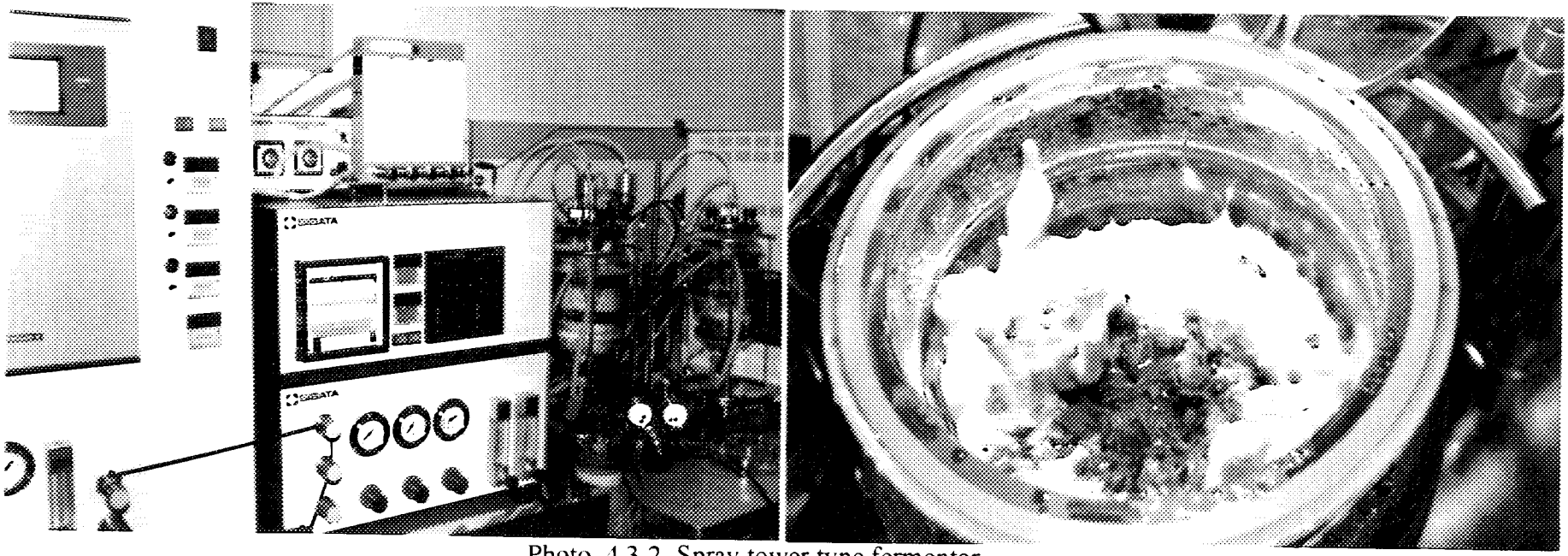

Photo. 4.3-2. Spray tower type fermentor

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4.4 Fermentation in Suspension

4.4.1 Introduction

Solid state fermentation is mainly used for the production of animal feeds from EFB. In the case of liquid (suspension) state fermentation, the protein contents can be increased in a short time but it is difficult to increase the substrate concentration because the EFB substrate is easily precipitated. It is, therefore, considered that the particle size of sample may affect on the fermentation in suspension. It is expected to produce the good seeds with homogeneous and high protein content using the fine powder of EFB prepared by Super Masscolloider.

In this study, fermentation of EFB by *Pleurotus sajor-caju* in suspension of EFB with various particle sizes has been investigated to obtain the basic data for the production of good seeds and the fermentation with higher protein concentration.

4.4.2 Materials and methods

(1) Preparation of EFB samples

Dry EFB obtained in Malaysia was cut to 2-3 cm length by straw cutter (Marumasu VL-56), chopped by Willey Mills (2 mm mesh) (WSX-200, Kiya), and finally ground by Super Masscolloider (Masuko, Paraboy-mini MKPB6-2). The EFB sample was sieved and separated according to the particle size.

(2) Analysis of waster extracts

EFB samples with various particle size were suspended in distilled water (1 g / 100ml) and stirred at 100 rpm for 48 hr. The suspension was centrifuged at 10,000 rpm for 30 min and the dry weight of extracts in supernatant was measured. Small molecular weight carbohydrates in extracts were analyzed by HPLC with Shodex SUGAR SH1821 column.

(3) Fermentation in suspension

EFB samples with various particle size were suspended in Mandel's medium (1 g / 100ml), inoculated with *P. sajor-caju*, and incubated at 30℃for 3 days.

4.4.3 Results and discussion

(1) Production of fine EFB powder

EFB powder with various particle sizes was obtained after grinding and sieving. Table 4.4-1 shows the dry volume, the volume in water and surface area for EFB particle with various sizes. By using Super Masscolloider, very fine powder with the size less than 0.074 mm were obtained. The volume of powder was decreased and the surface area was increased with the decrease in the particle size of EFB. Especially the larger surface area of fine powder suggests the effective fermentation because the fungi can easily contact with fiber.

(2) Analysis of extracts from EFB powder

The amount of water extracts was increased with the decrease in particle size (Table 4.4-2). The amount of extracts from fine powder (less than 0.074 mm) was more than twice of the value from larger particle (more than 1.0 mm). The sugar in extract was analyzed by HPLC with gel permeation column. As shown in Fig. 4.4-1, the peaks of smaller size sugar (may be monosaccharide and oligosaccharide) were increased in the extract from fine powder less than 0.074 mm. These results show that the extraction of sugar components increases with the increase in surface area.

(3) Fermentation of EFB in suspension

EFB with various sizes in suspension was fermented by *P. sajor-caju* and the protein contents were measured by Kjeldal method. Table 4.4-3 shows the protein contents in fermented products after 3 days incubation. The protein contents were significantly increased in fine powder fermentation and the values were 13.4% for smallest powder (less than 0.074 mm), 4.8% for 0.21-0.35 mm size, and 3.1% for the powder bigger than 1.0 mm. The result shows that the growth of fungi is faster on fine powder and the protein contents increase in a short time. Furthermore, the fermentation of smallest powder made the fine and uniform granules with ca. 2 mm. It is, therefore, considered that the fermented products are suitable as the seeds for the large scale fermentation.

EFB with various sizes in suspension was fermented by *P. sajor-caju* and protein contents were measured by Kjeldhal method. Figure 4.4-2 shows the effect of particle size of EFB on growth of *P. sajor-caju*. The protein contents was increased rapidly and reached a maximum after 4 to 5 days incubation. The growth of *P. sajor-caju* was highest in fine powder medium (less than 0.074 mm). The protein content in media

reached a maximum after 3 to 4 days. It is, therefore, possible to prepare the seed solution using fine EFB within 4 days.

It is pointed out that the activity of seed fungi will be decreased by subculture of fungi for many times using the substrate without cellulosic materials. At moment, we are using the substrate including EFB for the preparation of original seed in Erlenmeyer flask incubation because the same seed is used for several times. However, the substrate without EFB will be used for large volume incubation since the seed does not reuse in this condition. Further study is required to make clearer whether EFB is necessary to include in the media or not to obtain the good seed.

4.4.4 Conclusion

Two types of jar-fermentor were designed for the basic study of solid fermentation. It is considered that these fermentors are useful for basic study but the scale-up fermentation of EFB using jar-fermentor is difficult because the solid state fermentation takes a long time for 1 month. It is, therefore, concluded that the suitable system for scale-up fermentation of EFB is to increase the number of plastic bags for mushroom cultivation.

Table 4.4-1. Grainding of EFB by Masscolloider

Diameter of particle (mm)	Volume (dry) (ml)	Volume in water (ml)	Surface area (cm^2)
~ 0.074	5.2	9.2	1978
0.074 ~ 0.149	4.7	11.2	1508
0.149 ~ 0.21	4.8	10.4	870
0.21 ~ 0.35	5.6	9.9	531
0.35 ~ 1.0	6.6	10.5	233
1.0 ~	8.4	15.0	225

Table 4.4-2. Water Extracts from EFB with Various Particle Size

Diameter of particle (mm)	Water extracts (mg / g)
~ 0.074	83.5
0.21 ~ 0.35	52.5
1.0 ~	36.5

Table 4.4-3. Effect of Particle Size of EFB on Fermentation in Suspension by *P. sajor-caju*

Diameter of particle (mm)	Protein extracts (%)
~ 0.074	13.4
0.21 ~ 0.35	4.8
1.0 ~	3.1

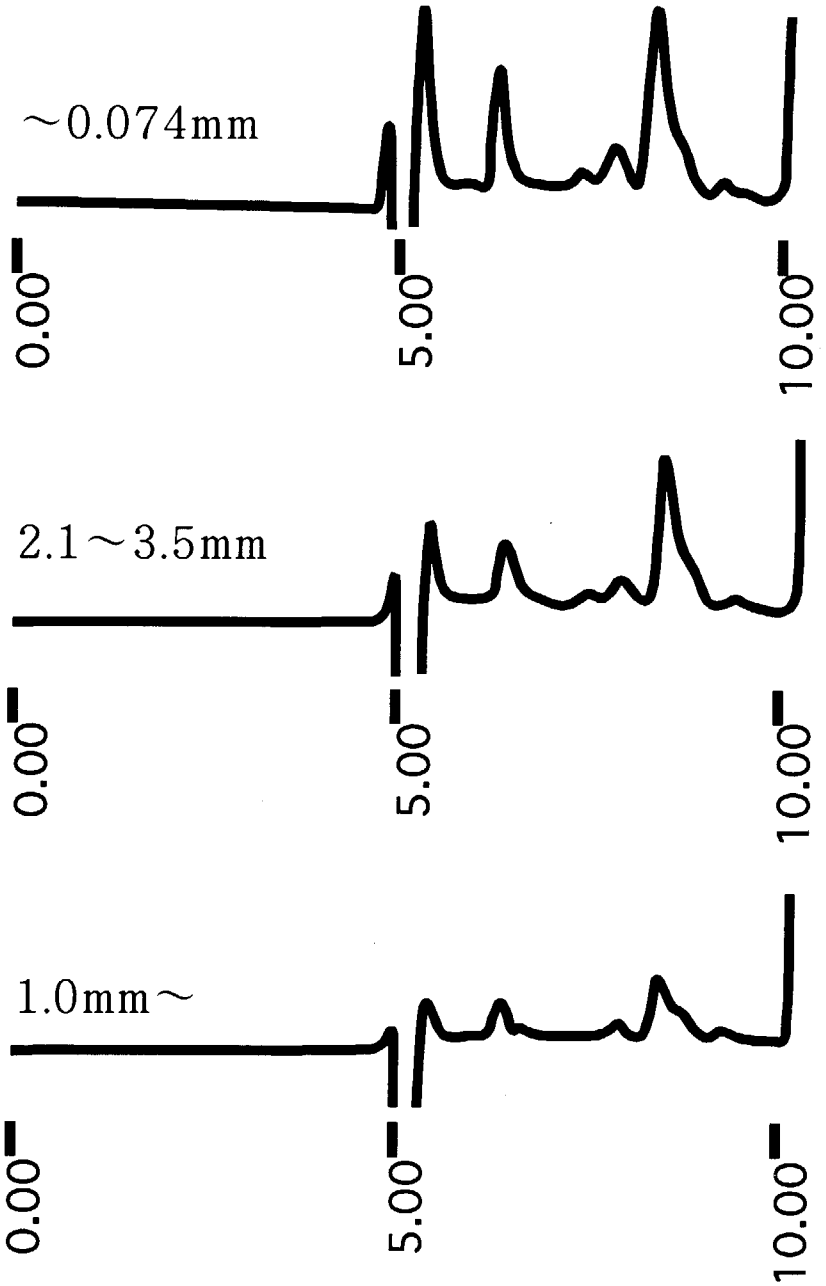


Fig. 4.4-1. Extracted sugar from EFB with various particle size

Grind EFB was suspended in water and extracted sugar was analyzed by HPLC.
column: Shodex SUGAR SH1821
mobile phase: 0.005N sulfulic acid
flow rate: 1.0 ml/min
detector: reflective index detector (JASCO 830RI)

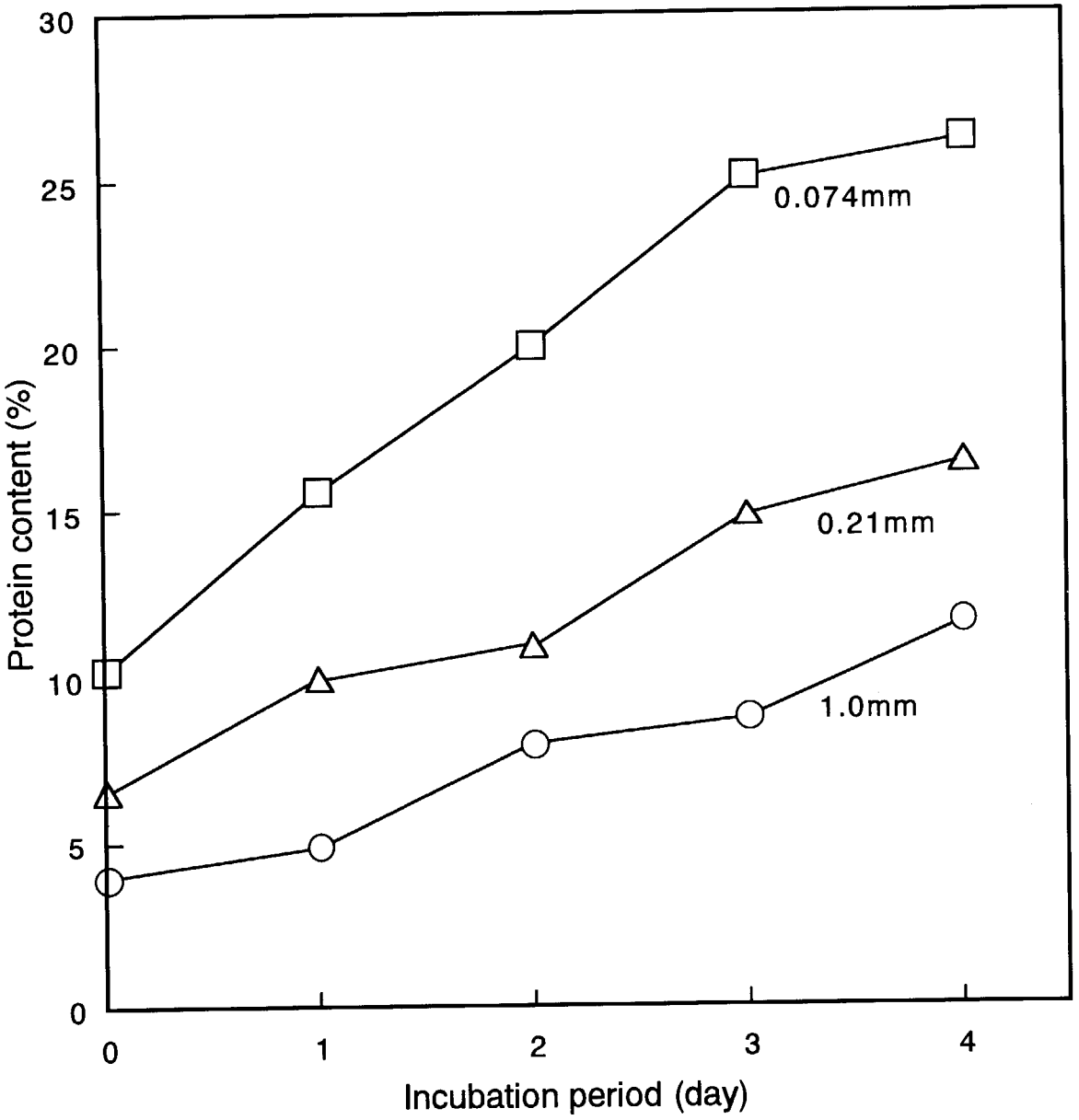


Fig. 4.4-2. Effect of particle size of EFB on fermentation in liquid media by *P. sajor-caju*

4.5 Inoculation System of Liquid Seed for Large Scale Fermentation

4.5.1 Introduction

It is especially required to establish the suitable seed preparation and inoculation system for pilot plant since a lot of plastic bags will be used for the fermentation. It is considered that the liquid seed is better than the solid seed for easier and rapid inoculation.

In this study, liquid fermentation system using big bottle was studied to prepare the homogeneous and high active seeds. Furthermore, the inoculation system of these liquid seed was also studied to develop the automatic inoculation.

4.5.2 Materials and methods

(1) Liquid substrate for seed preparation

Mandel's medium was prepared with 1% glucose for big bottle incubation or 1% EFB as carbon source instead of glucose for Erlenmeyer flask incubation. The EFB sample was prepared as described in chapter 4.4.

(2) Liquid fermentation

The media were inoculated with *Lentinus sajor-caju* seed and incubated at 30°C in a shaking incubator at 100 rpm. In the case of big bottle incubation, air was bubbled instead of shaking.

4.5.3 Results and discussion

(1) Liquid seed preparation using big bottle

As a lot of liquid seed is required for large scale fermentation, the liquid incubation system using big bottle was studied at JAERI. Figure 4.5-1 shows a fermentation bottle with attachments for aeration and auto-inoculation. The bottle (volume : 10 litter) was filled with 8 litter liquid medium. Seed solution of *P. sajor-caju* cultivated in Erlenmeyer flask was inoculated and air bubbled during fermentation. As the mycelium of fungi was precipitated without stirring even with a strong air blow (400 ml / min), the aeration system was modified to use the tube reached the bottom edge of bottle. Using this system, the mycelium did not precipitated but the big flock was formed. To prevent the formation of big flock, the stirring using magnetic stirrer was tried. The formation of flock was prevented by stirring during incubation but the stirring after incubation also enough to

break the flock. From these results, it is concluded that a magnetic rod is necessary to put in fermentation bottle and stir after cultivation.

It takes about 1 week incubation for full growth of mycelium at 25°C because there is no room to keep the incubator around 30°C in our laboratory. We hope that the incubation time becomes shorter at MINT because the ambient temperature is higher than thaat of Japan.

In this study, we used the chemicals for the preparation of liquid substrate (Mandel's medium) but it is important to find out the cheaper substrate for the practical seed preparation. Molasses from sugar cane, sludge from palm oil mill, potato extract, etc. have been tried to use as the substrate. Recently, it is also reported that the rubber waste with high concentration of proteins is useful as the substrate for fungi cultivation.

(2) Inoculation system

For large scale fermentation, auto-inoculation system is necessary because it is difficult to inoculate the seed in a huge number of fermentation bags by manual. Figure 4.5-2 shows the scheme of the inoculator designed originally. It was designed that the liquid seed was sucked to syringe and push out through needle, alternately. However, the glass stopper did not work well because the flock of liquid seed was stacked in the stopper. It was also designed that the seed suspension was sucked from the bottom to the top of bottle through the glass tube but the seed suspension could not suck easily.

From the results, the system was modified as shown in Fig. 4.5-3. In this system, the seed suspension is sucked from the bottom of bottle, and the tubes of inlet and outlet of syringe were stopped by magnetic switch, alternately. The inoculation volume and speed is changeable according to the inoculation conditions. Furthermore, the height of inoculator can be changed according to the size of sample. Using a foot switch for the inoculation, semi-auto inoculation system was developed (Fig. 4.5-4).

4.5.4 Conclusion

Liquid seed preparation and inoculation system were developed for large-scale fermentation. The system consists the liquid fermentor and injector. The seed was cultivated in liquid media in 10 ℓ bottle with air bubbling and stirring for 3 - 4 days. The seed suspension can be injected semi-automatically into the EFB substrate through needle.

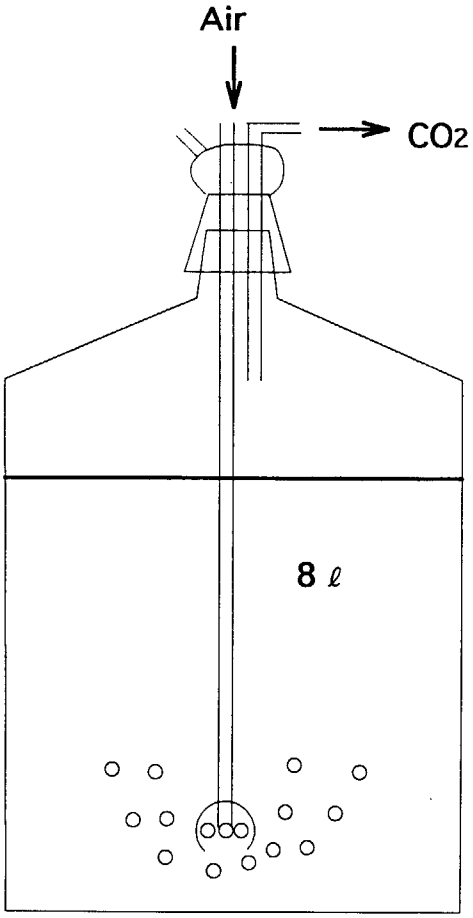


Fig. 4.5-1. Incubation bottle for liquid seed preparation

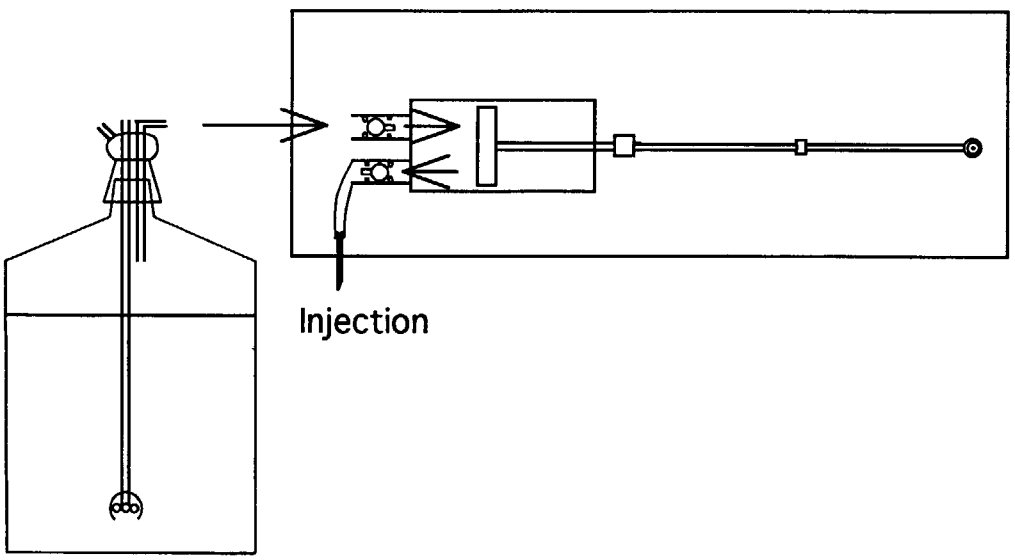


Fig. 4.5-2. First design of injection system

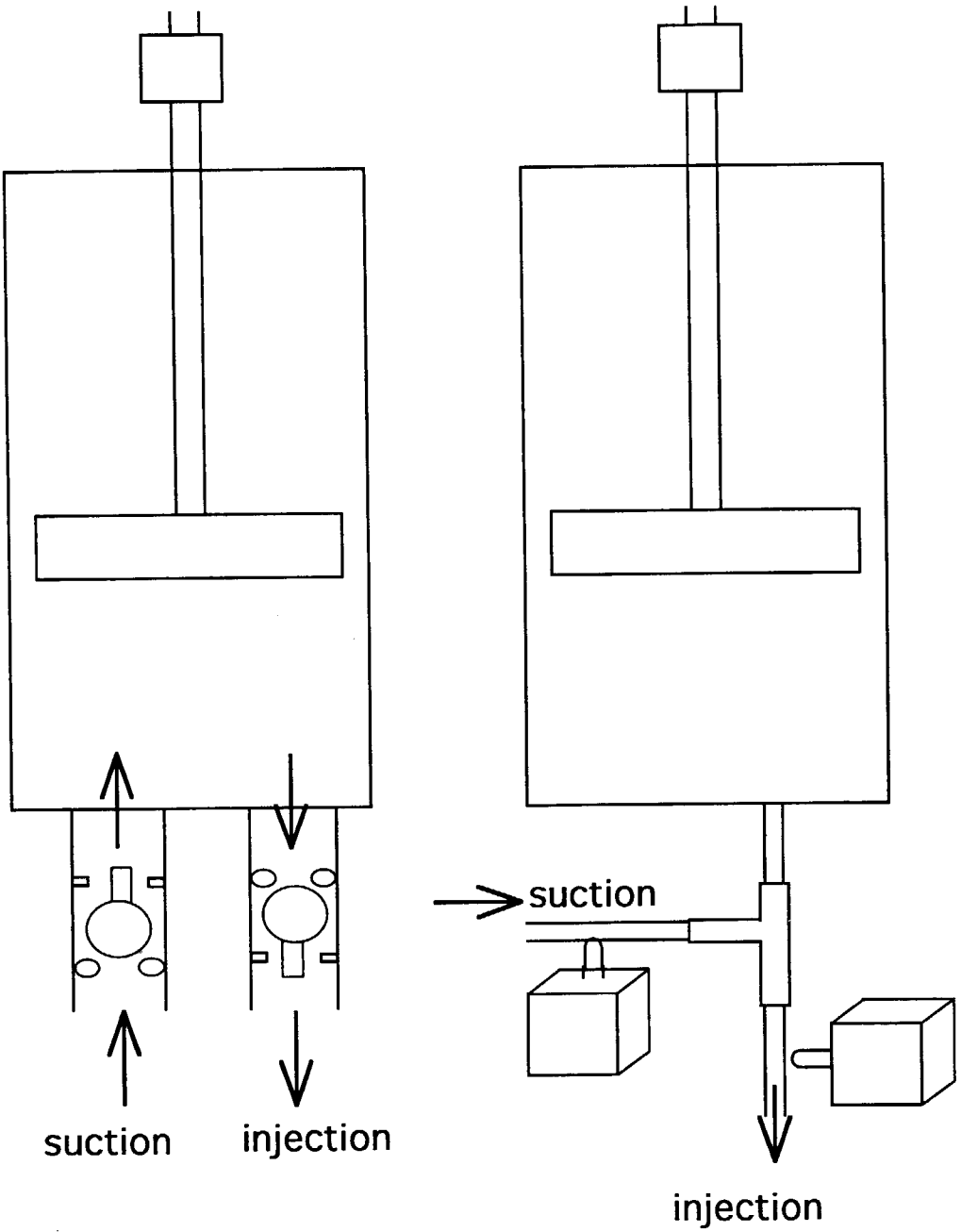
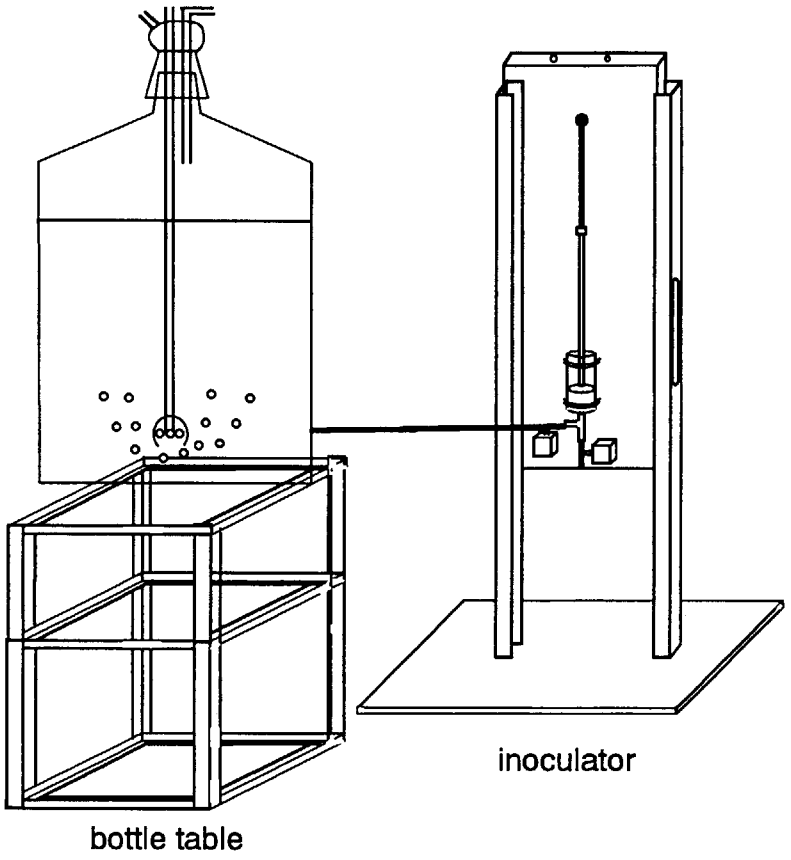
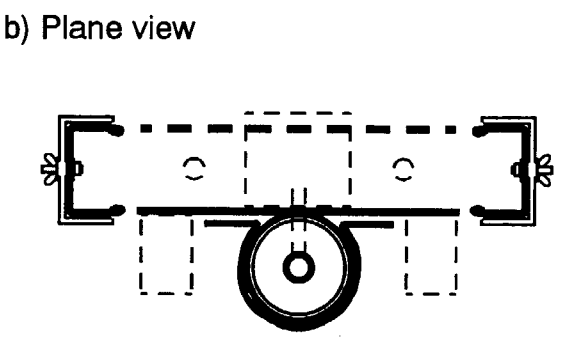


Fig. 4.5-3. Modification of injector for liquid seed





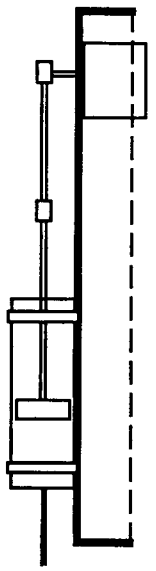


Fig. 4.5-4. View of inoculation system

5. STUDY ON QYALITY AND UTILIZATION OF FERMENTED PRODUCTS

5.1 Production of Animal Feeds

5.1.1 Introduction

C. cinereus and *P. sajor-caju* were the most suitable microorganisms for the fermentation of EFB based on their ability to degrade fiber and increase protein content. The optimum pH and temperature for the fermentation of EFB were 7 - 9 and 30 - 40℃, respectively (Chapter 4.1 and 4.2). *P. sajor-caju* was finaly selected for the seed strain on upgrading of oil palm waste because of the ability to decrease the lignin content, produce edible mushroom and easy cultivation than *C. cinereus*.

This chapter presents the evaluation of various products obtained from several manipulations to the EFB before fermentation; and their substratum during and after fermentation. They includes pretreatment (soaked and mixed) of EFB with lime, variation of fermentation conditions: prolonged incubation period, and post-fermentation manipulation such as harvesting mushroom out of the substratum. The evaluation are based on nutritional values by the method of AOAC and the pertinent *in vitro* dry matter digestibility (Gotto & Minson 1979), whenever appropriate. The evaluated products are compared and discussed.

5.1.2 Nutritional value

(1) Nutritional aspect of EFB

The variation in nutritional values of several fresh EFB collected at different time from at least two mills is shown in Table 5.1-1. EFB with on average high values of NDF (82.6%) compared to ADF (59.5%) indicated that material contains a high proportion of hemicellulose, a useful source of energy for ruminant feed. Lignin, an inert component present into a complex structure of a three-dimensional network with cellulose, had apparently protected cellulose from enzyme attack. The level of lignin in fibrous agricultural residues useful as feeds to ruminant animal must be below 20%. EFB contains 17.1% lignin, lower than the limiting level for it to be used as feed resources, indicating that the material was potentially useful and worth to be evaluated as ruminant feed.

The low protein contents (4.2%) also suggested that the material required further modification or supplement. Its direct utilization required additional protein as supplement. Despite the great potential as feed resume, the digestibility of material upon feeding to ruminant has been a major challenge.

The *in vitro* dry matter digestibility (IVDMD) data showed that only 15% of EFB is digestible. The data suggested that EFB cannot be used directly in the ruminant feeding system. The high CF values of the fiber (42.8%) reflect its high latent energy could not be fully benefited. In order for it to be effectively exploited, it must be treated, by a single treatment or combination of several treatments to improve its digestibility before use. The treatments carried out includes soaking or and mixing lime, followed by fermentation; a combination of physical and biological treatments.

(2) Treated EFB as ruminant feed

In the following particular study, the pretreatment was performed by lime soaking. The choice of lime treatment has been decided based on the earlier study on cultivation of mushroom on the pasteurized EFB. Table 5.1-2 shows the nutritional value of fermented EFB accomplished by *P. sajor-caju*. It was characterized after mushrooms had been harvested. The product showed a general decrease in CF (crude fiber), NDF, ADF and ADL (acid detergent lignin) regardless of pre-treatments involved: soaking and mixing. Both pre-treatments afforded products of relatively same quality. The CF had reduced substantially to 22%. Despite losing its fiber components, CP (6.9%) had increased slightly. The products had relatively higher in protein due to loss in weight the initial solid EFB media during fermentation. It occurred as if protein content become concentrated in the final product. The highest dry matter loss was 30% mainly as CO_2 gas.

Table 5.1-3 shows the nutritional data for the prolonged incubation period. It indicated that the prolonged incubation had allowed further digestion of lignin in fermented product despite retaining other nutritional quality.

In the post-fermentation condition where mushrooms had not been induced or harvested, the over all nutritional values of fermented products had increased over the mushroom harvested products, showed in Table 5.1-4. The values of CP had increased (8.4 %) due to mycelial biomass being remained in the product. The CF content was higher (31.5 %) compared to the fruiting substratum (22.2 %) indicating the fruiting had apparently allowed utilization of fiber components including lignin very effectively. Consequently the values of fiber components especially the NDF (initially value 77.5 %) had decreased (60.9 %) upon fruiting. Similarly the lignin, ADL (20.0 %) decreased upon fruiting (15.5 %). Since lignin was critical to the digestibility of the product, its level in the non-fruiting product was higher, therefore the fruiting could be better. However, the non-fruiting product was most useful in terms of energy. Obviously it had a higher energy content as reflected by the higher fiber content.

It was pointed out earlier, the importance of retaining fiber content for energy which had been produced by avoiding fruiting of mushrooms. However, the advantage that arise has been on the level of remaining lignin associated with this product.

A general comparison of chemical composition of EFB to other oil palm by-product is shown in Table 5.1-5. CF was lower in fermented EFB when compared to other oil palm by-product such as PPF, OPF and OPT. There was also an improvement in CP value (8.2%) of fermented EFB over the untreated EFB, OPF, OPT and PPF. The EE and ash content of POME is high due to the oil palm extraction process and probable soil contamination. POME is highly variable in EE and ash content and this can have profound effects on the feed quality. The ash value of fermented EFB was very much lower than that of POME, an indicator of batter quality material as a feed resource.

5.1.3 Animal feeding test

At the fermentation stage, about 300 gm of fermentation substrates were packed in polypropylene bags. A total of one ton samples were prepared for this test. The samples were irradiated with ^{60}Co (1.6 MCi) gamma-rays for 10 kGy and inoculated with 10% seed of *P. sajor-caju*. Samples were incubated in fermentation room at least 4 weeks at 25℃ with humidity controlled between 80 - 90 %.

Animal studies were started by comparing this sample to some typical oil palm by-products. The results of dry matter degradation in the rumen of cattle (nylon bag technique) were compared to other oil palm by-products (Table 5.1-6).

Degradation data showed that fermented EFB was moderately degradable. The degradation values of 56.7% for fermented EFB at 48 hr was comparable to most tropical grasses grown in the country. More than 95% of the degradable DM were degraded within 48 hr suggesting that prolonged incubation up to 72 hr did not significantly increase the extent of degradation. The high degradation value of 46.4% at 6 hours for fermented EFB reflect a high content of readily soluble material and chemical composition data showed that fermented EFB had about 34% cell contents. The highly degradable contents in fermented EFB may be due to the presence of fungi fruiting bodies and mycelia.

In the *in vivo* study, eight mature Kedah Kelantan bulls (4 animals/treatment) weighing approximately 250 kg were kept in individual pens and fed with basal diet (OPT silage + concentrate) and experimental diet (33% fermented EFB + basal diet). Feed intake and dry matter digestibility values were estimated by the substitution method as described by Schneider and Flatt (1975). The composition, dry matter

intake and dry matter digestibility of the basal and experimental diets are shown in Tables 5.1-7 and 5.1-8.

The dry matter intake of the experimental diet was comparable to the basal diet suggesting that fermented EFB was as palatable as the basal diet. *In vivo* digestibility values in cattle showed that fermented EFB had DMD of 34.6% which is lower than value reported for OPF but higher than values for PPF (21.8%). A comparison of the nutritive values of fermented EFB with other oil palm by-products is shown in Table 5.1-9.

Nutritive value data on TDN and ME were consistent with the degradation data. Higher TDN and ME values were obtained for PKC and POME with PPF having the lowest. Fermented EFB, OPF and OPT were moderate quality feeds for ruminants. Observations from feeding trials showed that fermented EFB was palatable and readily consumed by cattle. As long as the physical structure of the shredded wet fermented EFB is mixed with concentrate in complete ration for cattle, it is expected that a higher inclusion level of up to 50% can be incorporated in practical rations for rowing and fattening cattle.

5.1.4 Conclusion

Irradiation and fermentation treatments have been shown to reduce the fiber content (CF, NDF, ADF) and improve the digestibility of fermented EFB. Fermented EFB when added at inclusion levels of 33% in the ration was shown to be readily consumed by cattle with intake values comparable to OPF silage diets. Further studies need to be implemented to determine the optimal level of inclusion of fermented EFB in practical rations for ruminants and to study the long term effect on livestock, As long as the technology for producing this feed is economically feasible (collection, processing); there will be a tremendous potential for the utilization of EFB in the feeding system of ruminants due to the large amounts of EFB available in the country.

Table 5.1-1. Variation of chemical composition in fresh EFB

Chemical Composition in Fresh EFB (Dry Matter basis)	
Crude Protein (CP)	4.2 ± 0.3%
Crude Fibre (CF)	42.8 ± 4.6%
Neutral Detergent Fibre (NDF)	82.6 ± 5.5%
Acid Detergent Fibre (ADF)	59.5 ± 5.4%
Acid Detergent Lignin (ADL)	17.1 ± 2.7%

Table 5.1-2. Nutritional values of the lime treated fermented EFB

Treatments	Chemical composition (% dry matter basis)				
	CP	CF	NDF	ADF	ADL
Control	5.9	40.9	81.8	63.1	18.2
5 weeks Soaking	6.9	22.2	60.9	54.6	15.5
Mixed	7.0	22.1	59.8	46.7	11.6

After harvest the mushroom.

Table 5.1-3. Nutritional values of fermented EFB after various incubation periods

Incubation period (weeks)	Chemical composition (% dry matter basis)				
	CP	CF	NDF	ADF	ADL
CONTROL	5.9	40.9	81.8	63.1	18.2
5	6.8	22.2	60.9	54.6	15.5
6	6.9	20.6	58.5	50.1	12.8
7	7.3	20.6	62.0	51.9	11.9
8	7.1	17.5	58.1	46.6	11.5

Table 5.1-4. Comparison of nutritional values of fermented products before and after mushroom fruiting

Chemical composition (% dry matter basis)			
	Before fermentation	After fermentation at 25℃	
		Fruiting	Non-fruiting
CP	5.9± 0.1	6.9 ± 0.1	8.4 ± 0.5
CF	40.9±0.4	22.2 ± 0.3	31.5 ± 0.9
NDF	81.8±0.1	60.9 ± 0.2	77.5 ± 0.5
ADF	63.1±0.1	54.6 ± 0.9	58.8 ± 0.5
ADL	18.2 ±0.6	15.5 ± 1.3	20.0 ± 1.6

Table 5.1-5. Comparison of chemical composition of fermented EFB to other oil palm by-products

	Chemical composition (% dry matter basis)							
	DM	CP	CF	NDF	ADF	ADL	EE	Ash
Untreated EFB	89.6	3.9	51.4	94.2	85.9	n.a.	2.5	4.8
Fermented EFB	24.4*	8.2	25.7	66.0	63.2	n.a.	1.6	10.5
PKC	89.8	17.2	17.1	74.3	52.9	n.a.	1.5	4.3
PPF	91.2	5.4	41.2	84.5	69.3	n.a.	3.5	5.3
POME	89.0	12.5	20.1	63.0	51.8	n.a.	11.7	19.5
OPT	27.3	2.8	37.6	79.8	52.4	n.a.	1.1	2.8
OPF	25.5	4.7	38.5	78.7	55.6	n.a.	2.1	3.2

n.a., not analyzed; *dry matter of the fermented products before drying.

Table 5.1-6. Digestion of fermented EFB and other oil palm by-products in the rumen of steers

Feed	Degradation of DM (%)			
	Time of incubation (h)			
	8	24	48	72
FEFB	46.4*	52.0	56.7	55.8
PKC	27.0	46.7	69.3	78.6
PPF	13.8	17.6	19.8	21.8
POME	36.2	58.2	67.0	70.2
OPT	17.8*	25.9	39.3	41.1
OPF	12.8*	20.2	36.3	38.0

* Values taken at 6 h (Abu Hassan et. al., 1992)

Table 5.1-7. Composition of experimental and basal diet

Feed ingredient	(% DM basis)	
	Basal diet	Experimental diet
OPF silage	51.7	34.6
Concentrate*	48.3	32.4
Fermented EFB	-	33.0

*The concentrate consists of 60% PKC, 30% tapioca chips, 4.5% rice bran, 1 % limestone, 1% Dicalcium phosphate, 1% salt and 2.5 % urea (Abu Hassan et. al., 1992).

Table 5.1-8. Dry matter intake, dry matter digestibility of basal and experimental diet using feed materials fermented at 25℃

	Dry matter intake kg/ day			Total Dry matter intake (kg)	Dry matter digestibility of rations (%)	Digestibility by difference / fermented EFB (%)
	OPF silage	Concentrate*	fermented EFB			
Basal	2.52	2.36	-	4.88	54.44 ±1.60	
Experimental	1.59	1.48	1.51	4.58	47.88±3.01	34.56

*The concentrate consists of 60% PKC, 30% tapioca chips, 4.5% rice bran, 1 % limestone, 1% Dicalcium phosphate, 1% salt and 2.5 % urea.

Table 5.1-9. Estimated nutritive value of fermented EFB compared to other oil palm by-products for ruminants

Feed	TDN (%)	ME (MJ/kg)
Fermented EFB*	38.0	5.298
PKC [a]	75.8	11.129
PPF [a]	27.8	4.210
POME [a]	62.5	8.368
OPT [b]	39.3	5.952
OPF *	47.8	6.963

a : values from cattle; b: values from sheep,
* calculated values from *in vivo* trials

5.2. Production of Mushroom

5.2.1 Introduction

EFB (empty fruit bunch) is a solid oil palm industry by-product which has a high water holding capacity (Table 3.2-5). This chemical characteristics is essential for mushroom cultivation (Ganney 1990). The potential of this materials is to be used in industrial scale production due to it huge volume availability and the interest of using the residue to animal feed. The initial contamination of microorganisms in EFB is very high, and theregore the decontamination is required before fermentation. Substrate pasteurization by heat treatment takes 4 - 5 hr (Cairus 1990). It has also been reported that 15 hr is required to pasteurized the substrate at 70℃. Gamma irradiation offer a faster and uniform pasteurization technique without changing the physico-chemical properties of the material (Table 3.2-3). For 10 kGy pasteurization, it takes 1 hr with the dose rate of 10 kGy / hr. By using electron accelerator, a volume of 100,000 ton can be process.

Among many kinds of fungi, *P. sajor-caju* was selected as the most suitable strains for the fermentation of EFB. Mushroom of *P. sajor-caju* is called as oyster mushroom and cultivated popularly in south Asia. Thus, the objective of this study is to utilize this easily available by-product of the agro-waste industry by radiation pasteurization for cultivation of mushroom for culinary purposes at industrial scale.

5.2.2 Materials and methods

(1) Empty fruit bunch (EFB) of oil palm

Freshly shredded EFB fiber of mean gross length 9.1 ± 3.4 cm was obtained from Southern Realty Mill, Kapar, Klang, Malaysia. The fiber were left under shade to be air dried with residual moisture content of 12% (the moisture content of sun-dried fibers was 5% and it was increased to 10 - 12% when left at room temperature under shade for storage). The fiber length was further reduced to a mean gross length of 3.5 ± 1.3 cm.

(2) Preparation of substrates

About 40 kg of cut fiber was placed in a fiber glass water tank of 100 gallon capacity and filled with tap water. For mixing of each batch, the wet fiber with 2% $CaCO_3$ (moisture content 70%) was transferred into a mixer and mixed thoroughly with 5% (0.59 kg) rice bran, added portion wise to the mixing fiber. The homogenized fiber was piled up on the floor, covered with a polyethylene sheet or returned back to fiber glass

water tank and covered. This fermentation substrate is used after 3 days.

(3) Seed preparation

Pleurotus sajor-caju (Fr.:Fr.)Fr. and other fungi such as *Pleurotus djamor* (Fr.:Fr.) Boedijin. (=*Pleurotus flavellatus*), *Pleurotus cystidiousus* and *Auricularia polyticha* were incubated on the sterilized wheat grain of about 150 g per 500 ml bottle and used as seed.

(4) Cultivation of Mushroom

1) JAERI procedure

EFB was mixed with rice bran (ca. 20%) and the moisture content was adjusted to 60%. The EFB substrates (ca. 420 g) were put into polypropylene bottles (880 ml) and sterilized by 30 kGy irradiation or by autoclaving at 121℃ for 40 min. The inoculated samples were incubated at 25℃and 65% RH for mycelium growth and then moved to the mushroom cultivation room controlled at 22℃ and 90% RH for fruiting.

2) MINT procedure

About 300 g of fermentation substrate were packed in a polypropylene bag and closed with a perforated plastic cap of 2.5 cm diameter, padded with cotton wool. The bag was pasteurized by ^{60}Co (1.6 MCi) gamma-rays at 10 kGy, then directly inoculated with 30 g seed of *P. sajor-caju*. The inoculated substrate was incubated at 25℃ for 4 - 10 weeks. For fruiting, the mushroom bags fully grown with mycelium were fully open and placed horizontally on the fruiting rack, arranged in layers of four bags per row (Photo. 5.2-1). The bags were sprayed with tap water twice a day in the morning and afternoon. The number of mushroom fruit bodies and their fresh weight per bag appeared on the fifth or sixth days were recorded.

Biological Efficiency (BE) was defined as the ratio of fresh weight of mushroom to dry weight of substrates (Chang, 1982). BE calculated in this work were based on first flush of mushroom production. The yield per bag is the average weight of fresh mushroom in gram.

(5) Analysis of fermented products

The product was freeze-dried before analysis. The crude protein was determined by Kjeldahl method and fiber components were determined by using Fibertec system 1010 (Tecator, Sweden).

5.2.3 Results and discussion

(1) Mushroom production by *P. sajor-caju* obtained at JAERI

The mushroom cultivation of *P. sajor-caju* was examined prior to produce animal feeds using various conditions of EFB substrates. Table 5.2-1 shows the results of mushroom cultivation of *P. sajor-caju*. Mushroom cultivation on sawdust of *Cryptomeria* (Japanese cedar; sugi) media was also included for the comparison. The growth of mycelium was faster on autoclaved EFB substrate than the irradiated one, and the total time for cultivation was also slightly shorter. However, the yield of mushrooms from irradiated EFB substrate was higher and quality of cultivated mushrooms was better than that of autoclaved substrate. *P. sajor-caju* can grow well and produce mushrooms on EFB substrate without pH adjustment or washing although the yields decreased. It is very helpful for the practical purpose if the pH adjustment and washing are not required. Further study will be continued to establish the suitable conditions for high yields and the easier handling. The yields of mushroom from sawdust substrate were lower than that of EFB substrate. Usually the hole in center of the substrate is made for the easier growth of mycelium when the bottle is used for the cultivation. However, the hole in both substrates were not made in this experiment because it was difficult to make hole in EFB substrate. The mycelium in some bottles on sawdust did not reach the bottom because of compression of the substrates due to small size of its particles. EFB substrate is fibrous in nature which provided enough aeration for the growth of fungi to the bottom.

The contents of crude fiber and crude protein in residual substrates after mushroom cultivation were 23 - 28% and 9 -11%, respectively. These residues still contain high protein and can be used as animal feeds. From these results, it can be concluded that the EFB is a good substrate for mushroom and animal feeds production. Although further study is necessary to clarify the reason for the delay of mycelial growth on irradiated substrates, it is considered that the radiation treatment is suitable to sterilize a large amount of substrates continuously.

(2) Study at MINT

1) Selection of suitable mushroom strain

P. sajor-caju, *P. cystidiosus* and *Auricularia polytricha* are strains of edible mushroom i.e. gray oyster mushroom, abalone mushroom and black jelly mushroom, respectively. Table 5.2-2 shows BE of *P. sajor-caju*, *P. flabellatus*, *P. cystidiosus* and *Auricularia polytricha* mushrooms grown on EFB substrate soaked in lime solution. *P.*

sajor-caju has the highest BE of 63.7% which indicates a good yield out of the EFB substrate. This is better than cultivation on sawdust with 50.5% yield (Lee 1992). Other mushrooms that can also be grown were *P. flabellatus* (40.4% BE) and *P. cystidiosus* (47.5% BE). *A. polytricha* however, only achieved 5.8% B.E.

It has been reported that the production yields using commonly used substrate such as waste paper and cotton fibers for gray oyster mushroom was 40%, black jelly mushroom 50% and white oyster mushroom 35% (Noor 1992, Lee 1992). Cost analysis of their work indicates that the project is viable for with this production condition. From the results in Table 5.2-2, *P. sajor-caju* was selected for further study to establish the cultivation conditions on EFB.

2) Effect of aeration

The effects of aeration on the fruiting of *P. sajor-caju* are shown in Table 5.2-3. With limited aeration, through a cap of 2 cm diameter, BE was 38.1% after incubating for four weeks. However, for the same incubation period, BE of 63.7% was recorded when the top of the polypropylenebag was fully opened (Table 5.2-2).

Aeration is one of the factors for production of mushroom. It is necessary, especially during cropping once temperatures are elevated because of an increase in CO_2 production (Georgina 1991, Chang 1982).

3) Effect of pre-treatment of EFB with lime

For the comparison of lime treatments on cultivation, the growth of mycelium has been analyzed. The results indicate that, the treatment with lime solution made EFB better for the growth of mycelium. The full growth of the mycelium (*P. sajor-caju*) in propylene bag (packed with 400 g EFB substrate) took only 10 days. It was observed that the mycelium growth was thicker as it propagated over the substrate. However, in the lime powder mixed EFB, the mycelium grew slower - 12 to 13 days. The mycelium growth was thinner and slowly became thicker after it has fully grown on the substrate.

Table 5.2-4 shows the production of *P. sajor-caju* cultivated on the lime-soaked EFB substrate, incubated for 4, 5, 6, 7, 8, 9 and 10 weeks. The BE of *P. sajor-caju* was 73.8%, after 4 weeks incubation. Prolonging the incubation period further has no significant effect on the production of the mushroom. This indicates that soaking the EFB in the lime solution has conditioned the substrate faster, suitable for the growth of the mycelium. As a result the mycelium fully utilized the nutrient in the substrate within 4 weeks.

However, an attempt to grow mushroom on the EFB substrate mixed with lime powder has resulted in a lower yield. BE of the mushroom cultivated on this substrate was 48.1 % (4 weeks incubation) as shown in Table 5.2-5. The BE increased to 54.6 %, after 5 weeks incubation. About 60% BE was achieved by incubating for 6 weeks, and BE was 62.3% for 7 weeks incubation. Evidently, with the lime powder treatment the conditioning process of the substrate surface was slower resulting in a slower growth of the mycelium.

From these results, it can be concluded that the lime treatment soaked in solution is effective for the cultivation of mushroom and the BE was reached a maximum after 4 weeks incubation.

5.2.4 Conclusion

P. sajor-caju was selected from other mushrooms i.e. *P. flabellatus*, *P. cystidiosus* and *A. polytricha* based on the Biological Efficiency (BE) from trial growth on lime soaked EFB substrate. The substrate treatment, aeration for fruiting and incubation period of the selected mushroom i.e. *P sajor-caju* on the EFB substrate was further investigated. BE of the *P. sajor-caju* grown on the lime-soaked EFB substrate and incubated for four weeks was 73.8%. The study also showed that four weeks incubation period was sufficient for fruiting. Aeration was also found necessary for fruiting and gave a better yield.

Table 5.2-1. Mushroom Production from EFB by P. sajor-caju and Composition of Residue

Sample	Sterilization method	Mushroom production			Composition of residue	
		Full growth of mycelium (day)	Cultivation time (day)	Yields (g)	Crude fiber (%)	Crude protein (%)
EFB, pH adjusted	30 kGy	29.6	44.5	87.9	23.9	9.7
EFB, pH adjusted	Autoclave	25.1	38.9	80.9	24.9	11.0
EFB, Washed	30 kGy	22.4	39.1	62.1	27.6	9.2
EFB, Untreated	30 kGy	31.3	45.3	72.0	22.8	10.0
Sawdust	30 kGy	25.8	37.2	54.2		
Sawdust	Autoclave	21.8	33.7	51.8		

Table 5.2-2. Production of mushrooms on EFB substrate

Mushroom	Weight of EFB (g)	Moisture (%)	No. of bag	dry matter /bag (g)	Mushroom yield/bag (g)	Biological efficiency (%)
P. sajor-caju	500	68.0	10	159.9	101.9 ± 39.3	63.7 ± 24. 6
P. flabelatus	500	68.0	10	159.9	64.6 ± 18.4	40.4 ± 11. 5
P. cystidiosus	500	68.0	10	159.9	75.9 ± 17.2	47.5 ± 10. 8
A. polyticha	500	68.0	10	159.9	9.2 ± 7.2	5.8 ±. 4.5

Table 5.2-3. Production of mushroom (*P. sajor-caju)* on the lime soaked EFB with limited aeration

Incubation period (w)	Weight of EFB (g)	Moisture (%)	No. of bag	Dry weight /bag	Mushroom yield (g)	Biological efficiency (%)
4	50, 000	70	294	149.7	57.0	38.1 ± 9.1
5	75, 000	70	450	146.7	41.9	28.6 ± 2.9

Table 5.2-4. Production of oyster mushroom (*P. sajor-caju*) on EFB substrate soaked in lime solution

Incubation period (w)	Weight of EFB (g)	Moisture content (%)	No. of bag	Dry matter /bag (g)	Mushroom yield/bag (g)	Biological efficiency (%)
4	15,000	69.9	30	150.5	111.1 ± 9.2	73.8 ± 6
5	15,000	69.9	30	150.5	95.7 ± 6.5	63.6 ± 7.5
6	15,000	69.9	30	150.5	109.8 ± 6.5	72.4 ± 7.5
7	15,000	69.9	30	150.5	107.6 ± 0.3	71.5 ± 0
8	15,000	69.9	30	150.5	91.5 ± 3.0	60.8 ± 0
9	15,000	69.9	30	150.5	104 .9 ± 5.5	69.7 ± 6.3
10	15,000	69.9	30	150.5	92.2 ± 9.0	61.2 ± 0. 6

Table 5.2-5. Production of *P. sajor-caju* on EFB substrate mixed with lime powder

Incubation period (w)	Weight of EFB (g)	Moisture content (%)	No. of bag	Dry matter /bag (g)	Mushroom yield/bag (g)	Biological efficiency (%)
4	15,000	66.5	30	167.5	80.5 ± 3.8	48.1 ± 4.0
5	15,000	66.5	30	167.5	91.4 ± 5.1	54.6 ± 5.3
6	15,000	66.5	30	167.5	99.9 ± 2.0	59.6 ± 2.1
7	15,000	66.5	30	167.5	104.4 ± 3.3	62.3 ± 0.7
8	15,000	66.5	30	167.5	96.5 ± 3.0	57.6 ± 3.1
9	15,000	66.5	30	167.5	114.1 ± 9.8	68.1 ± 0.1
10	15,000	66.5	30	167.5	99.3 ± 18.1	59.3 ± 8.7

5.3 Microscopic Study of Fermented EFB

5.3.1 Introduction

By the chemical analysis, it is difficult to judge where the mycelium grow and which part is digested by fermentation. In this chapter, the differences of structures between raw EFB and fermented EFB were studied by using scanning electron microscopy.

5.3.2 Materials and methods

(1) EFB sample

EFB collected in Malaysia was cut with straw cutter (WSX-200, Kiya Seisakusho) and Willey mill (Marumasu VL-56). The final length was bellow 1 cm.

(2) Alkali treatment of EFB

A part of EFB was soaked in 1% (w/v) NaOH solution for 1 hr at room temperature for the analysis of alkali effect on enzymatic digestion of EFB. Then neutralized with HCl and dried in the air.

(3) Irradiation

EFB samples were put into plastic bags and irradiated at 50 kGy with Co-60 gamma-rays. Fermentation medium for *P. sajor-caju* which contains 1 % EFB was irradiated 30 kGy. The dose rate used was 5-10 kGy/hr.

(4) Enzymatic digestion

Five g of commercial enzyme, Driselase (from *Plyporus tulipiferae*, Kyowa Hakko Co. Ltd.) was suspended into 50 ml of distilled water and kept for 10 min at room temperature to precipitate the insoluble materials. Supernatant of 5 ml was added to 0.1 g of EFB samples and the mixtures were incubated at 30 °C for 2 days. After digestion, enzyme solution was removed by decantation, and remained fibers were washed with distilled water several times and dried.

(5) Fermentation of EFB by *P. sajor-caju*

One g of EFB was suspended into 100 ml of the Mandel's medium in a 300 ml volume of conical flask. The mixed medium was irradiated at 30 kGy. One piece of *P. sajor-caju* pre-fermented on Potato-Dextrose-Agar plate was inoculated to the sterilized medium.

Then incubated in the rotary shaker (100 rpm) at 30 °C for one week. After fermentation, EFB fiber was corrected by filtration and freeze-dried.

(6) Scanning electron microscopy

EFB samples were snapped in liquid nitrogen. The broken EFB samples were fixed vertically on metal stages by Dotaite reagent and then coated with Au. Samples were evaluated in the SEM at 15 kV.

5.3.3 Results and discussion

Scanning electron microscope was used for the observation of effect of alkali and/or irradiation to increase the enzymatic digestibility of EFB and growth of *P. sajor-caju* on EFB and digestion of the fiber. Figures 5.3-1 (A) - (C) show the section, vascular bundle and surface of low EFB, respectively. In Fig. 5.3-1 (A) showing the section of EFB fiber, a few large tubes (app. 50 - 100 μm diameter) and a lot of small (< 10 μm diameter) tubes were observed in the fiber. Both of them were presumed xylem and phloem. However, cortex tissue could not observed in the fiber. Figure 5.3-1 (B) shows the fine structure of vascular bundles. Xylem was covered with packing tissue, such as parenchyma. Parenchyma filled the space among the xylem. The surfaces of the tubes (inside) was not rough. Figure 5.3-1 (C) shows the surface of EFB fiber. The surface of the EFB was smooth. In this picture, microorganisms were observed on the surface of the fiber.

The digestibility of Driselase for untreated EFB was analyzed. Figure 5.3-2 (A) shows the vascular digested by Driselase. The thickness of xylem was smaller than that of before digestion and inside of the xylem became more rough than that of row EFB (Fig. 5.3-1 (B)). Parenchyma among the xylem was also digested by Driselase. The surface of the EFB fiber also digested by Driselase but the degree of digestion was little (Fig. 5.3-2 (B)). These results show that two days digestion was too short to obtained enough degradation of EFB.

Figure 5.3-3 shows the Driselase digestion of 50 kGy irradiated EFB. The degree of digestion was almost same as the unirradiated EFB (Fig. 5.3-2 (B)).

Figures 5.3-4 show before and after Driselase digestion of sections and surface of EFB fiber which treated with 0.1% NaOH and 50 kGy irradiation. Figure 5.3-4 (A) shows the effectiveness of alkali treatment on EFB. The surface of the tubes became more rough than that of low EFB (Fig. 5.3-1 (B)). Figure 5.3-4 (B) shows the section of EFB fiber after Driselase digestion. The thickness of the xylem was decreased by enzymatic digestion, and there were a lot of space between xylem. Inside of the xylem also digested by Driselase and

became uneven. These photos show that alkali treatment on EFB is effective to increase the enzymatic digestibility. The surface of EFB fiber also digested by Driselase (Fig. 5.3-4 (C)). Driselase digestion of EFB is mainly occurred at parenchyma but xylem is difficult to digest by enzyme. However, changes of the surface were smaller than that of inside of the fiber. Therefore, it presumed that surface tissue of EFB has rigid structure.

Figures 5.3-5 show the growth of *P. sajor-caju* on EFB fiber. Fig. 5.3-5 (A) shows the section of fermented EFB fiber. Mycelia of *P. sajor-caju* invaded into the vascular bundles and digest the parenchyma tissue. Most of the tubes presented at the middle were digested by mycelium. Fig. 5.3-5 (B) shows growth of *P. sajor-caju* and digestion of vascular bundles by *P. sajor-caju*. This picture shows *P. sajor-caju* grew well in the bundles and digest most of the parenchyma. The thickness of the bundles became very thin, but xylem elements still remained. This result shows that the parenchyma tissue is easy to digest and good substrate for *P. sajor-caju*, but the xylem element is too hard for *P. sajor-caju* to digest. The shapes of mycelia in this picture were string or sheet and the mycelia crossed bundles to bundles. Compare with this, mycelium of *P. sajor-caju* could observed at small space of the surface of EFB. Fig. 5.3-5 (C) shows the growth of *P. sajor-caju* on the surface of EFB fiber. This mycelium did not cover all of the surface of EFB, and the shape was different from the inside-mycelium (Fig. 5.3-5 (A) and (B)). Fig. 5.3-5 (D) shows the expanded *P. sajor-caju* mycelium on the surface of the fiber. A lot of globular particles presented on the mycelium. It speculates that they are immature sporophore. It means that this mycelium was matured tissue. These photos (A) and (B), and (C) and (D) show the two types of mycelium shape. One is like string or sheet which in the vascular bundles. Most of the mycelium belonged to this type (Fig.5.3-5 (B)). Another is shapeless mycelium which has immature sporophore. There were not so many number of this type on EFB (Fig. 5.3-5 (D)). It is possible to presume the propagation of *P. sajor-caju* on EFB fiber from these photos. *P. sajor-caju* digests parenchyma tissue first, and then comes out to the surface of EFB fiber and matures at there, and forms sporophore. On the other hand, another process is possible to presume. *P. sajor-caju* attaches to the surface of the EFB fiber, then comes into the vascular bundles, digests the parenchyma, and forms soporiferous.

5.3.4 Conclusion

The results show that *P. sajor-caju* digests mainly vascular bundle, especially parenchyma tissue of EFB. The remains after mycelial digestion is xylem element. Xylem element is quite hard for *P. sajor-caju* to digest. The surface of EFB also difficult to digest. *P. sajor-caju* attacks inside or sprit of EFB fiber, and then propagate to the surface and

digest. Finally, *P. sajor-caju* forms sporophore on the surface of EFB. Microscopic study is useful to observe the growth of mycelium on EFB and degree of digestion of EFB.

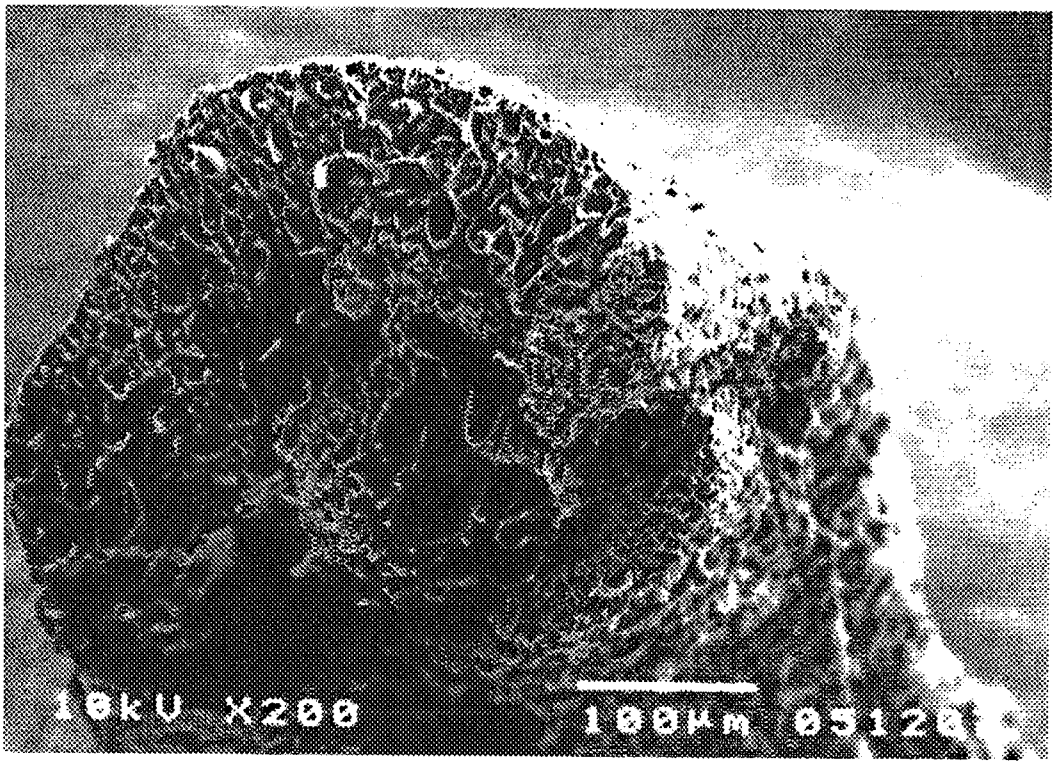


Fig. 5.3-1(A). Scanning electron micrographs: Section of raw EFB

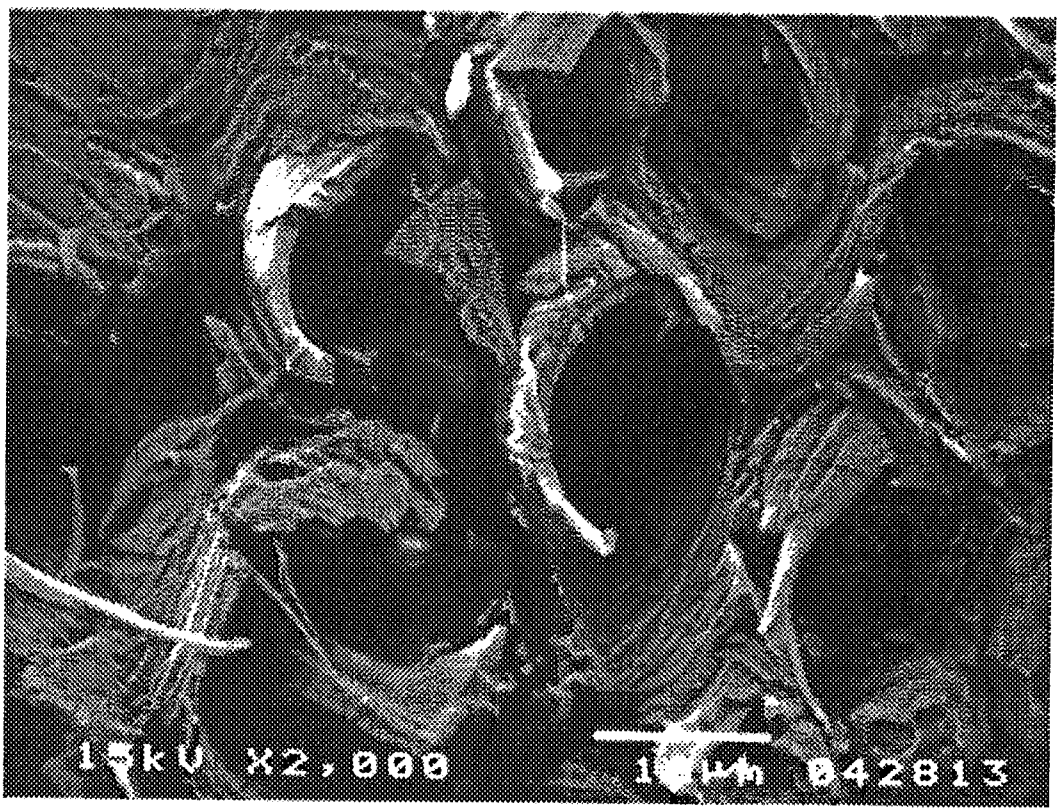


Fig. 5.3-1(B). Scanning electron micrographs: Fine structure of raw EFB section

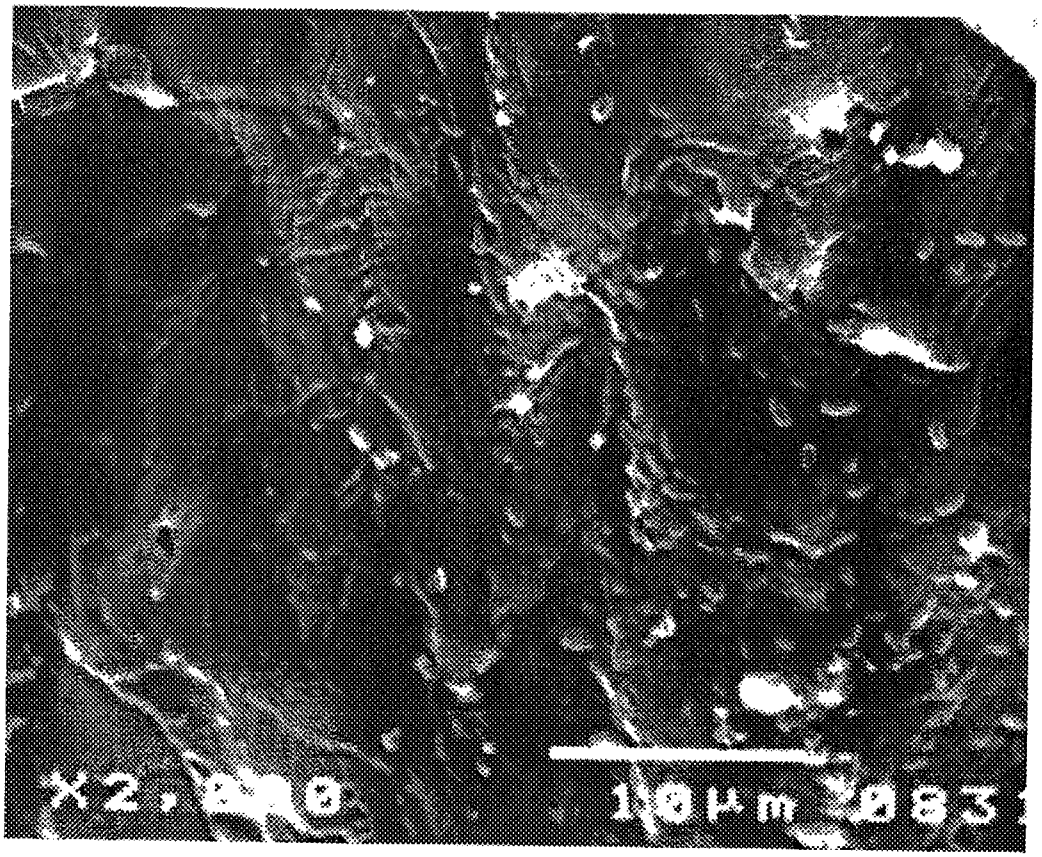


Fig. 5.3-1(C). Scanning electron micrographs: Surface of raw EFB fiber

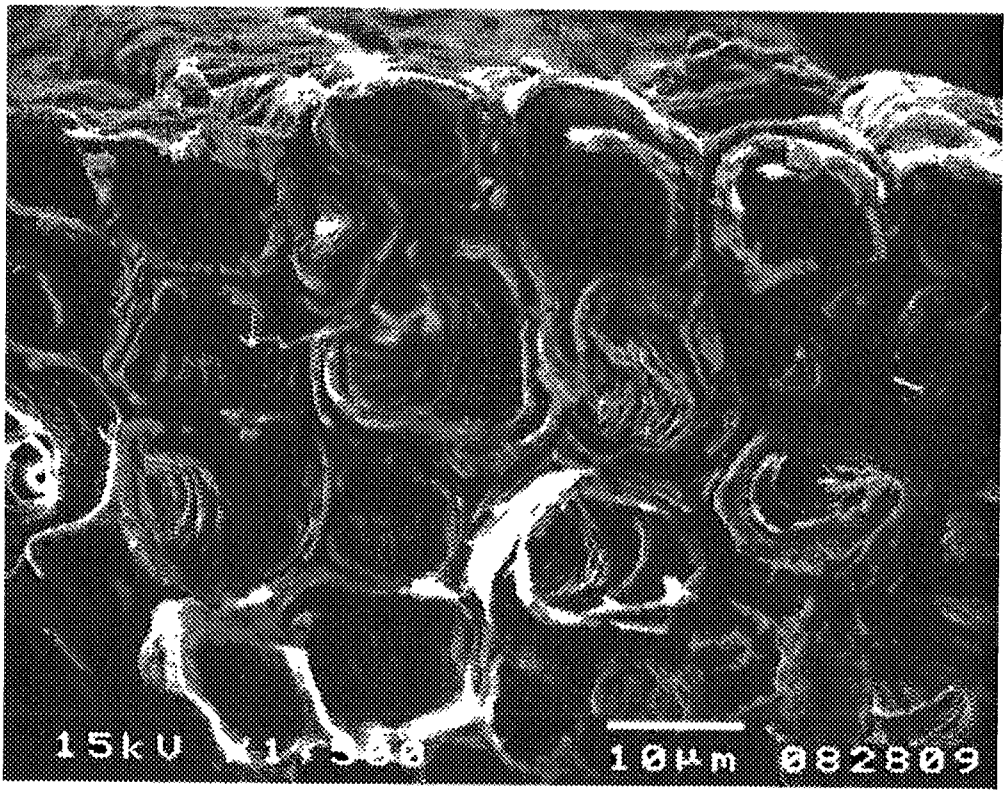


Fig. 5.3-2(A). Scanning electron micrographs: Section of Driselase digested raw EFB

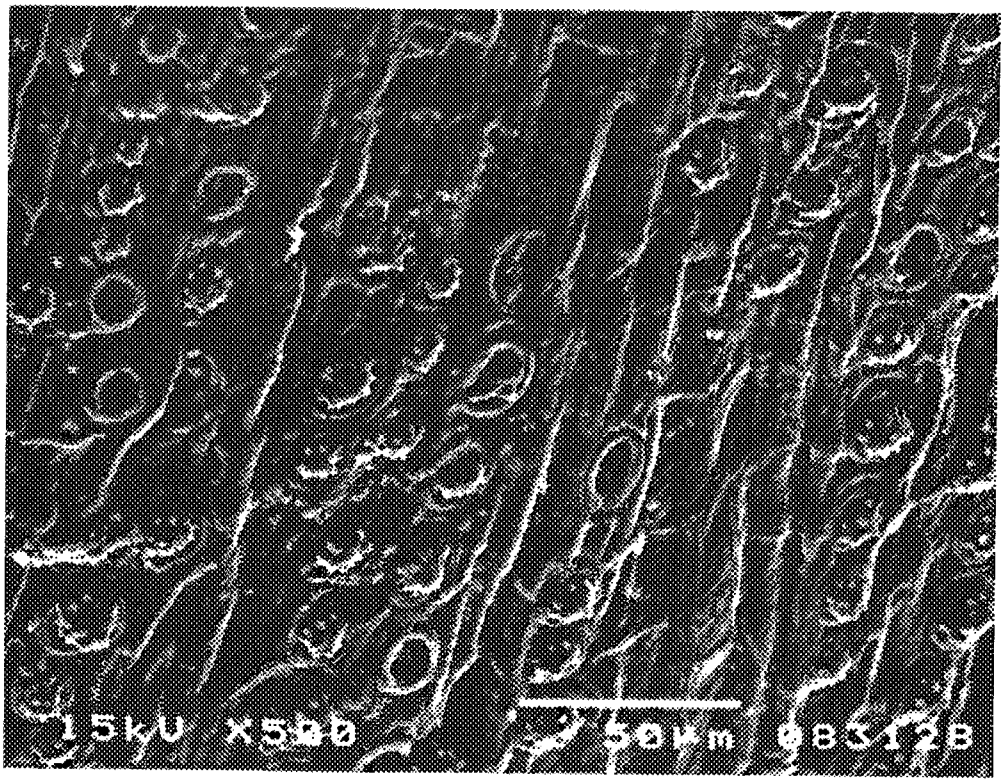


Fig. 5.3-2(B). Scanning electron micrographs: Surface of raw EFB fiber digested by Driselase

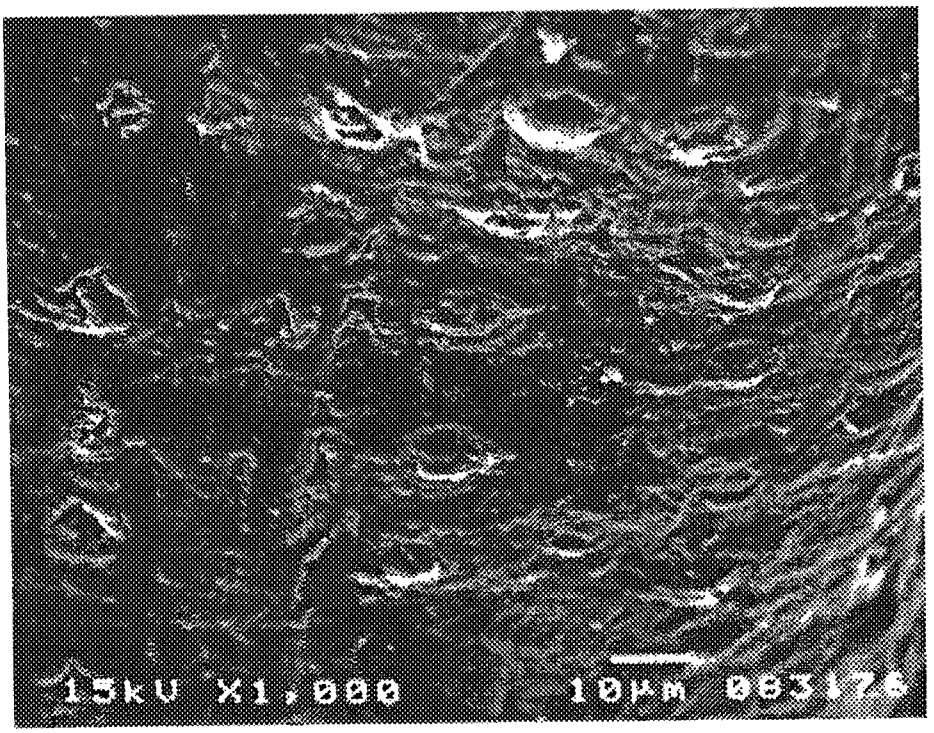


Fig. 5.3-3. Scanning electron micrographs: Surface of 50 kGy irradiated EFB fiber digested by Driselase

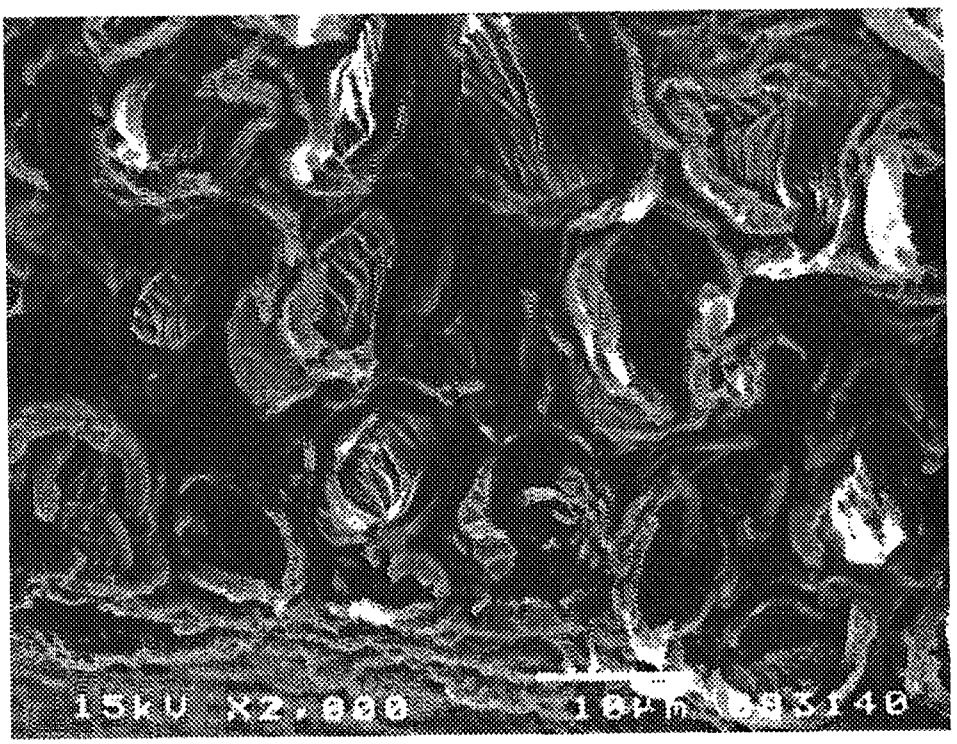


Fig. 5.3-4(A). Scanning electron micrographs: Section of alkali and irradiation treated EFB

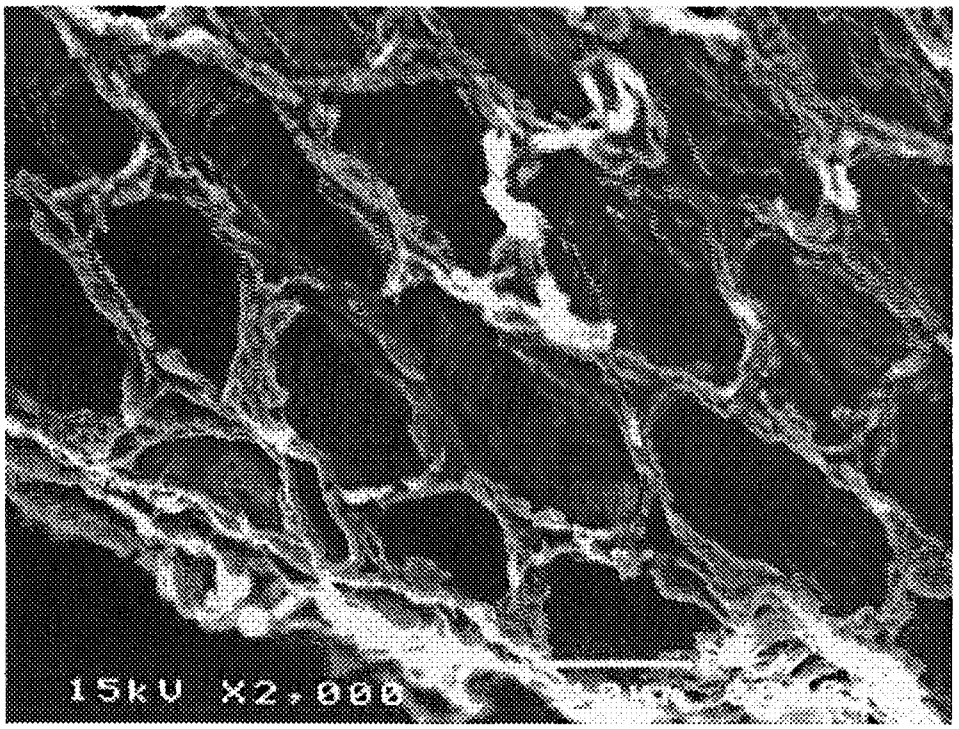


Fig. 5.3-4(B). Scanning electron micrographs: Residues of alkali treated EFB digested by Driselase

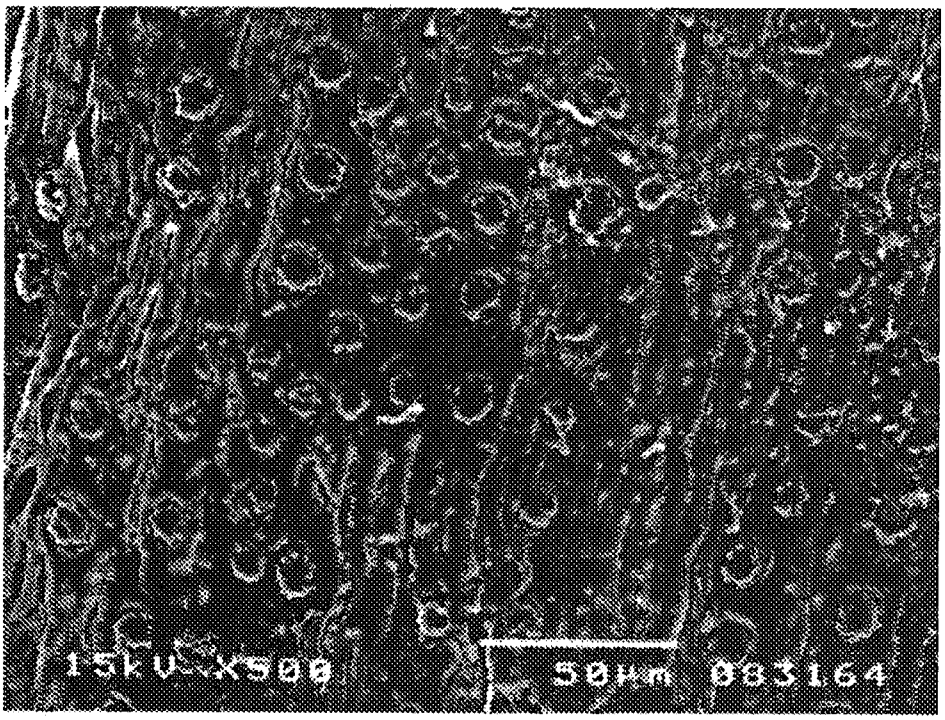


Fig. 5.3-4(C). Scanning electron micrographs: Surface of alkali treated EFB digested by Driselase

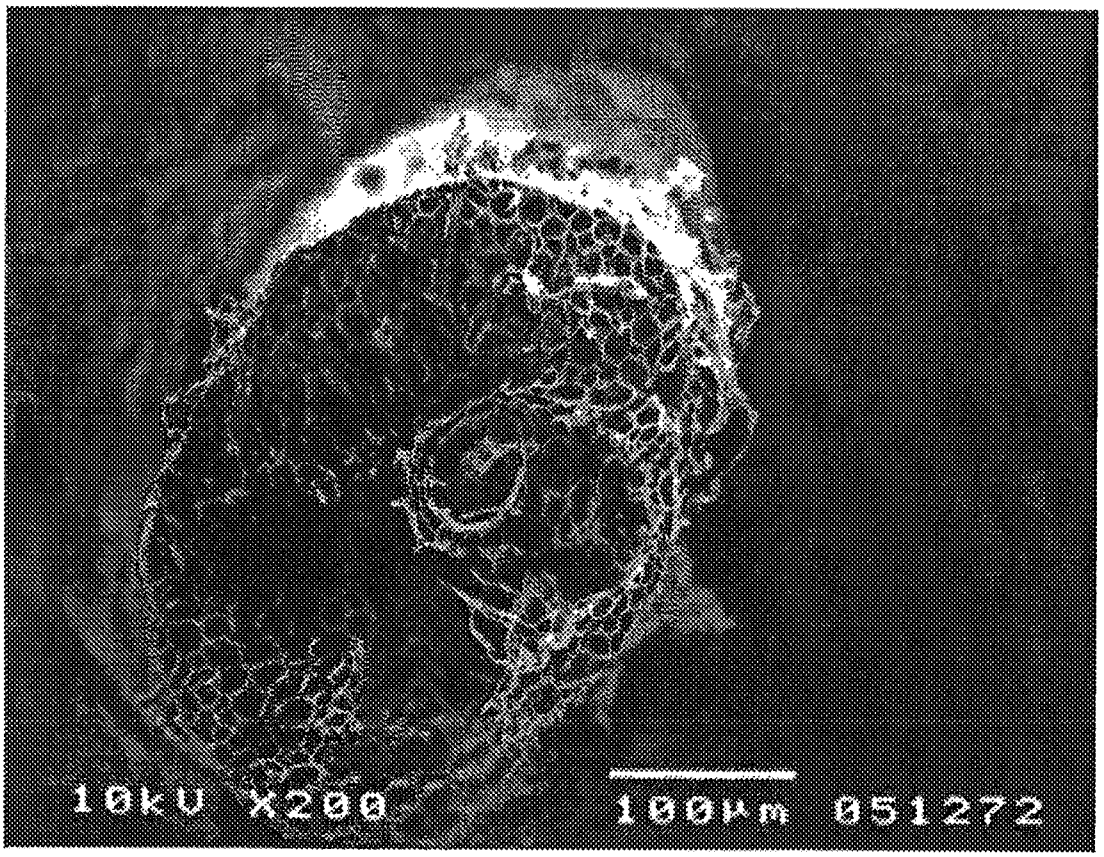


Fig. 5.3-5(A). Scanning electron micrographs: Section of fermented EFB by *P. sajor-caju*

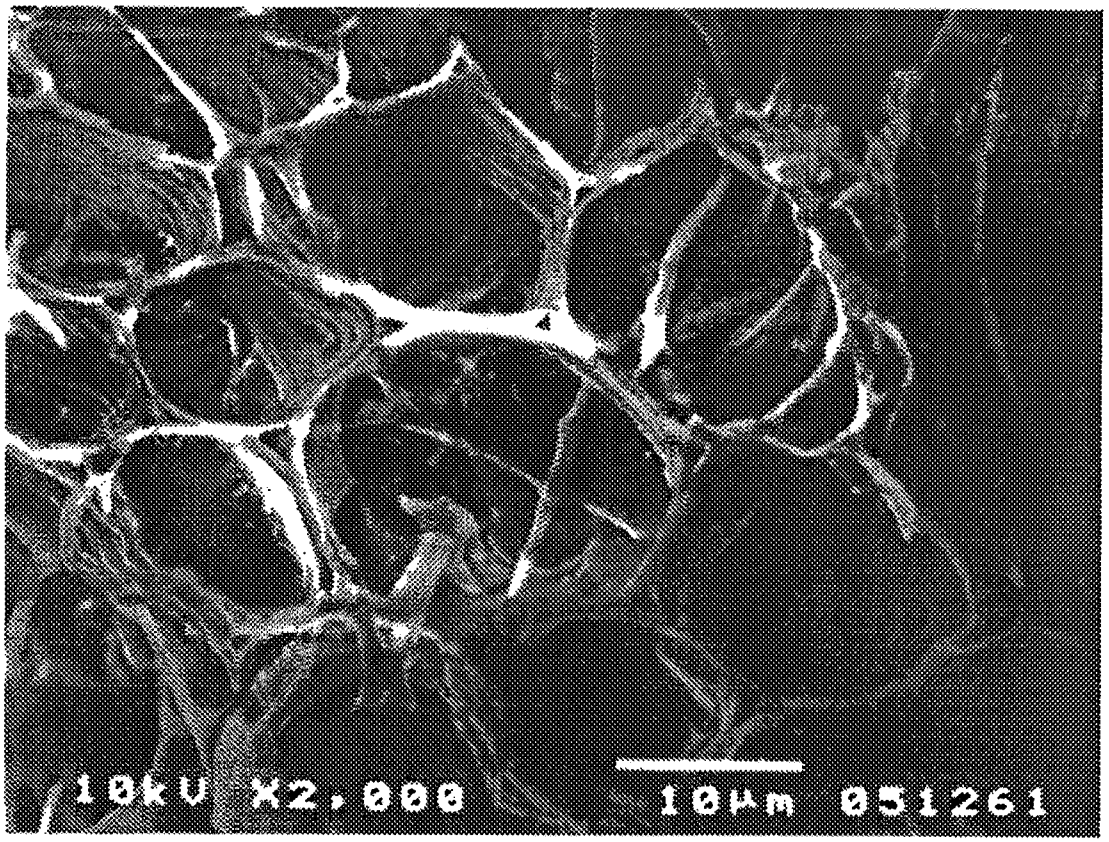


Fig. 5.3-5(B). Scanning electron micrographs: Digestion fo parenchyma by *P. sajor-caju*

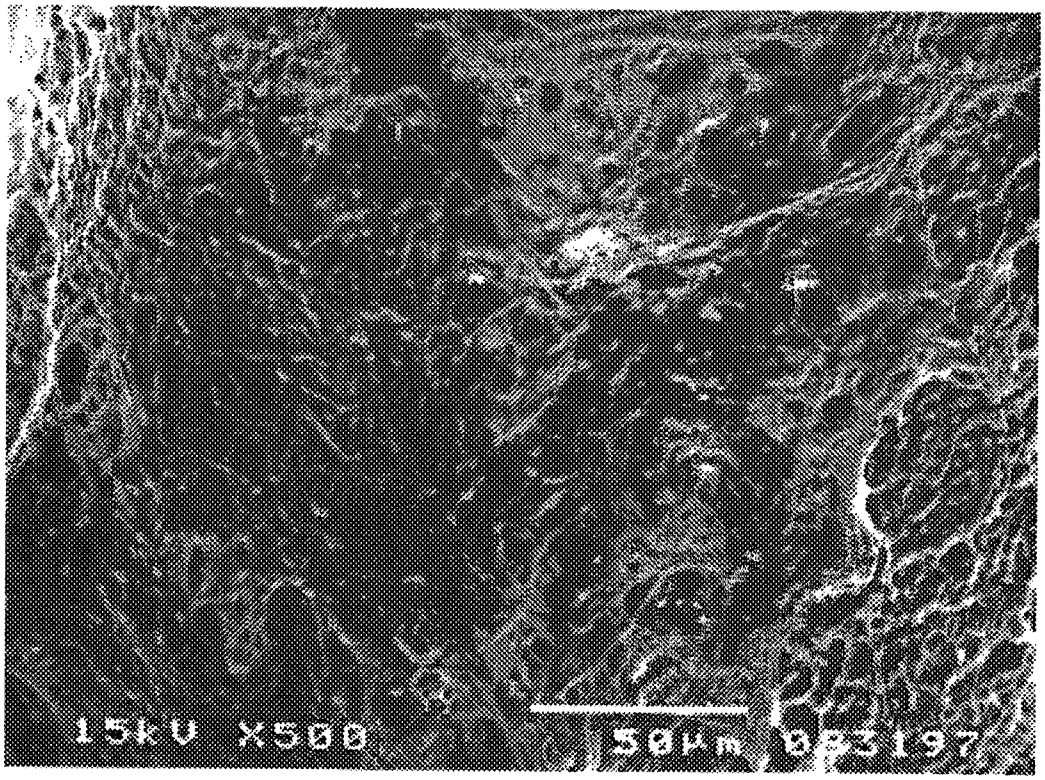


Fig. 5.3-5(C). Scanning electron micrographs: Growth of *P. sajor-caju* on surface of EFB

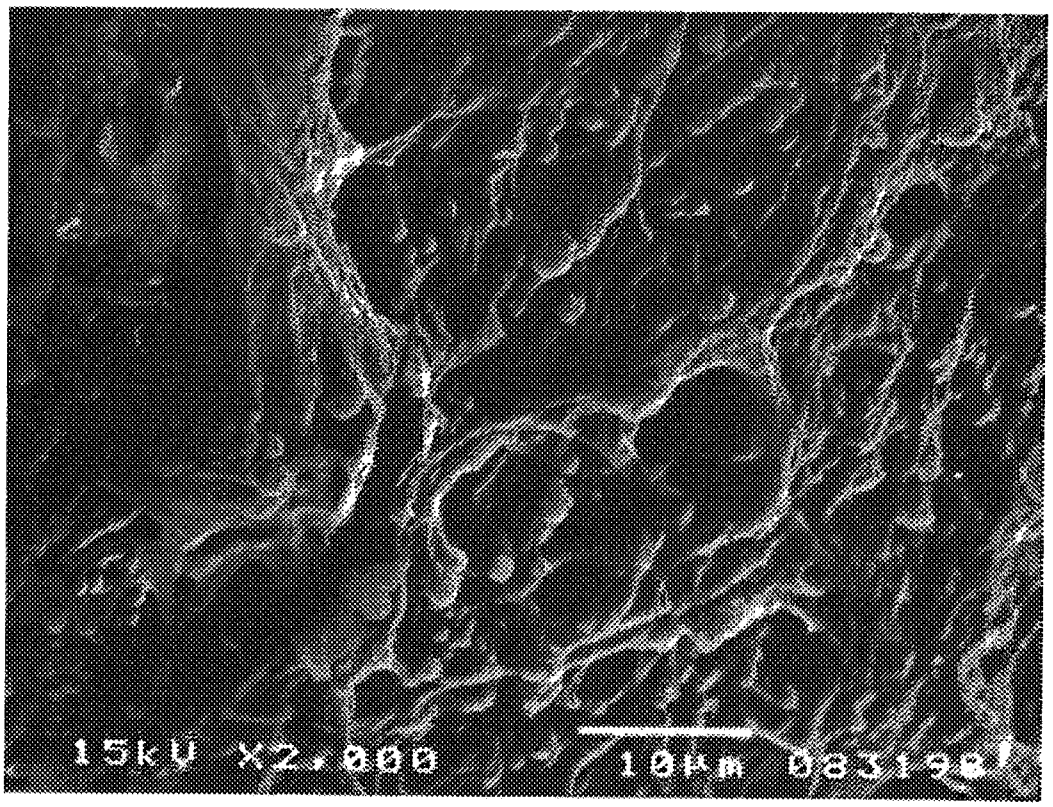


Fig. 5.3-5(D). Scanning electron micrographs: Immature sporophores of *P. sajor-caju*

6. PILOT SCALE STUDY AT MINT

6.1 Facilities at Sterifeed Pilot Plant

6.1.1 Introduction

The pilot plant named "Sterifeed" at MINT was opened on 13 June, 1996. The purpose of the plant is to produce 50,000 ton of animal feed to determine its commercial viability and to test its acceptability to animals, toxicity and nutritional value with MARDI (Malaysian Agriculture Research Development Institute) and UPM (Malaysia Agricultural University).

6.1.2 Facilities

The Sterifeed pilot plant consists with 1) physical treatment room, 2) inoculation room, 3) incubation room, 4) quality control room and 5) animal feed processing room. The plane view of pilot plant was shown in Fig. 6.1-1 and Photo. 6-1. The area of building is 1260 m^2 and the cost for construction was as follows:

Building:	1.0 Million RM
Earth work:	0.43 Million RM
Land:	0.138 Million RM
Mechanical and electrical:	0.5 Million RM
Total	2.068 Million RM

The EFB substrate prepared at physical treatment room are sent to SINAGAMA ^{60}Co- γ irradiation plant for pasteurization (or sterilization). After irradiation treatment, the samples are sent back to the Sterifeed pilot plant and inoculated with seed under sterile condition. Then the samples are incubated for 1 month under environmental conditions and the fermented products are pelletalized as the final products for anima feed.

For the process of EFB upgrading to animal feed, the Sterifeed pilot plant are equipped with various machines. The equipment and their specification are as follows:

Physical processing

- EFB cutting machine: rate, 15 kg/hr; power, 2.2 kW
- Mixer for substrate: rate, 30 kg/hr
- Hydraulic press
- Packaging (Manual)

Inoculation and incubation

Inoculation system: rate 100 kg/hr

Incubation facilities (rack + irrigation system): capacity, 15 ton/month

Product processing

Pellet mill: rate 0.5 ton/hr, power 20 hp

Mixer for preparation of EFB substrate is shown in Photo. 6-2. The liquid seed inoculator and inoculation room with clean bench are shown in Photo. 6-3 and 6-4. Photo. 6-5 and 6-6 show the incubation room and the mushroom products cultivated in the Sterifeed pilot plant after 1 month incubation. Photo. 6-7 shows the pellet machine and Photo. 6-8 shows the final products for animal feed by using the pellet machine.

6.1.3 Monitoring and establishing suitable environmental condition of the incubation house

One of the most important technical data required for pilot was environmental conditions, namely temperature and humidity, favorable for fungi or mushrooms to grow on EFB. Without any control, this facility will be regulated by natural condition with temperature variation between 23 - 33℃ and humidity between 60 - 100% as recorded at the other side mushroom house. However, under natural condition, the growth of *P. sajor-caju* was unfavorable (especially for temperature above 30℃). The optimum temperature for growth was 28℃ with the moisture content of substrate being maintained between 60 - 70%. In order to achieve these conditions, some elements of controls must be introduced but minimum investment. As reflected by the moisture content (60 - 100%) of the substrate, the humidity of the facility should be maintained at 60% or above, to avoid evaporation of water from substrate. This data confirmed that controlling humidity was not a problem as the environmental humidity always be at least 60% or above. Therefore, controlling temperature was necessary. One of the most practical technique is by spraying water to the blind, during high intensity of sun light.

6.1.4 Conclusion

The construction of pilot plant named "Sterifeed" at MINT was completed in 1996 to produce a large amount of feed for animal test and to test its acceptability to animals, toxicity and nutritional value. The plant is operating to produce 50,000 ton of animal feed. Through the pilot plant study it is also testing its commercial viability.

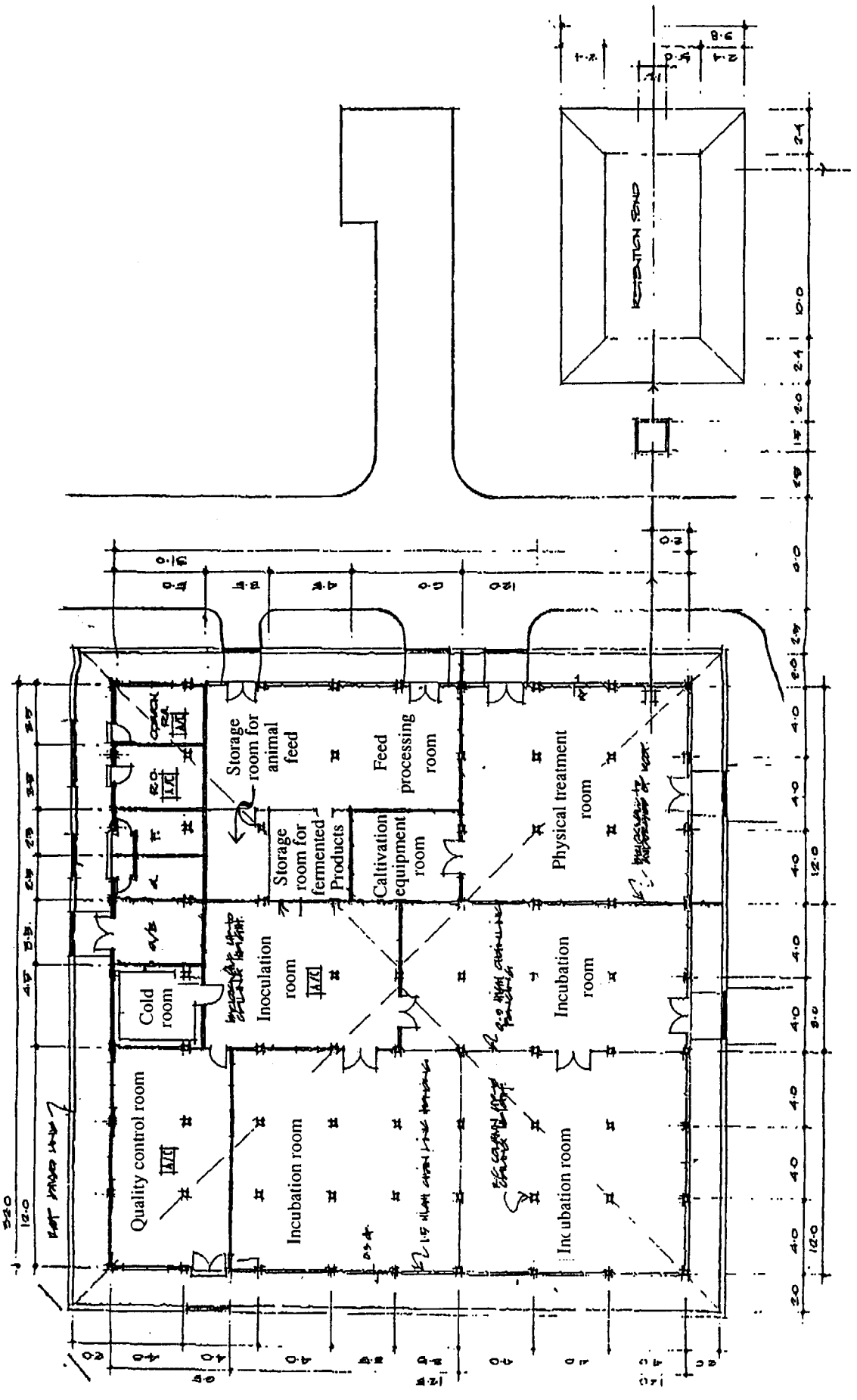


Fig. 6.1-1. Plane View of Sterifeed pilot plant

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Photo. 6-1. View of Sterifeed pilot plant

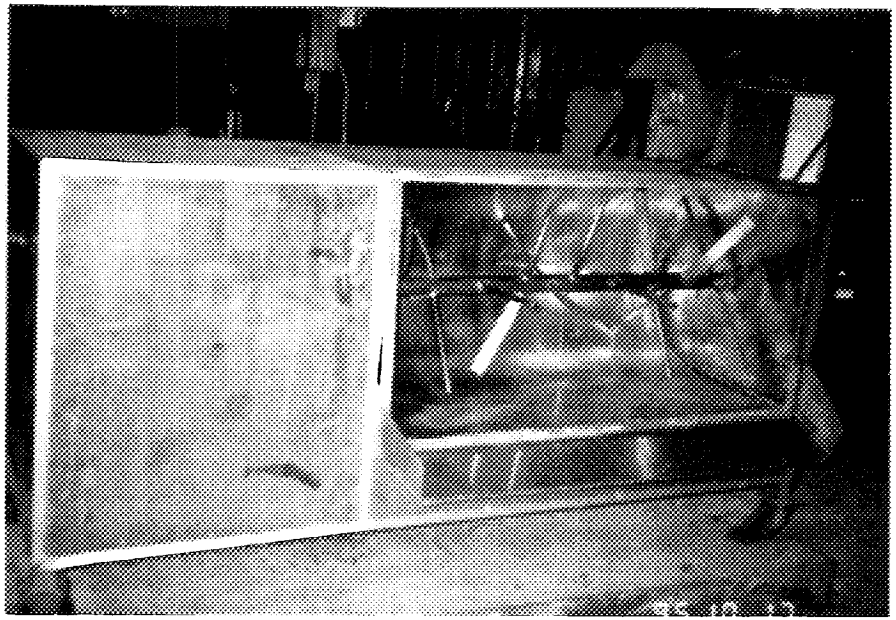

Photo. 6-2. Mixer for preparation of EFB substrate

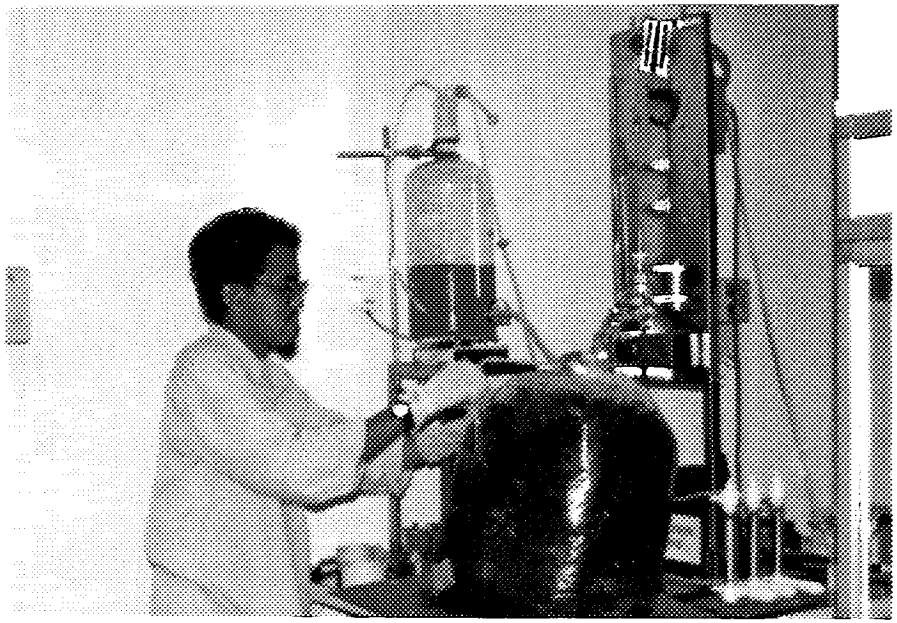

Photo. 6-3. Liquid seed inoculator

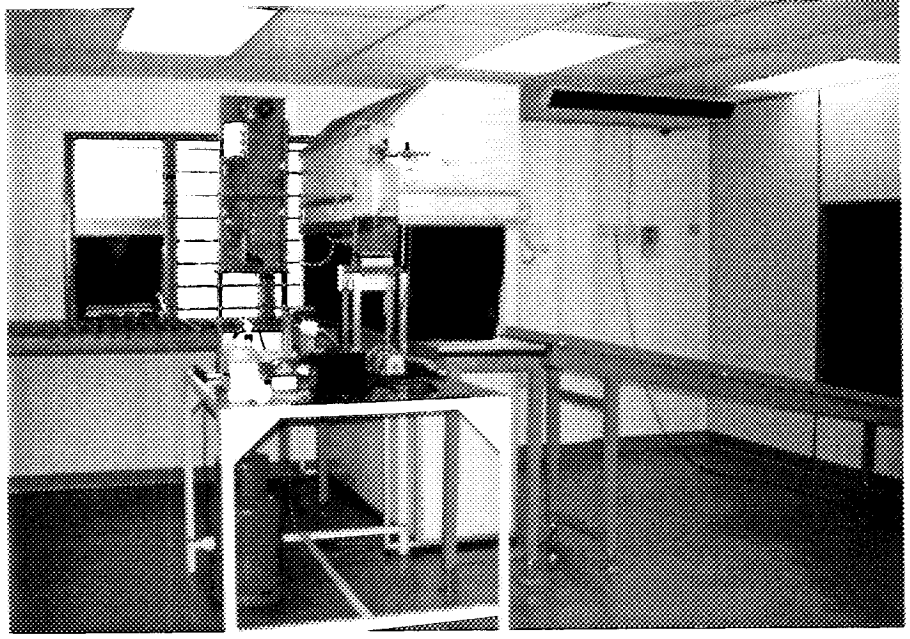

Photo. 6-4. Inoculation room

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Photo. 6-5. Incuvation room

Photo. 6-6. Cultiovation of mushroom

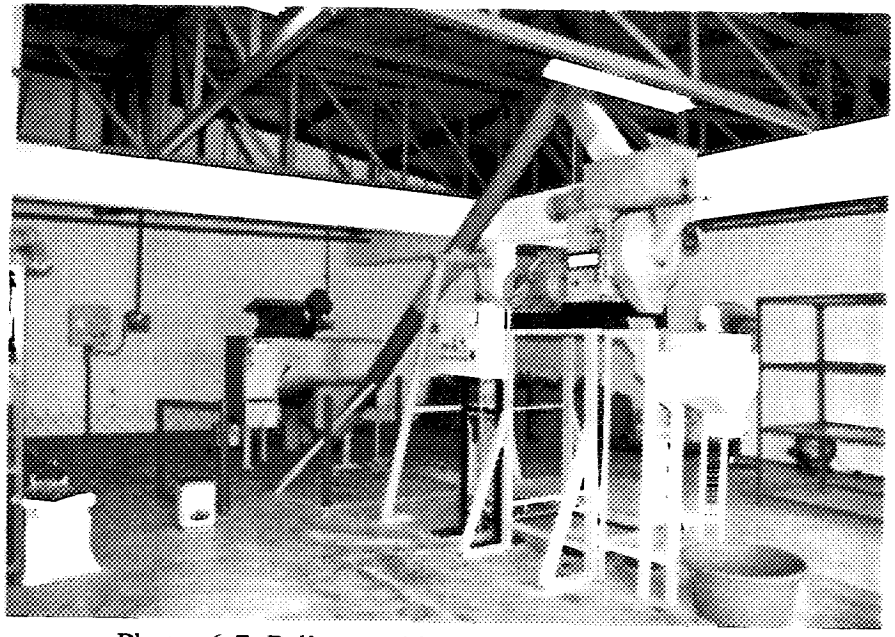

Photo. 6-7. Pellet machine for animal feed

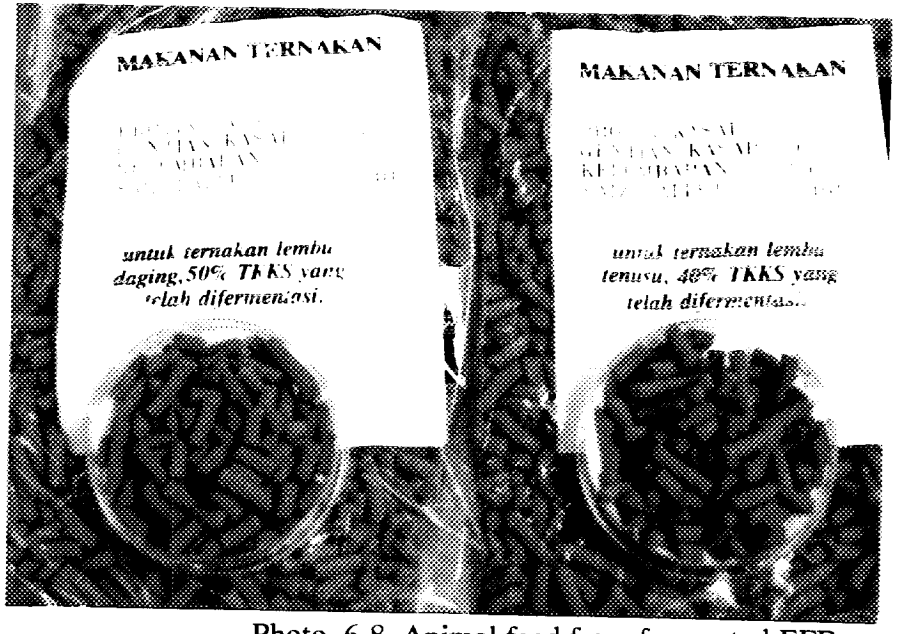

Photo. 6-8. Animal feed from fermented EFB

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6.2 Processing of EFB at Sterifeed Pilot Plant

6.2.1 Introduction

Fermented oil palm empty fruit bunch now known as Sterifeed has been characterized by physico-chemical properties. It has also been proven to have an improved digestibility (by *in vitro* and *in vivo* test) over the original material. The remaining important aspect of feed to be examined is the long term effect of feeding this material to animals.

The size of fermentation media bags used was 500 g - 1 kg / bag. In the large scale production of this materials, the numbers of bags were increased. The production at pilot scale level reinvestigated the basic processing parameters for the 1 kg / bag media and also performed a trial run for different sizes of bags. These includes:

* investigation on the growth of fungi on fermentation media subjected to different treatment times and the non treated media;
* evaluation of the processing rate and
* trial run processing of 25 - 50 ton oil palm EFB into feed
* processing of different sizes of bags.

6.2.2 Process

The fermented EFB or Sterifeed was processed by radiation fermentation treatment as described previously and summarized as follows:

(1) Preparation of fermentation media

Six batches of EFB, each of which weigh 30 kg (15% moisture content) were soaked in water at various treatment period; 0 (non-soaking), 1, 3, 6, 12 and 24 hr. After the treatment, the moist samples were made to fermentation media by mixing with lime (2%) and rice bran (5%). The non-treated sample requires addition of appropriate amount of water before adding in lime and rice bran. The solid media obtained were packed in polypropylene bags with an average weigh of 500 g per bag. The fermentation bags were set for irradiation by placing in 30 bags of solid media in a craft paper box (53 x 43 x 28 cm).

(2) Pasteurization

Boxes of solid media (12 boxes/carrier) were loaded in a hanging carrier of ^{60}Co SINAGAMA facility and irradiated for 25 kGy by γ - rays.

(3) Inoculation

Solid media in polypropylene bags were inoculated with 1 - 2% liquid seed of *Pleurotus sajor-caju*.

(4) Fermentation

Samples were placed on incubation rack at normal ambient temperature for 1 month. The fermented solid media, now referred as Sterifeed were obtained in fresh form and ready to be used as animal feed.

(5) Calculation of Sterifeed production rate

The production rate was measured based on production of Sterifeed per hour (kg/hr, fresh weight basis). It is a ratio of total weight of Sterifeed produced to the total processing time.

In order to meet the target production of 50 metric tones (MT), the plant production rate must be at least 1 ton / week or 30 kg/hr. However, during the conditioning period the plant the production target rate is only 15 kg/hr.

6.2.3 Results and discussion

(1) Preparation of Sterifeed

The dry EFB (30 kg, moisture 15%) absorbs water during soaking treatment of media preparation. The number of bags obtained from each batch of samples varies between 180 to 190 bags (Table 6.2-1) with the resulted weight gains of approximately three times of the dry weight. The estimated solid media weight, calculated from the numbers of bags obtained were 90 - 97 kg, with moisture content between 71 - 74% (Table 6.2-2).

The inoculated fungi on pasteurized media showed, generally good growth. It also showed no difference in growth of fungi for the different treatment time of 6, 12 and 24 hr soaking. The other three batches have yet to be observed. The contamination of samples were also very minimal (Table 6.2-3). However, the non-treated samples and the briefly soaked samples (1 - 3 hr) were prone to contamination, especially those of larger bags (20 kg / bags samples).

(2) Production rate of Sterifeed

The process involved can be categorized into three parts; Process 1, 2 and 3. The overall processes and current rates are depicted in Fig. 6.2-1.

1) Process 1 (cutting, soaking and mixing)

In the Process 1 which involves cutting, soaking and mixing additives, the processing rate was low (10 - 15 kg/hr) as indicated in Batches 4 and 5. When the cutting process was excluded as in the case of ready cut samples being used, the rate of processing increased (20 - 30 kg/hr) as indicated by Batches 1, 2 and 3. In the case of both cutting and soaking were ignored by using the ready cut samples and the process involved only direct mixing of moist fiber with additives, the rate of processing increased tremendously (50 kg/hr), as in Batch 5.

2) Process 2 (packing)

Packing of media into polypropylene bag was accomplished manually. The rate of processing were consistently low (below 10 kg/hr) for all batches. This data is representative for the current process and it certainly require improvement. The improvement in packing efficiency can be achieved by introducing automatic packing machine. The nature of substrate from the long fiber may have to be changed into ground form according the requirement. of packing machine. However grinding EFB required a powerful machine.

In the larger bags, an additional processing line was introduced. It consists of a loading and unloading equipment for handling of EFB coupled with a mixer and a semi-automatic packing machine linked-up in line with conveyor belts. This media packaging facility are able to pack fermentation media from 5, 10 - 20 kg per bag.

3) Process 3 (preparation of seed and inoculation)

The process was performed by semi-automatic operation. The overall process rate was low, especially at the stage of preparing liquid media. The inoculation stage was moderately efficient. It was performed at 25 kg/hr (approximately 100 bags / hr / 2 persons). In order to increase the efficiency, the sample handling system - a conveyor type sample feeder with a fully automatic inoculation machine must be installed.

Table 6.2-1. Processable EFB into fermentation media subjected to different soaking time and non-soaking media

Date of completion	Soaking time (hr)	Total fibre (kg)	Moisture (%)	Total Fibre (kg, DM basis)	Total (bags)
18-Apr Batch 1	24	30.00	15	25.5	180
3-May Batch 2	12	30.00	15	25.5	182
4-May Batch 3	6	30.00	15	25.5	181
7-Jun Batch 4	3	30.00	15	25.5	183
7-Jun Batch 5	1	30.00	15	25.5	180
8-Jun Batch 6	0	30.00	15	25.5	194
		180.00		153	1100

Table 6.2-2. Estimated weight of solid media and water content from each batch of process

Samples	Estimated values		
	Media wt (kg)	Water wt (kg)	Moisture (%)
Batch 1	90.00	60.00	71.67
Batch 2	91.00	61.00	71.98
Batch 3	90.50	60.50	71.82
Batch 4	91.50	61.50	72.13
Batch 5	90.00	60.00	71.67
Batch 6	97.00	67.00	73.71
Total	550.00	370.00	
Average	91.67	61.67	72.16

Table 6.2-3. Perfect growth of fungi in fermentation bags

Samples	Soaking time (hr)	Total growth (bags)	Contaminated (bags)	No-growth	Missing bag	Available 12/7/96
Batch 1	24	178	2	0	10	168
Batch 2	12	180	2	0	0	180
Batch 3	6	181	0	0	1	180
Batch 4	3	173	7	3	11	162
Batch 5	1	160	20	0	33	127
Batch 6	0	158	30	6	48	110

Table 6.2-4. Processing time and rate

Samples	Time 1 (hr)	Time 2 (hr)	Time 3 (hr)	Total time (hr)	Average rate (kg/hr)	(kg/day)
Batch 1	4.00	17.00	16.34	37.34	2.41	16.87
Batch 2	3.00	22.75	11.65	37.40	2.43	17.03
Batch 3	4.00	13.50	11.65	29.15	3.10	21.73
Batch 4	5.67	11.50	4.34	21.51	4.25	29.78
Batch 5	10.92	12.00	4.33	27.25	3.30	23.12
Batch 6	1.75	13.25	4.25	19.25	5.04	35.27
Total	29.34	90.00	52.56	171.90	20.54	143.81
Average	4.89	15.00	8.76	28.65	3.42	23.97

Table 6.2-5. Processing rate at three processing stages

Samples	Rate of process 1 (kg/hr)	(kg/day)	Rate of process 2 (kg/hr)	(kg/day)	Rate of process 3 (kg/hr)	(kg/day)
Batch 1	22.50	157.50	5.29	37.06	5.51	38.56
Batch 2	30.33	212.33	4.00	28.00	7.81	54.68
Batch 3	22.63	158.38	6.70	46.93	7.77	54.38
Batch 4	16.14	112.96	7.96	55.70	21.08	147.58
Batch 5	8.24	57.69	7.50	52.50	20.79	145.50
Batch 6	55.43	388.00	7.32	51.25	22.82	159.76
Average	25.88	181.14	6.46	45.24	14.30	100.08

Table 6.2-6. Overall current and projected rate

Samples	Processing time (hr)	Processing rate (kg/hr)	(kg/day)	Projected rate (kg/hr)	(kg/day)
Batch 1	37.34	2.41	16.87	5	35
Batch 2	37.4	2.43	17.03	10	70
Batch 3	29.15	3.10	21.73	15	105
Batch 4	21.51	4.25	29.78	20	140
Batch 5	27.25	3.30	23.12	25	175
Batch 6	19.25	5.04	35.27	30	210
Average		3.42	23.97	17.50	122.50

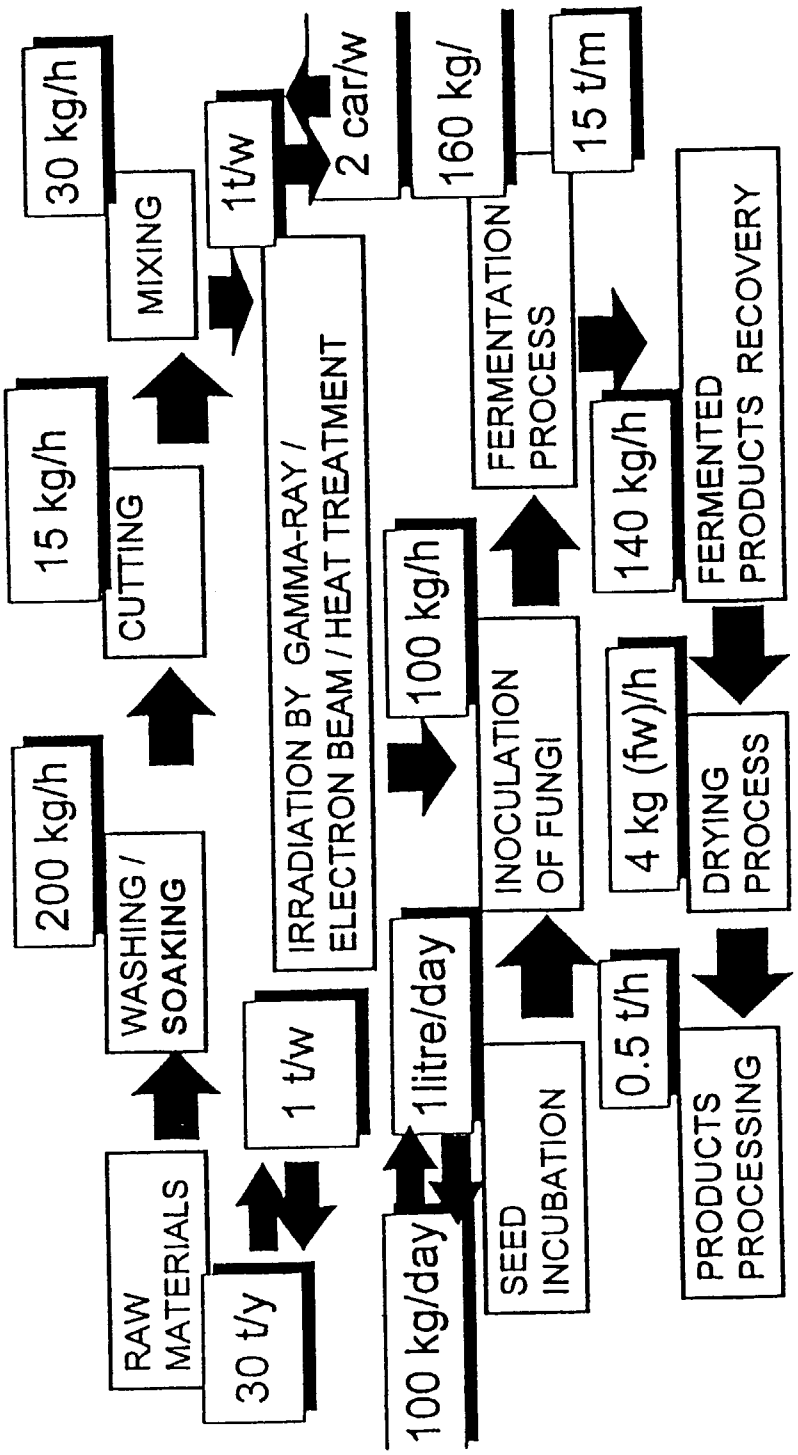

Fig. 6.2-1. Processing stage and rate at Sterifeed pilot plant

6.3 Electron Beam Treatment

6.3.1 Introduction

The pasteurization of EFB by irradiation is one of the important step for a large scale fermentation. The contamination of microorganisms in EFB was high, and it required more than 15 kGy to eliminate aerobic bacteria and 10 kGy for fungi to undetectable level. The γ-rays was used for the pasteurization of EFB substrate because of its high penetration power, but it is considered that electron beam (EB) is more suitable for the irradiation in a large amount of sample even though the penetration power is low. As it is estimated that minimum 18,000 ton of EFB are produced per mill annually, the irradiation conditions in a large scale using γ-rays and EB were discussed.

6.3.2 Materials and methods

(1) Materials

EFB was cut to ca. 2 cm length and soaked in water overnight. Wet EFB (100 g with 70% moisture content) was packed in 20 x 12 cm plastic bag. The thickness of each pack of the samples was measured and the density was calculated as ca. 0.3 g/cm^3.

(2) Irradiation

Irradiation was carried out using Dynamitron type accelerator (3MeV, 1mA) with beam width 60 cm at JAERI. The distance between beam window to surface of samples was set at 20 cm. The dose was measured using CTA (cellulose triacetate) film.

(3) Microorganisms count

Irradiated EFB samples (25 g) were analyzed for microorganisms using nutrient agar for total aerobic bacteria and MYG-chroramphenicol agar plates for fungi.

6.3.3 Results and discussion

(1) Comparison of γ-rays and EB for pasteurization of EFB

γ-rays has been used for the pasteurization of EFB substrate because of its high penetration power. Irradiation by ^{60}Co γ-rays has undoubtedly performed a good pasteurization of fermentation substrates. On the other hand, it is considered that EB

is more suitable for the irradiation in a large amount of sample than γ-rays because of the high dose rate of 500,000 kGy/hr compared to 20 kGy/hr of γ-rays. Therefore, the effect of irradiation on EFB by EB was studied to compare with γ-rays.

Fig. 6.3-1 shows that the effect of irradiation on the survival of total aerobic bacteria and fungi in EFB by γ-rays and EB. The dose rates used were 10 kGy/hr for γ-rays and 1 kGy/pass/sec for EB. The radiation sensitivities in both total aerobic bacteria and fungi by EB were a little lower than that by γ-rays. The results show that EB is also effective for the pasteurization of EFB substrates though a little higher dose is necessary.

(2) Dose distribution and pasteurization effect on EFB by EB irradiation

Figure 6.3-2 shows the dose distribution in EFB irradiated using Dynamitron type accelerator with 3MeV, 1mA. The EFB sample of 70% moisture content was packed in 200(W) x 120(L) x ca. 10(D) mm plastic bags (density: 0.3 g/cm^3) and piled up. When the EFB samples with 8 cm thickness were irradiated from both sides, the dose at 4 cm thickness was minimum. The dose uniformity ratio (D_{max} 22.3 kGy / D_{min} 14.3 kGy) was calculated as 1.56. As the dose decreased to half of surface dose at thickness of ca. 3 cm, the uniform irradiation is expected when EFB sample with 6 cm thickness is used. However, the condition of dose uniformity of 1.56 obtained 8 cm thickness is also applicable for the practical irradiation to pasteurize the EFB substrates.

The ^{60}Co irradiation facility at MINT has 1.6 MCi maximum loading. The throughput capacity for EFB was calculated as 12,000 ton/year with 3MCi source. This volume is definitely insufficient to treat even EFB discharged from a smallest mill producing 18,000 ton/year. It is, therefore, considered that the EB is more suitable for the irradiation of EFB because of its high throughput capacity.

(3) EB machine at MINT

The specification of EB machine at MINT is as follows:

Accelerator voltage:	3 MeV (0 - 3.0 MeV), stability $\pm$2%
Beam current:	30 mA (1 mA - 30 mA) , stability $\pm$2%
Beam width:	120 cm (30 - 120 cm)
Dose uniformity:	$\pm$5%
Conveyor speed:	1 - 20 m/min

In order to calculate the processing capability of EFB by EB, a study on dose distribution was carried out using EB at MINT. The result showed that the depth dose into EFB samples was 1.1 kg/cm^2. Based on the dose depth, this facility is

capable of treating 100,000 ton/year EFB, with treated material packing density of 0.16 g/ cm^3.

6.3.4 Conclusion

Two types of radiation source, γ-rays from ^{60}Co and EB used for the radiation processing, were compared for a large scale EFB treatment. The radiation processing by EB can be expected to have advantage with high dose rate of 500,000 kGy/hr compared to 20 kGy/hr of γ-rays. Radiation processing of EFB in a large scale can be performed effectively to utilize the advantage of EB treatment.

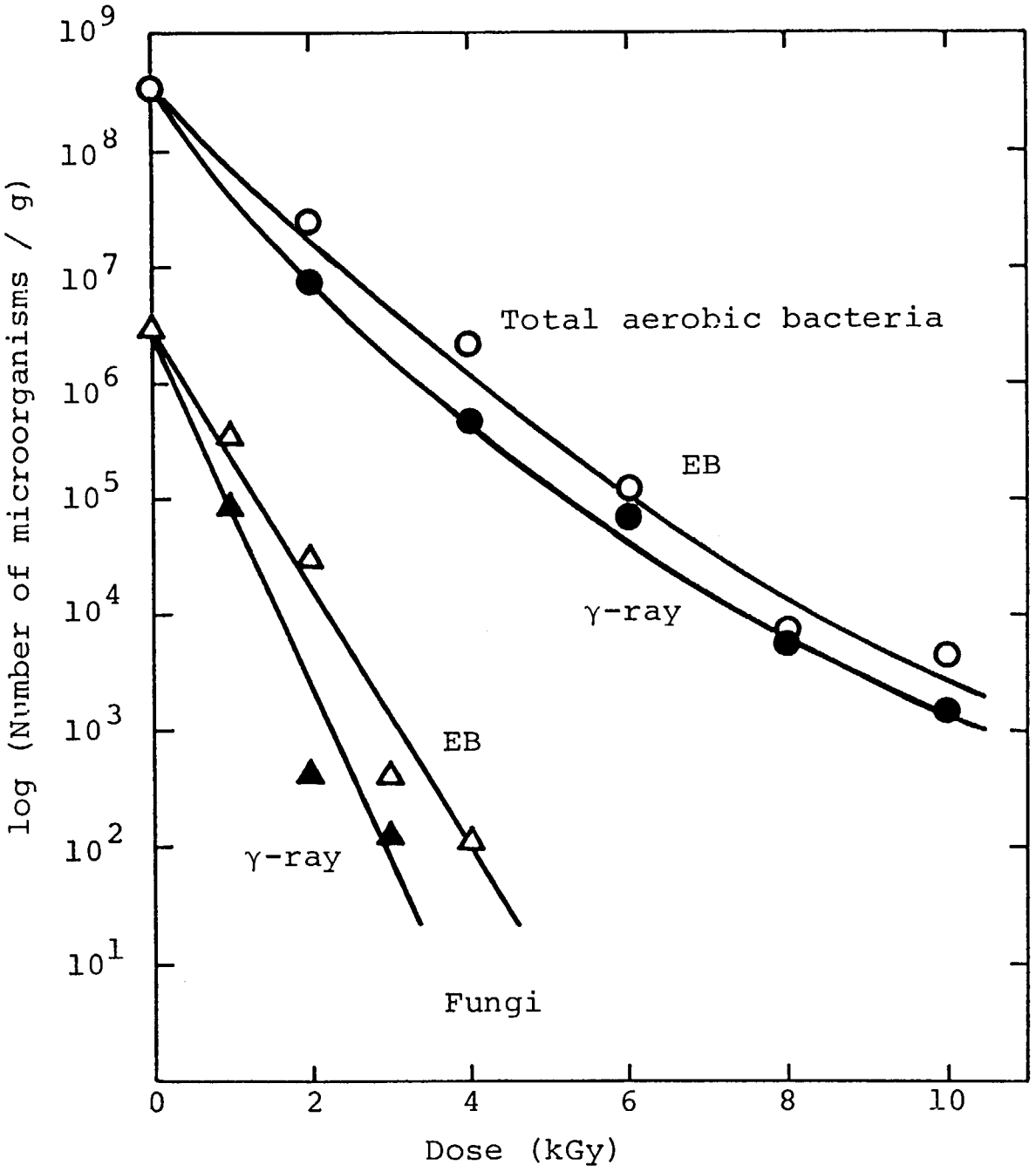

Fig. 6.3-1. Decrease in number of microorgnisms contaminating in EFB by γ-rays and EB irradiation

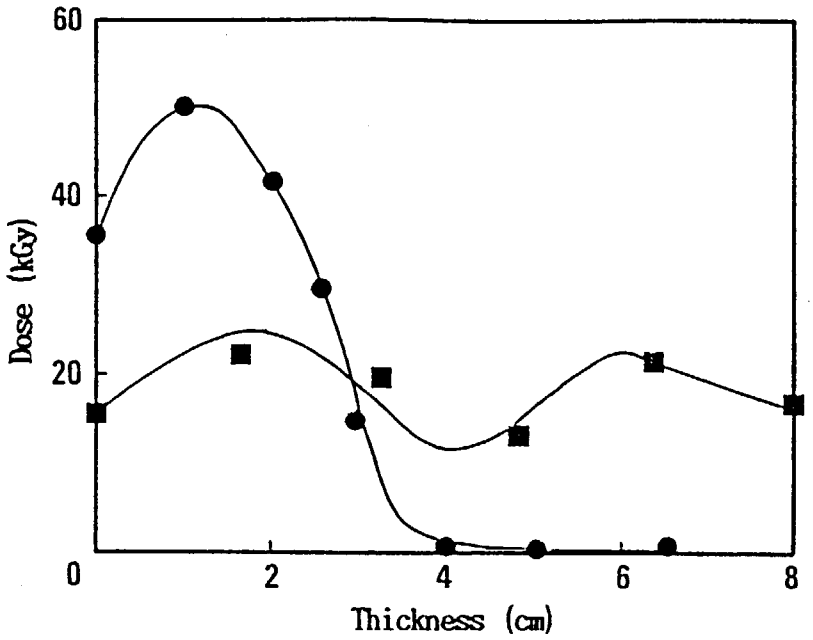

Fig. 6.3-2. Dose distribution in EFB by EB irradiation
Energy: 3 MeV, Current: 1 mA
Conveyor speed: 1.37 m/min (4 pass)
Irradiation: one side (●), both sides (■).

7. Techno-Economical Evaluation

7.1 Conceptional Evaluation of Large Scale Processing using EB

7.1.1 Introduction

The purpose of this investigation is to compare the process of production system to produce cattle feeds from oil-palm waste and evaluate the economical aspects through comparing the construction cost and operation cost with the conventional methods. In order to produce cattle feeds from oil-palm waste, electron irradiation was applied to pasteurize the EFB. This technology can be applied effectively for utilization of under-utilized agricultural products and agricultural wastes. For the irradiation, radioactive isotope such as ^{60}Co and electron generated by accelerators are generally used. In this economical evaluation, electron beam method is adopted because of economical advantage of this plant system are described. This section was prepared using the report by NKK Corporation under the Consignment Research of JAERI in 1993.

7.1.2 Outline and fundamental condition of the process

(1) Outline of process flow diagram

The process flow diagram of the oil-palm processing for cattle feed is shown in Fig. 7.1-1. Raw material is washed with water, cut and mixed with rice bran as preprocessing after collection. It is irradiated with electron, inoculated by seeds and transferred to the fermentation process. After fermentation for one month, the fermented products are recovered, dried, packed and shipped.

(2) Fundamental condition

1) Property of raw material

* Components and composition:	Protein 2-3%
* Crude fiber:	more than 50%
* Density:	0.8g/cm^3 (before cutting)
	2g/cm^3 (after cutting)
* Water content:	60% (before and after cutting)

2) Treatment amount

* 3 cases of 30,000 t/year, 100,000 t/year and 300,000 t/year (operation: 24 hr/day,

300 day/year).

* The investigation and evaluation were made mainly based on 100,000 t/year case, and the 30,000t/year and 300,000 t/year cases were also economically analyzed.

3) Pre-treatment condition

* Water washing

Soak in water cell during 1 day (every day batch treatment, volume of water cell is about 2 times of raw material)

* Cutting

Size 2cm

* Mixing

Rice bran mixing

- Mixing ratio 3.0% wet
- Water content of rice bran 15%

* Container packing

- Packed into container after irradiation
- Thickness of container is about 40 cm for the best fermentation

4) Irradiation condition

The dose required for pasteurization, which is dependent on the initial number of contaminating microorganism and their radiosensitivity is 10kGy according to the basic research (section 3.1).

The relation between dose and penetration depth is shown Fig. 7.1-2. Generally, irradiation thickness of specified electron energy is determined to be the same dose at the incoming surface of raw material as shown in Fig. 7.1-2.

According to the Fig. 7.1-3, the thickness of raw material is determined as follows, based on the penetration depth and raw material density of 0.2 g/cm^3.

at 2.0 MeV 35 mm
at 2.5 MeV 43 mm
at 3.0 MeV 58 mm

Generally high energy creates deep penetration and high productivity. But it results also thick shielding wall and high investment cost. In this investigation, following parameters were adopted:

Radiation dose:	10 kGy
Electron energy:	2.5 MeV
Thickness of material:	43 mm

5) Fermentation condition

Based on the basic research, the fermentation condition was determined as follows:

* Seed: *P. sajor-caju*
* Inoculation method: Liquid seed slurry
* Best inoculation rate: 1% of raw material
* Temperature: 30℃
* Ventilation: Natural circulation
* Duration: 1 month
* Density after fermentation: 0.2g/cm^3
* Water content after fermentation: 60%

Seeds incubation is based on the following assumptions.

* Seeds are incubated in liquid. After fully grown, the volume of liquid is increased and the seeds are incubated in larger cell. Cutting of seeds is necessary as they grew to be lumps.
* Temperature: room temperature
* Incubation period: 10days

6) Post-treatment condition

* Drying: Reduce water content from 60% to less than 10%
* Property of Products
  - Protein content: more than 10%
  - Crude fiber content: less than 25%
* Goods: Pellet, packed in 25kg package

7) Others

Since this process is to treat the waste produced in palm-oil factory, it is profitable to construct the plant near the palm-oil factory. Therefore the construction place should be plain and good ground condition near palm-oil factory. Water system required in this process should be introduced from the factory.

7.1.3 Economical evaluation

(1) Construction cost

The estimation of construction cost is divided into 4 groups, namely the cost of machinery facility, the cost of electrical facility, the cost of civil engineering and building.

1) Machinery facility: The costs of each equipment are accumulated and added with the cost of installation and piping. Expenses are included in each equipment cost.
2) Electrical facility: The costs of electricity distributor, control panel and their installation and cabling.

3) Civil engineering and building: The structure of the irradiation building is RC concrete and other building is prefab. The cost of land is included is civil engineering.

In the estimation of 3), the costs are estimated as Malaysian price. Malaysian dollars are calculated as 55 Yen. The costs are shown in Table 7.1-1.

(2) Operation cost

Operation cost is divided into utility cost, material cost, labor cost, amortization and maintenance cost.

1) Utility cost: Unit prices of electricity, fuel and water are necessary.

2) Material cost: Rice bran and seed media(Morasses and urea) are necessary.

3) Labor cost: Labor cost is calculated using the unit price of labor 800,000 Yen/man/year(Malaysian price).

4) Amortization

Amortization cost is calculated as follows:

Amortization/year = Construction cost x (Average interest rate + Amortization rate)

Average interest rate:

Machinery, Electricity	Amortization 15 years
Civil, Building	Amortization 50 years

Machinery, Electricity $= 0.07/2 \div \{1- 1/(1+0.07/2)^{2\times 15}\} = 0.054$

Civil, Building $= 0.07/2 \div \{1- 1/(1+0.07/2)^{2\times 50}\} = 0.036$

Amortization rate:

Machinery, electricity $= (1- 0.1) \div 15 = 0.06$

Civil, Building $= (1- 0.1) \div 50 = 0.018$

Therefore, Amortization /year = (Cost of machinery and electricity) x (0.054 + 0.06) +(Cost of civil and building) x (0.036 + 0.018)

5) Maintenance

Maintenance cost is calculated as follows:

Maintenance cost/year = (Total construction cost = installation and piping) x 0.02

6) Operation cost

The operation cost based on the the amount of utility, labor and material is shown in Table 7.1-2.

(3) Products cost

According to the above investigation, the products price is shown in the following

Table. But in the actual oil-palm factor, empty fruit bunch must be abandoned and the cost of this abandonment is about 2.4 Yen/kg. In this process this expense is not necessary and it should be deducted from the calculated products cost. The deducted price is also shown in Table 7.1-3. For the comparison with the price of this process, the prices of popular cattle feed are shown in Table 7.1-4.

7.1.4 Conclusion

In the report, in order to realize the process to produce cattle feeds from oil-palm waste using electron irradiation, the conceptual design and the specification of each process are investigated and the economical aspects are evaluated through the comparison with conventional cattle feeds. The results showed that this process of productivity more than 100,000 t/y has economical advantage compared to the conventional method. It is also showed that several technologies must be overcome to realize this process.

For instance:

1) Development of cutter which can process the row material of actual amount
2) Development of seed incubation equipment of actual amount
3) Development of protection method from mixing of harmful seeds at the inoculation or fermentation
4) Development of dryer

To solve these problem efficiently, it is required to construct a pilot-plant of 1/10 scale and demonstrate this technology.

The process to produce cattle feed from oil-palm waste, which by-produce edible mushroom was planned to be economically evaluated. But the evaluation was not carried out in this investigation, since the production condition of the mushroom has not been established yet. If the mushroom is produced, it creates economically advantageous situation. The future development of the mushroom production is greatly expected.

In addition to electron beam, gamma rays from ^{60}Co was to be investigated as a radiation source. Since the cost of gamma rays from ^{60}Co is higher than electron beam, and the maximum amount of ^{60}Co in a facility, which is 3,000,000Ci, can not treat the oil-palm waste of 30,000t/y, gamma rays from ^{60}Co was excluded in this investigation.

Table 7.1-1. Construction cost

Item	30,000 t/y	100,000 t/y	300,000 t/y
Machinery facility	3,668,440	6,853,500	12,949,300
Electrical facility of electricity	62,500	120,840	233,600
Civil and building	1,016,210	1,964,500	3,797,740
Total cost	4,747,150	8,938,840	16,980,640

(Note) Cost of electron irradiation ÷ total cost=0.048 (4.8% for 100,000 t/y), and it is very low compared to the total.

Table 7.1-2. Operation cost

Item	30,000 t/y	100,000 t/y	300,000 t/y
Utility cost	85,680	164,940	260,300
(Electricity)	(56,160)	(108,000)	(150,120)
(Fuel)	(26,880)	(51,840)	(100,320)
(Water)	(2,640)	(5,100)	(9,860)
Material cost	62,500	120,840	233,600
(Rice bran)	(30,600)	(102,000)	(306,000)
(Seed media)	(3,900)	(13,000)	(39,000)
Labor cost	80,000	160,000	312,000
Amortization cost	480,200	901,160	1,707,930
Maintenance cost	70,620	131,760	248,730
Total cost	751,000	1,472,860	2,873,960

Table 7.1-3. Products price

Item		30,000 t/y	100,000 t/y	300,000 t/y
Operation cost	(1,000 Yen)	751,000	1,472,860	2,873,960
Products cost	(Yen/kg)	25.0	14.7	9.58
Products cost including waste	(Yen/kg)	22.6	12.3	7.18

Table 7.1-4. Price of popular cattle feed

Item	Protein content (%)	Price (Yen/kg)	Notes
PKC	15	17.5	Malaysian price
Mixed feeds for milk cow PKC 70% Soybeans 10% Maize 20%	17	21.5	Malaysian price
Chicken feeds	20	40	Malaysian price
Pig feeds	20	40	Malaysian price
This process	13	22.6	30,000 t/y
		12.3	100,000 t/y
		7.2	300,000 t/y

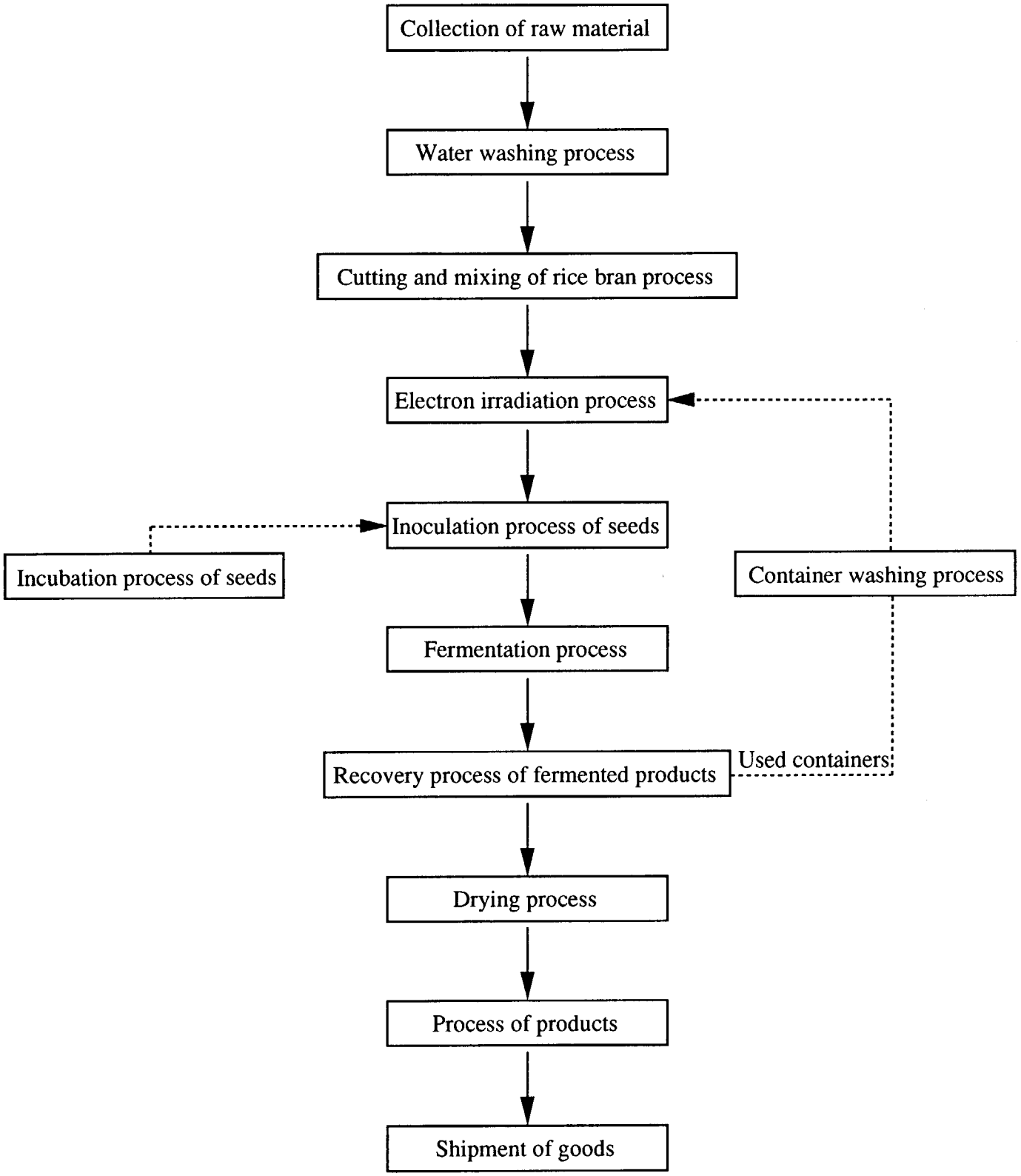

Fig. 7.1-1. Flow diagram of EFB treatment

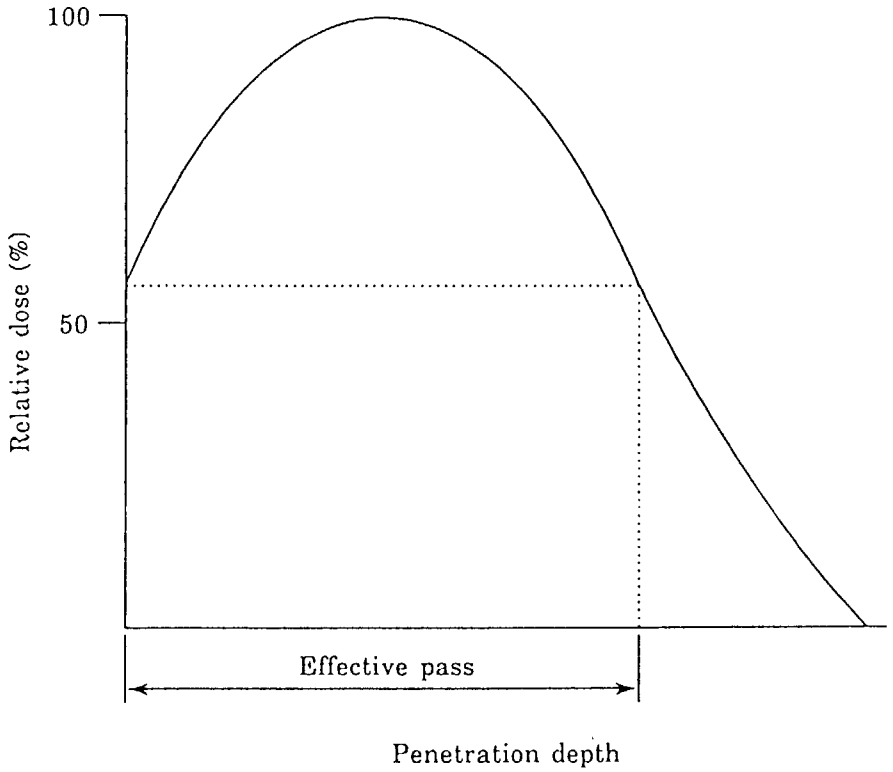

Fig. 7.1-2. Relation between dose and penetration depth of EB

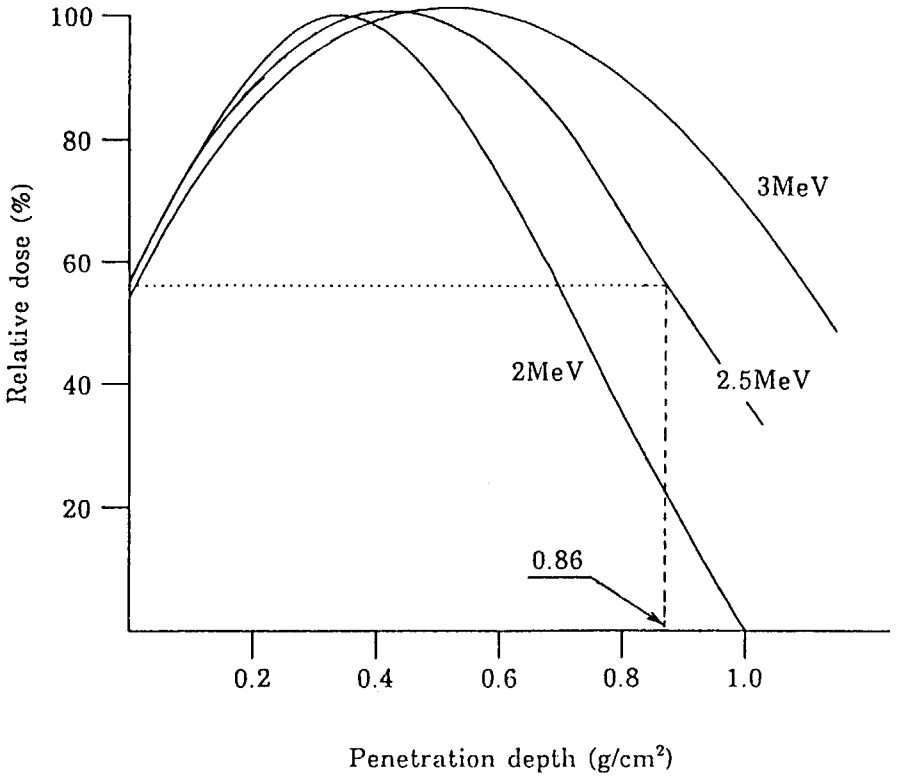

Fig. 7.1-3. Energy and penetration of EB

7.2 Evaluation Based on Pilot Plant Study

7.2.1 Introduction

Irradiation of EFB at doses above 10kGy followed by fermentation can reduce crude fiber (CF) content from 50% to 20-30% and crude protein (CP) content elevated from 2% to 10-15%, respectively. The end-product of fermentation displayed all the characteristics of animal feed, and at these levels of CF and CP can be utilized for feeding ruminants. Further reduction of CF and raising of CP can result in the products suitable for feeding non-ruminants such as poultry and pigs.

7.2.2 Techno-Economical Feasibility

Following the successful conversion of raw EFB into feeding stuff for ruminant in the laboratory, there is an urgent need to evaluate whether such products could be mass-produced economically at larger scale for further feeding trials. A pilot plant has been set up at MINT to simulate the actual commercial production process before any technology transfer can be undertaken.

The main objective of this section is to report firstly, the economic and financial feasibility of the production process at pilot level. Secondly, preliminary evaluation on the cost of production of animal feed from EFB.

The economic and financial feasibility of the project were examined using capital budgeting analysis (4). All computations were developed in Lotus 123 spread sheet.

Capital budgeting involved Discounted Cash Flow analysis (DCF) and utilizes investment criteria like Net Present Value (NPV), Internal Rate of Return (IRR), Net Benefit Cost Ratio (BCR) and Pay back Period (PBP) to make accept / reject decision. The Unit production cost (UPC) was computed based on 'Break-even analysis'.

7.2.3 Major Assumptions:

The computation of UPC and analysis of data in the DCF are based on the following assumptions:

(1) The project will have a useful life of 15 years and discounted at the rate of 4% i.e. the lending rate for capital investment offered by many financial institutions for new industry such as Asian Development Bank (ADB), Malaysian Industrial Development Fund (MIDF) etc. Pilot plant would be set up on 1 acre of land. Building included all infrastructures such as storage and processing facilities etc.

(2) The revenue was computed based on the projected production for the first year of 5000 ton (1500 ton fermented EFB plus 3500 ton basal ration) for complete diet and 300 ton of fresh mushroom; and increased by 5% a year. The sale prices / market prices for each are M$0.80 and M$5.00 per kg respectively and there is no increase in the sale prices of these products throughout the 15 year project.

(3) Irradiation of EFB were carried out at service facility with average dose approximately 12.0 kGy. The cost of irradiation per kg EFB was M$0.549 / kg (M$87.84 / cm^3).

7.2.4 Project Costs and Financing

The items which made up the investment and their costs for the project is as shown in Table 7.2-1. In the initial year, the total investment required for the project is about M$4.3 mil. which made up of 38.15 % fixed cost and 61.84% variable cost.

Further scrutiny of the variable costs showed material cost and irradiation charge represent 55% and 31% respectively of the total variable cost. There is scope to reduce these costs in order to keep UPC low (see below for discussion on UPC).

7.2.5 Economical and Financial Indicators

This project is an independent project. The economic viability, can be measured in term of their financial indicators like NPV, IRR, PBP and Net Benefit-cost Ratio (B/C). This project is highly viable and profitable in the short, and long terms provided all the assumptions made are reliable and sales of the animal feed and mushroom meet the target to generate the projected revenues. The NPV at 4% cost of capital is highly positive, RM$41.5 mil; IRR, 175%; B/C, 26 times; and initial investment can be recouped at the end of first year operation. However, the project is sensitive to the fluctuation in the cost of capital and any increase would result in the decline of the net worth of the project as indicated by the decline in NPV (Fig. 7.2-l).

7.2.6 Unit Production Cost

Unit production cost must be able to cover repayment of the capital (own equity and borrowing) initially invested, variable / operational cost and generate a certain percentage of mark-up / profit.

The repayment of capital / fixed cost is calculated based on 4% cost of capital and amortized for 15 years i.e. the useful life of the project. Variable costs increase in tandem with the volume of animal feeds produce.

$$\text{UPC} = \frac{(\text{Repayment of Capital} + \text{Variable Cost} + \text{Profit})}{\text{Volume of animal feed produced (in kg)}}$$

The UPC for producing complete animal diet is about M$0.615/kg based on the volume of 5 000 ton a year. If mushroom is included, the UPC is reduced to M$0.581/kg. The market price for complete diet for non-ruminants is about M$0.80/kg, hence, our cost of production is still competitive. Conservative estimates of the profit based on the above selling price will be M$(0.8-0.615) = M$0.185/kg.

Unit production cost included the element of repayment of capital which is influenced by the market cost of capital. In contrast to NPV, increasing cost of capital would result in the increase of UPC (Fig. 7.2-2). The cut-off point for cost of capital is 30% before it has adverse effect on the UPC.

The main components which made up substantial proportions of the variable costs are material cost and irradiation charge (Table 7.2-1). They are therefore directly related to the UPC. The ability to reduce their costs during the course of production would inevitably reduce UPC. Material costs could be reduced by for instance replacing seed bottles with plastic bags and changing the procedure of composting; and irradiation charge by installing dedicated facility such as Electron Beam Machine / Co-60 to decontaminate EFB.

7.2.7 Conclusion

The above analysis indicates that on financial ground and with the assumptions made, this project is highly viable. Net Present Worth/NPV and UPC are very sensitive to cost of capital. There is a need to source financing from low interest bearing loan in order to maximize return on investment and reduce risks. Other observations and cost reduction required in the operating cost are materials and irradiation costs which affect UPC.

(7.2 Appendix)

In economic and financial evaluations, the indicators used to test feasibility of the project are Net Present Value (NPV), Internal Rate of Return (IRR), Pay Back Period (PBP) and Benefit -Cost Ratio (B/C).

(a) NPV: The NPV of a project is defined as the value obtained by discounting separately for each year, the difference of all cash outflows and inflows occurring throughout the life of a project at a fixed, predetermined interest rate. The NPV is therefore the present value of net cash inflows minus the initial investment cost. The difference is discounted to the point at which the implementation of the project is supposed to start.

The Formula for computation of NPV is

$$NPV = \sum_{t=1}^{N} Rt / (1+k)^t - I$$

where Rt, net cash inflow at time period t; I, initial cost of the project; k, discount factor of cost of capital; N, projects expected useful life. Project showing positive NPV is considered viable.

(b) IRR: The internal rate of return is the discount rate at which the present value of cash inflows is equal to the present value of cash outflows. It is the rate at which the present value of receipts from the project is equal to the present value of investments i.e. NPV is zero. The IRR represents the exact profitability of the project.

IRR = i1 +PV (i2 - i1) / PV + NV

where i1 = low discount rate,

i2 = high discount rate,

PV = NPV (positive) at low discount rate of i1

NV = NPV (negative) at high discount rate of i2.

IRR indicates the actual profit rate of the total investment outlay and if required, of the equity capital. Investment proposal may be accepted if IRR is greater than the cut-off rate, which is the lowest acceptable investment rate for invested capital.

(c) PBP: PBP is defined as the period required to recuperate the original investment outlay through the profit earned by the project. It is a rough indicator of choosing between investments in business enterprises, especially where there is a high degree of risk involved. The PBP however has two major weaknesses firstly, it fails to consider

earnings after PBP and secondly, it fails to take into consideration differences in timing of proceeds. Nevertheless, it is a crude criterion to indicate length of time from starting of the project before the net benefits returns the cost of capital investment.

(d) B/C ratio: The B/C is the ratio of present worth of benefits to the present worth of costs.

$$\text{Net B / C ratio} = \frac{\text{Present value of benefits}}{\text{Present value of costs}}$$

The formal decision criterion is to accept a project with a ratio of 1 or grater. It is generally accepted that, the NPV method and B/C ratio give the same accept / reject signal. However, in many cases NPV is preferred because it expresses in absolute term the expected contribution of the project. In contrast, B/C merely expresses the relative profitability.

Table7.2-1. First year estimates of the fixed and variable / operational cost for production of complete animal diet from EFB

Fixed cost		
Land	1,068,047.00 RM	(64.43%)
Building	424,000.00	(25.58%)
Machineries	86,540.80	(5.22%)
Contigencies (5%)	78,929.39	(4.79%)
Sub-total	1,657,517.19	(100%)
Variable/operational cost		
Materials	1,472,100.00	(54.79%)
Salary / wages	96,600.00	(3.59%)
Utilities	35,000.00	(1.30%)
Irradiation charge	823,500.00	(30.65%)
Other miscl. expenses	15,000.00	(0.55%)
Contingency (10%)	244,220.00	(9.00%)
Sub-total	2,686,420.00	(100.00%)
Total investment	4,343,937.19	

The projected increase in variable / op. costs per year for the 15-years project are as follows:

material costs, utilities and irradiation charge increased by 5%;
salary / wages increased by 7%.

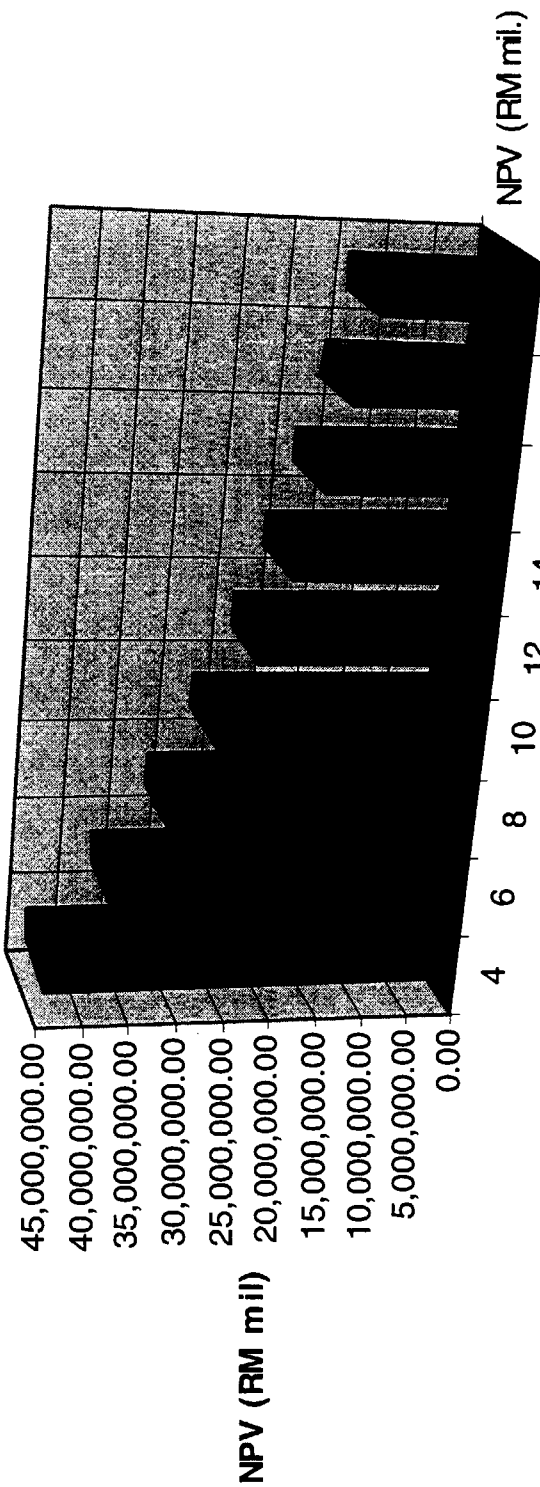

Fig. 7.2-1. Relationship of NPV with cost of capital

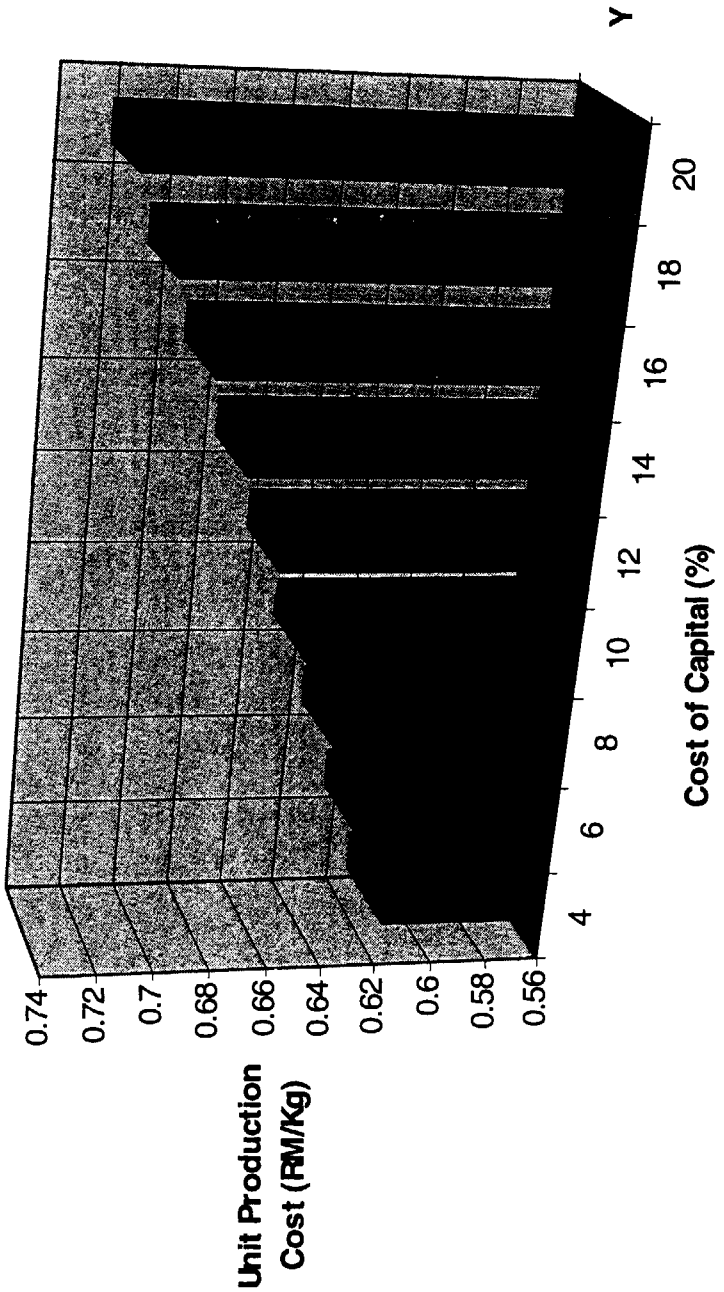

Fig. 7.2-2. Unit production cost *vs.* cost of capital

8. PRELIMINARY STUDY FOR USEFUL PRODUCTS

8.1 Enzymes Induced in Fungi

8.1.1 Introduction

EFB is a cellulose waste, consisting of 40 - 60% cellulose with the balance of hemicellulose, lignin and other materials. Cellulase is a complex of enzymes containing chiefly endo- and exo-glucanases as well as cellulase, hemicellulace, etc. (Mandel et al, 1976). Studies on cellulase produced from *Trichoderma viride* is well known and widely reported. The enzyme system from this fungi is considered as a complete composition of cellulase; and it was reported to be able to hydrolyze a more resistant portion or crystalline portion of cellulose, but at a slow rate.

Pleorotus sajor-caju and *Coprinus cinereus* were found from previous work to be easily grown on EFB. In this study, the enzyme system derived from liquid state fermentation by these fungi utilizing EFB as carbon source were investigated. The quality of this enzyme system was characterized based on its activity on filter paper, salicin and xylan. These activity tests would revealed the ability of cellulase enzyme system to break down insoluble cellulose, and hydrolyzing salicin as cellobiase and xylanase for breaking down hemicellulose.

8.1.2 Materials and methods

(1) Preparation of liquid inoculum

About 200 ml Mandel's media containing 1% glucose was dispensed in 500 ml conical flask and autoclaved at 121℃ for 20 min and then allowed to cool down to room temperature. The media was inoculated with *P. sajor-caju* seed grown on potato dextrose agar (PDA). The inoculated media was incubated at 30℃ in a shaking incubator (Bio-shaker BR-3000L, TAITEC) at 100 rpm. A fully grown mycelium was obtained ready to be used after 4 days to 1 week. The liquid inoculum of *C. cinereus* was also prepared in a similar manner.

The liquid inoculum of both strains of fungi were propagated and maintained by inoculating 5 ml inoculum in 200 ml freshly prepared, autoclaved Mandel's media containing 1% glucose (w/v) in the same manner described above. Alternatively, the liquid inoculum can also be prepared by inoculating one piece of fungi agar plate in a sterilized media either autoclaved or irradiated Mandel's media containing 1% (w/v)

suspension of ground EFB instead of glucose. The liquid inoculum obtained using EFB as carbon source produced coarser mycelial balls. Before use, the mycelium must be homogenized (Excel-Auto Homogenizer, Nihonseki Ltd.). The seed was a cut of agar plate using a cork borer of 1mm in diameter from a fully grown *P. sajor-caju* on PDA plate. Ground EFB consist of cut fibrous of sizes not more than 2 mm and moisture content about 10%.

(2) Liquid state fermentation

About 100 ml Mandel's media was dispensed in 300 ml conical flask and consequently suspended with 1% (w/v) or 1 g ground Empty Fruit Bunch (EFB). Alternatively in the two-fold volume fermentation system, about 200 ml media was dispensed in 500 ml conical flask and suspended with 1% (w/v) or 2 g ground EFB. The samples were autoclaved at 121℃ for 20 min or irradiated with ^{60}Co gamma-rays for 30 kGy. The media was inoculated with 5% (v/v) or 5 ml of liquid inoculum *C. cinerous* and incubated at 30℃with shaking speed of 100 rpm for 20 days. The product was centrifuged in 250 ml tube (100 ml/tube) at 10,000 rpm for 20 min. The supernatant was filtered (Filter Paper Quantitative ADVANTEC Toyo, No. 5A) and collected as enzyme broth. The solid fermented product was freeze-dried for enzyme extraction.

(3) Concentration of broth

About 2500 ml enzyme broth from *C. cinereus* and 3200 ml from *P. sajor-caju* were concentrated using a combination of membrane filters (Hollow Fiber System, Amicon CH_2 Concentrator and Ultra filtration cell, Model 8200) in order to obtain enzyme concentrates and other protein of molecular weight higher than 10,000. The Hollow fiber system allowed concentrating about 10 - 20 times of the original sample volume. The concentrated enzyme broth was collected by displacing with 100 ml of 0.1 M acetate buffer (pH 5.0) twice. Further concentration was performed on membrane ultra-filtration cell system (Amicon, Model 8200) leaving about 10 ml concentrated enzyme solution.

(4) Extraction of enzyme

1) Extraction from solid residue

About 2 g freeze-dried sample was added to 10 ml of 0.1 M acetate buffer (or 2 g/8 ml for freeze-dried sample of solid plus broth extracted without homogenizing and sonifying). The slurry was mixed using homogenizer (Nissei Bio-mixer); and further disrupted with sonifier (Bronson Sonifier, Cell Disruption 200) for 2 minutes 3 times.

The sample was centrifuged for 20 min at 10,000 rpm, Sakuma refrigerated centrifuge, Model 50A-7, 85 ml tube or for 5 min at 10,000 rpm, Kubota 1700, 1.5 min eppendorf vial. The clear supernatant was collected in a test tube or filtered through 0.45 μ m filter into an eppendorf tube.

2) Extraction from mycelium

Mycelium prepared in liquid medium was centrifuged at 10,000 rpm for 20 min. The supernatant was filtered into a conical flask. The wet residual mycelium was directly sonified and centrifuged at 10,000 rpm for 20 min. This supernatant from intracellular mycelial cell break down was collected.

(5) Analyses of enzymatic activity

1) Filter paper degradation activity (FPA) test

This activity test was carried out according to the method described by Yakult Company. Five ml enzyme solution (1 g enzyme was dissolved in 300 ml distilled water. Fifty ml of enzyme solution was mixed with an equal volume 0.1 M acetate buffer) was dispensed in an L-shaped tube. The tube was placed in water bath incubator shaker at 40℃ and kept for 5 min to achieve the bath temperature. The test filter paper (Toyo 51B, 1 cm x 1 cm, 97 - 100 mg) was added to the enzyme solution and immediately shaken at 72 rpm. This procedure was tested using commercial enzyme (Cellulase Onozuka). However, the activity test for enzyme from liquid media and its extract from solid part was carried out by dissolving an equal volume of acetate buffer to the enzyme solution. Fuve ml enzyme solution in buffer was tested for filter paper activity.

2) β-glucosidase and xylanase

The enzyme activity of β-glucosidase and xylanase were tested according to a modified method based on Mandel et al (1976) and Omiya (1992) procedures. In this test, the enzyme solution from fermentation system was reacted with salicin {(2-[Hydroxymethyl]phenyl beta-D-glucopyranoside), Sigma} and xylan from birchwood (Sigma) to afford glucose and xylose respectively. The reducing sugar produced as glucose and xylose was determined by the method of Somogyi-Nelson.

Five ml of 1% (50 mg) salicin or xylan solution was added to 0.5 ml of enzyme solution dissolved in 1 ml of phosphate citrate buffer, pH 6.3 in a 25 ml graduated test tube. The reaction solution was incubated in an incubator shaker for 30 min. The glucose produced was determined by the method of Somogyi-Nelson.

(6) Gel electrophoresis

Qualitative detection of enzyme and other proteins in liquid and solid samples was carried out according to the method of Laemmli (1970). Gel sheets were mainly stained with Coomassie Brilliant Blue R-250.

(7) Ion-exchange chromatography

The samples containing enzymes were purified through the ion-exchange column of Bio-Rex 70. The eluent with 100 ml acetate buffer was called unabsorbed eluent. The eluent with 100ml of 0.5N NaCl in 0.1 M acetate buffer was called the absorbed portion.

8.1.3 Results and discussion

(1) Enzymatic activity induced in fermented products

1) Filter paper activity

The filter paper activity tests in all samples were negative. These results implied that the enzyme activity in the liquid enzyme preparation directly from fermentation system and their concentrates had low cellulase activity. For reference cellulase Onozuka from *Trichoderma viride*, was used for the test and it was found the filter paper activity as 13 International Unit (IU)/ml.

2) Activity of β-glucosidase and xylanase

The β-glucosidase activity in the original liquid preparation and their concentrates of *C. cinereus* waas not detected. It was not detected also in the liquid enzyme preparation of *P. sajor-caju* but the activity was detected rather low, 100 μ g/ml as glucose in its concentrates than expected from its huge reduction in volume (Table 8.1-1). Apparently, their activity in the enzyme concentrates of *P. sajor-caju* was lost during handling process for concentrating and extracting.

Interestingly, the xylanase activity was found in significant amount in the enzyme preparation of *C. cinereus* and also detected in the enzyme preparation of *P. sajor-caju* (Table 8.1-2). Xylanase activity of *C. cinereus* was detected as 266 μ g/ml xylose and *P. sajor-caju* produced only 45 μ g/ml. In the liquid concentrates, the xylanase activity of *C. cinereus* had decreased and became 235 μ g/ml and in the case of *P. sajor-caju*, the activity increased about two-fold to 111 μ g/ml. The activity of enzyme

from both fungi had not increased so much as compared to their reduction in volume in the concentrating process. These results were consistent to the activity of β-glucosidase where its activity was lost in the concentrating process. In the solution of solid extract of *P. sajor-caju* only 30 μ g/ml of xylose was detected.

3) Isolation of enzyme/protein components

Enzyme preparation in liquid state fermentation can be divided into two parts, namely enzyme solution or broth and its insoluble parts. In the solution, extracellular enzymes were released from the cells in the preparation. In the solid portion, the enzyme was released upon destruction of cells and intracellular type enzymes were obtained.

In the original enzyme preparation of liquid state fermentation, the protein from *C. cinereus* and *P. sajor-caju* enzyme components were not observed on gel electrophoresis. However, upon concentrating the samples, by freeze-drying the whole enzyme preparation, and consequently extracted with buffer solution (1 g sample/ 5 ml acetate buffer) about 12 components of protein were observed for *P. sajor-caju*. Using silver stain instead of Cooomassie Brilliant Blue staining the protein bands can be observed much clearer.

In solid state fermentation, the bands of protein components (intracellular enzyme were not obvious even using the most concentrated samples. In the extracellular enzyme concentrates (after 145 times concentration) the protein bands were not so clearly seen on the gel but strong band appeared after the protein band developed by silver stain. Several component of protein bands also appeared for *C. cinereus* extracellular enzyme concentrates after 56 times concentration. The enzyme components of liquid state fermentation were observed on the gel both in *P. sajor-caju* and *C. cinereus* concentrates but not obvious in the original enzyme preparation which may be due to very low concentration.

Since the cellulase preparation described herein lack in exo-beta-glucanase, it may not be suitable to carry out the Filter Paper Activity test by the technique described in this report. It was, therefore, considered that other substrate such as carboxy-methl-cellulose may be used instead. The incubation time of the modified methods of Mandel's and Omiya's described in this report need to be cross-checked because the optimum reaction time was not thoroughly monitored. However, a quick check of 10 min incubation indicated that higher values of reducing sugar were produced. These results indicated that 10 min incubation or less could be sufficient. The long incubation time might have allowed other enzyme component to decompose glucose. Therefore,

the optimum incubation time suggested was between 5 to 30 min.

(2) Isolation of xylanase

The β-glucosidase activity was not detected in mycelium of *C. cinereus* but the xylanase activity was found in significant amount in extracts from *C. cinereus* mycelium (Table 8.1-3). Then the enzyme in the extract was purified by ion-exchange chromatography. Unabsorbed portion and absorbed portion which were eluted by 0.5N NaCl in acetate buffer were collected. Absorbed portion have xylanase activity but mycelium of unabsorbed portion have no xylanase activity. However, the enzyme activity (xylanase) reduced after passing from ion-exchange column. Further study is required to establish the suitable condition for ion-exchange column. After separation using ion-exchange chromatography, 2 main bands were observed in absorbed sample and several different main band were observed in unabsorbed sample. It is considered that the main bands is the xylanase because xylanase activity is including absorbed sample.

(3) Effect of carbon source on induction of xylanase

Table 8.1-4 shows the induction of xylanase activity during growth on various carbon sources. It was found that the specific xylanase activity of cells cultivated in the presence of EFB was higher than that of xylan, cellulose and glucose. It means that EFB induced the xylanase production.

Figure 8.1-1 shows the protein bands of samples. Lanes 1 - 5 were standard marker, glucose, EFB, cellulose and xylan bands, respectively. Only one clear band can be detected in lane 5. The molecular weight is expected at 45,000. The others clear bands in other samples cannot be detected.

8.1.4 Conclusion

It was concluded from the results that, xylanase, an enzyme which can break down hemicellulose can be produced by the method described in this report. Xylanase was a very interesting enzyme for EFB utilization; and also the work in this field had not been widely reported as yet.

Table 8.1-1. β -glucosidase activity in broth from liquid state fermentation using EFB

Fungi	Activity (Glucose produced, μ g/ml)		
	Enzyme broth (outside cell)	Enzyme broth concntrates	Enzyme extract (inside cell)
Coprinus cinereus	10	10	not analysed
Pleurotus sajor-caju	not analysed	145	192

Table 8.1-2. Xylanase activity in broth from liquid state fermentation using EFB

Fungi	Activity (Glucose produced, μ g/ml)		
	Enzyme broth (outside cell)	Enzyme broth concntrates	Enzyme extract (inside cell)
Coprinus cinereus	266	235	not analysed
Pleurotus sajor-caju	45	111	30

Table 8.1-3. Xylanase in extracts from *Coprinus cinereus* mycelium

	Volume (ml)	Protein content (mg/ml)	Glucose produced (μ g/ml)
Before ion-exchange	8.5	1.7	230
After ion-exchange			
Unabsorbed	2.8	1.5	0
Absorbed	2.8	0.7	142

Table8.1-4. Xylanase activity of crude intra-cellular enzyme of *P.sajor-caju*

Carbon sources	Xylanase activity		
	Total protein (mg)	Total activity (U) *	Spesific activity (U/mg)
EFB	0.2	135.9	679.5
Glucose	0.9	416.3	462.6
Cellulose	0.1	46.8	468.0
Xylan	0.4	249.3	623.3

P.sajor-caju was grown in Mandel's media supplemented with various carbon sources at 1% (wt/ vol).

* Released xylose μ g / 0.2 ml enzyme solution.

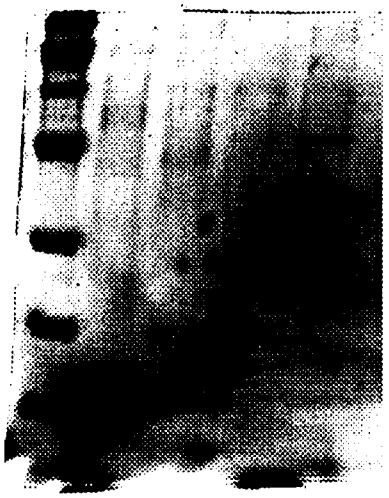

Fig. 8.1-1 SDS PAGE of protein samples (Coomasie Brilliant Blue R 250)

8.2 Biological Activities of Extract from Fermented EFB on Plant

8.2.1 Introduction

A large amount of cellulosic wastes, such as empty fruit bunch (EFB) from palm oil industry, generated in Malaysia. Normally these kind of materials are use as fermentation medium or substrate. We developed fermentation process of mushroom on cellulosic material by fungi for production of mushroom or animal feed. After harvesting mushroom, the residual substrate contains the degraded cellulosic compounds and mycelium of fungi. In this study, we used the fermented EFB as a source of bioactive materials and tried to find out the bioactivities against plants. The extraction of fermented EFB were assayed for their ability to interfere the pea stem elongation with indol acetic acid (IAA) and to induce the glyceollin from the soybeans cotyledons.

8.2.2 Materials and methods

(1) Preparation of extracts from fermented EFB

Mandel's medium which contained 1% EFB and 5% rice bran was irradiated at the dose of 30 kGy by gamma-rays and fermented by *P. sajor-caju*. Fermented EFB was extracted with hot water at 100°C for 4hr, and then with 85% ethanol at 80°C for 3hr 3 times. Both extraction were concentrated by the vacuum evaporator.

(2) Cotyledon assay for elicitor activity

The cotyledon assay was done according to Sharp *et. al.* (1984). The soybean was sterilized with 10% of $NaClO_3$ for 10min, washed with distilled water, and germinated on the barmiculite base for 7days at 25°C in dark. After 7days cultivation, the surface of cotyledons was sterilized with 10% $NaClO_3$ for 5min and washed with distilled water. A thin slice (7mm length x 5mm width) of the under surface of each cotyledon was removed by a sterilized razor. Ten cotyledons were placed on moist filter paper in petri dish. For each dish of 10 cotyledons, 80ml of extraction (aliquots of them were added to 1ml of buffer of 4mM sodium acetate and 3mM sodium bicarbonate) was placed on the wound surface of each 10 cotyledons and incubated at 26°C for 24hr in dark with enough humidity. Then the cotyledons were extracted with 80ml of 80% ethanol overnight at 4°C. The extracts were concentrated by vacuum evaporator, filtered by ultra membrane filter (exclusion limit of MW < $1x10^4$, Millipore co. Ltd., Japan). And the glyceollins in the extraction were analyzed by HPLC with column of Inertsil ODS-3 (GL Sciences Inc., Japan).

(3) Indole acetic acid assay (IAA Assay)

For IAA assay (Branca *et. al.*, 1988), pea seeds were washed in running tap water for 24hr and then germinated at 25°C in dark. After a week, a segment (1cm length) was cut just below the hook that had 2-3 cm length third internode. Batches of 10 segments were weighed and placed in petri dishes containing 10ml of 0.1mM IAA and fermented EFB extraction. After 3hr at 25°C in dark, the segments were harvested, put on filter paper to remove excess solution, and weighed again. The percentage of inhibition was calculated by the formula of 100 x (C-T) / C, where C is the increase weight of control (without EFB fermented extracts) and T is the increase in fresh weight of the sample treated with extracts.

8.2.3 Result and Discussion

(1) Inhibition of stem segment elongation (IAA Assay)

Water soluble and alcohol extract fractions were applied for their activity of interfere with elongation of pea stem induction by Indol acetic acid (IAA). Table 8.2-1 and 8.2-2 show the inhibition ability of water extract fraction against IAA inducing pea stem elongation. Tables show the pea stem elongation activity of IAA. Addition of 0.1 mM IAA caused 4 times larger increase in relative fresh weight of pea stem. Only the addition of 100 ml of extract suppressed 20% of the elongation. The results show that water soluble fraction of fermented EFB did not have any effect on pea stem elongation both +IAA or -IAA conditions. The ethanol extract fraction inhibited pea stem elongation. The results of inhibition activity test with alcohol extract fraction were shown in Table 8.2-3 and 8.2-4. At the presence of IAA, alcohol extract fraction have little inhibition activity of pea stem elongation by IAA (Table 8.2-3). However, without IAA condition, pea stem elongation was inhibited by the ethanol extract fraction (Table 8.2-4). Especially, addition of 100 μl extract caused 43.7% of inhibition of elongation. The elongation-inhibition activity of 200ml of ethanol extract was slightly weaker than that of 100 μl. At 300 μl extraction, disappeared the inhibition activity of pea stem elongation. The conclusion of inhibition activity of pea stem elongation by extracts of fermented EFB was shown in Fig. 8.2-1. The pea stem elongation by IAA is stable with both fraction of water and ethanol extract. And Both extract did not work as inhibitor or accelerator of IAA. When the water extract applied to the pea stem without IAA, only the amount of 200 μl caused 20% inhibition of elongation. The ethanol extract showed strong inhibition of pea stem elongation in 100 to 200 μl.

(2) Cotyledon assay for elicitor activity

Water and ethanol soluble fraction were applied for glyceollin induction assay. Figure 8.2-2 shows the HPLC chromatograms of induced components from soy bean cotyledon. In the figures, position of arrow (a) and (b) show the glyceollin estimated from previous study. The chromatogram of water extract fraction has 2 peaks of glyceollin (a) and (b). On the other hand, the chromatogram of ethanol extract fraction has only glyceollin(a) but glyceollin(b) was not observed. Glyceollin induction activity of water soluble fraction is higher than that of alcohol fraction, this is showed by size of peak (b) of in water extract which is more than 3times larger than that of peak (a) in ethanol extract. It is suggested that the components in water fraction can act as elicitor stronger than the soluble substances in alcohol fraction. The result of this bioassay test revealed that the EFB by-product can release compound capable of inducing glyceollin production in soybean cotyledon. This is due to the composition of EFB, which contains plant cell wall oligogalacturonides. According to Sharp *et. al.* (1984), oligosaccharides of plant cell walls are one of the elicitor of biotic origin. Furthermore, there is an evidence that oligogalacturonides derived from the pectic polysaccharides of plant cell walls can serve as regulatory molecules that induce glyceollin accumulation in soybean (Davis *et al.*, 1986).

8.2.4 Conclusion

The results of preliminary study on biochemical activity of fermented EFB suggest that fermented EFB contains quite unique component as bioactive materials. The IAA assay with ethanol extract fraction showed the interest result. Normally these kind of component act as inhibitor of IAA. The fraction did not show any activity with IAA, but without IAA, it acts as elongation inhibitor of pea stem. It means that the components in ethanol extract fraction do not compete with IAA activity. The gryceollin assay showed that water extract fraction had strong induction activity of gryceollin in soy bean cotyledon. Therefore, it was considered that fermented EFB is useful not only animal feed but also the course for the bioactive materials.

Table 8.2-1. Effect of different volumes of water soluble fraction on increase in fresh weight in the presence of 0.1mM IAA

H_2O extract (μl)	Initial FW (g)*	Final W (g)	Relative FW (g)	% C**
0	0.309	0.371	0.062	100
100	0.303	0.365	0.062	100
200	0.300	0.360	0.060	96.7
300	0.327	0.389	0.062	100

* Relative FW (fresh weight) is increase in FW after 3h in dark at 25°C.
** %C is percentage of increase in FW above increase of control (no sample added).

Table 8.2-2. Effect of different volumes of water soluble fraction on increase in fresh weight in the absence of 0.1mM IAA

H_2O extract (μl)	Initial FW (g)*	Final W (g)	Relative FW (g)	% C**
0	0.357	0.372	0.015	100
100	0.328	0.340	0.012	80
200	0.349	0.364	0.015	100
300	0.336	0.351	0.015	100

Table 8.2-3. Effect of alcohol extract fraction on pea stem elongation in the presence of 0.1mM IAA

EtOH extract (μl)	Initial FW (g)	Final W (g)	Relative FW (g)	% C
0	0.343	0.394	0.051	100
100	0.353	0.402	0.049	96.1
200	0.392	0.447	0.052	102
300	0.348	0.401	0.053	104

Table 8.2-4. Effect of different volumes of alcohol extract fraction on increase of fresh weight in the absence of 0.1mM IAA

EtOH extract (μl)	Initial FW (g)	Final W (g)	Relative FW (g)	% C
0	0.365	0.381	0.016	100
100	0.365	0.374	0.009	56.3
200	0.357	0.370	0.013	81.3
300	0.388	0.404	0.016	100

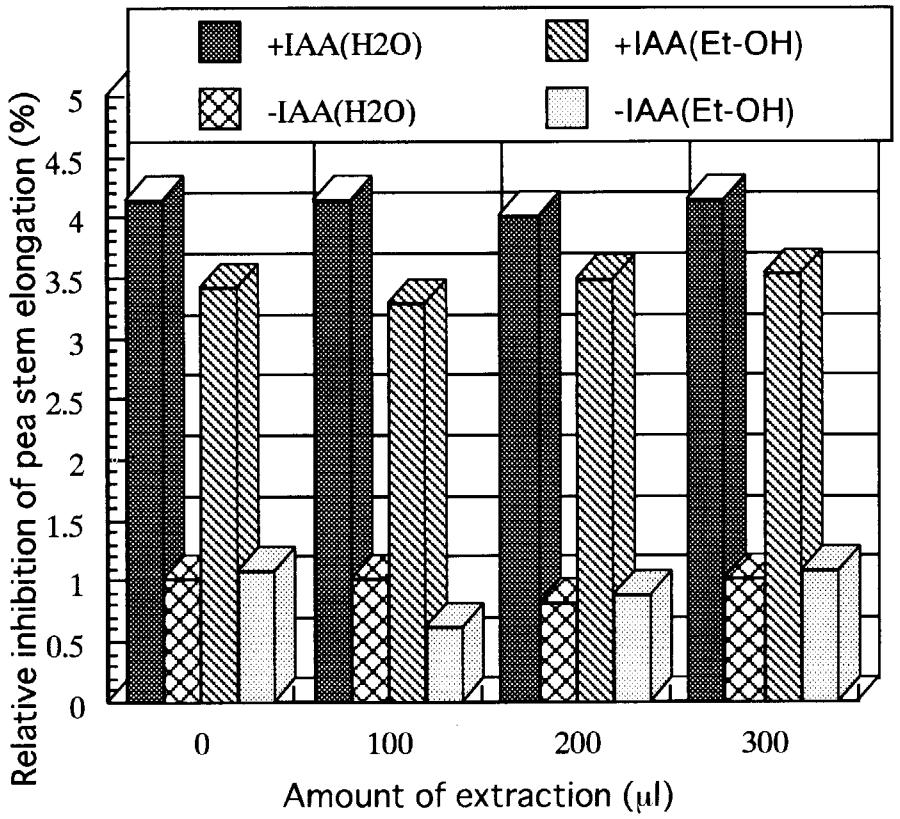

Fig. 8.2-1. Inhibition of IAA inducing pea elongation by extraction of fermented EFB

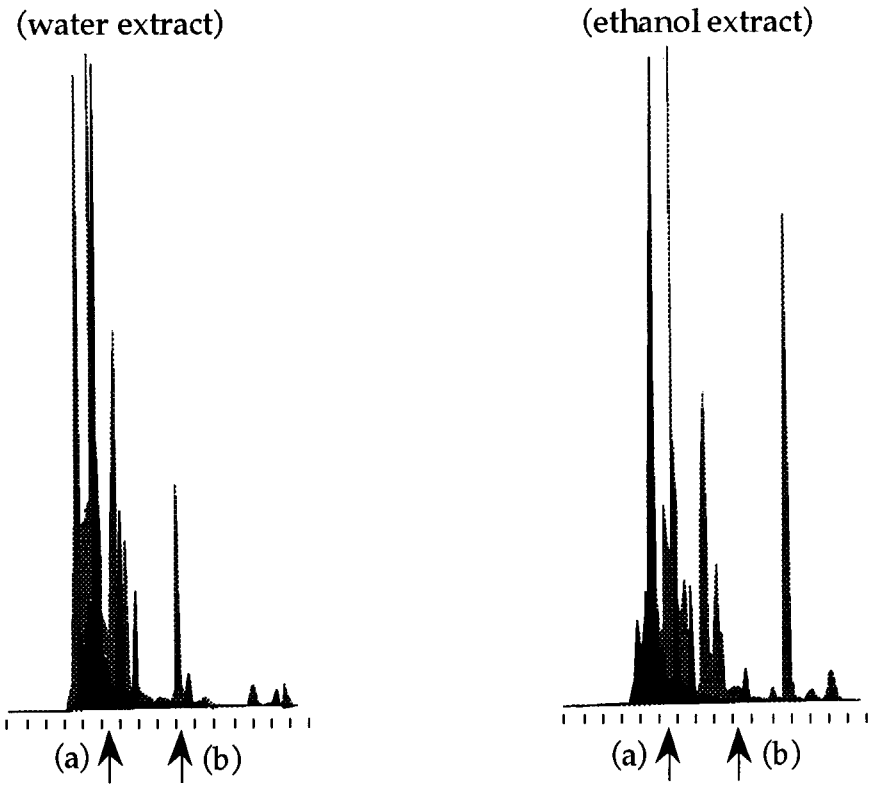

Fig. 8.2-2. HPLC analysis of induced components of soy bean cotyledons by water and ethanol extraction of fermented EFB

8.3 Effect of Rubber Waste on Fermentation

8.3.1 Introduction

Upgrading of oil palm wastes to animal feeds has been studied using rice bran as the nutritional additive for EFB fermentation. On the other hand, it is reported that the rubber wastes obtained from deposition of solid suspension of effluent in rubber processing industry enhance the growth of fungi and the yield of mushrooms.

In this study, the effect of rubber waste on liquid and solid state fermentation by *P. sajor-caju* was investigated to clarify the possibility of utilizing the rubber wastes for EFB fermentation instead of rice bran.

8.3.2 Materials and methods

(1) Liquid state fermentation

One batch of 100 ml of Mandel's media in a 300 ml conical flask was added 1% EFB powder (size : < 2 mm) or 1% glucose with 2% rubber waste. The media were sterilized by autoclaving at 121°C or irradiation at 30 kGy. The sterilized media were inoculated with 5 ml homogenous liquid seed of *P. sajor-caju*. The media were incubated at 30°C in an orbital shaker at 100 rpm. The samples were collected in duplicate after 1, 2, 3, 5 and 7 days incubation. The fermented products were collected by filtration through filter paper No. 5c and rinsed with distilled water two times. The samples were dried at 100°C for 4 hr and weighed.

(2) Solid state fermentation

Three types of EFB substrates with moisture content of 65% were prepared; 1) only EFB (size : < 2mm), 2)EFB with 5% rice bran (dry matter basis), 3)EFB with 5% rubber wastes. About 100g of EFB substrate was put into a 500 ml conical flask and covered with aluminum foil. The samples were sterilized by autoclaving at 121°C or irradiation at 30 kGy. The sterilized substrates were inoculated with 5 ml liquid seed of *P. sajor-caju* and incubated at 30°C. The samples were collected in duplicate and dried at 100 °C after 0, 1, 2, 3 and 4 weeks incubation.

(3) Preparation of seeds

Seven strains such as *P. sajr-caju*, *P. flavellatus*, *Coprinus phlytidosporus*, *Favolus arcularius*, *Volvariela volvacea*, *C. cinereus* and *Hevelom vinosophyllum* were selected as

mushroom seed fungi. These seeds were cultivated on potato dextrose agar (PDA) plates and incubated at 30°C for 5 to 12 days.

8.3.3 Results and discussion

(1) Liquid state fermentation

Table 8.3-1 shows the increase of protein content in fermented EFB with and without 2% rubber wastes after 1, 2 ,4 ,5 and 7 days incubation. The increase of protein content in fermented EFB with rubber wastes was a little higher than that without rubber wastes. The contents of crude protein in fermented EFB with rubber wastes increased from 4.3% to 14.5% after 4 days incubation and then leveled off. While the protein content in fermented product without rubber wastes increased from 4.1% to 10.0% after 4 days incubation, and up to 11.8% after 7 days. It is, therefore, considered that the presence of rubber wastes for the fermentation of EFB could increase the protein content in a short time.

Table 8.3-2 shows the production of mycelium and total protein in liquid fermentation by *P. sajor-caju* using Mandel's media containing 1% glucose and 1% glucose with 2% rubber wastes. The mycelium and its total protein in fermentation media containing rubber wastes increased rapidly than that in fermentation media without rubber wastes. The total protein contents were increased from 0.6 g/l to 6.4 g/l after 7 days incubation while the contents in fermentation media without rubber wastes increased only a small amount after the same incubation period.

(2) Solid state fermentation

Table 8.3-3 shows the protein contents of fermented EFB; 1) without any nutritional additives, 2) with 5% rice bran, 3) with 5% rubber waste. It was found that the protein contents in fermented EFB with 5% rubber waste were highest among these conditions. The result suggests that the rubber wastes are better than rice bran as nutritional additives for EFB fermentation. Further study is necessary to clarify the effect of rubber wastes with different concentrations.

From three different conditions of fermentation, it is considered that the rubber wastes have a potential to be used as an nutritional additives in preparation of liquid seed or EFB fermentation.

(3) Effect of rubber wastes to the growth of fungi on agar plate

Six types of agar plates without rubber waste were prepared by mixing 1% carbon sources such as glucose, glucose and peptone, EFB, cellulose, xylan and xylose into

Mandel's solution. The solutions were sterilized by autoclaving at 121°C. Another two series of agar plates containing 0.5% and 1.0% of rubber wastes were prepared. The agar plates were inoculated with various seeds (inoculum diameter, 9 mm) and incubated at 30°C.

Figures 8.3-1, 2, 3 show the growth rate of *P. sajor-caju* on various media containing different concentration of rubber waste. In the case of *P. sajor-caju*, the growth was increased with higher concentration of rubber wastes (1.0% > 0.5% > 0) in Figs. 8.3-3, 2 and 1. Similar tendency was observed with various fungi but the different results were also obtained with some strains. The effect of substrates was not clear. Further study is necessary to clarify the effect of substrates and additives such as rubber wastes.

8.3.4 Conclusion

Effect of rubber wastes on EFB fermentation was investigated to clarify its growth factor. The growth rate of fungi was increased with 0.5 - 5% rubber waste and the enhancement of growth was higher than that of rice bran. The results suggest that the rubber waste can be used as nutritional additive for EFB fermentation.

Table 8.3-1. Crude Protein contents of fermented EFB by *P. sajor -caju* in liquid state

Incubation Period (Days)	Protein content (% of dry matter)	
	A	B
0	4.1 ± 0.1	4.3 ± 0.3
1	4.9 ± 0.08	6.6 ± 0.0
2	7.7 ± 0.3	11.2 ± 0.3
4	10.0 ± 0.4	14.5 ± 3.6
5	10.9 ± 0.5	14.2 ± 0.4
7	11.8 ± 0.2	14.3 ± 0.8

A : 1 % EFB in Mandel's media, B: 1% EFB + 2 % rubber waste

Table 8.3-2. Yield of mycelium and protein contents in EFB substrate by *P. sajor-caju* in liquid fermentation

Incubation period (days)	Dry weight of mycelium (g/l)		Total protein produced (g/l)	
	G	GR	G	GR
0	0.8	1.6	0.2	0.58
1	0.95	1.2	0.23	0.55
2	2.2	2.7	0.65	1.10
4	4.4	10.8	1.17	3.94
5	6.4	17.9	1.65	5.78
7	7.5	22.1	2.00	6.36

G: Mandel's medium containing 1 % glucose
GR: Mandel's medium containing 1 % glucose and 2 % rubber waste.

Table 8.3-3. Protein content of Fermented EFB by *P. sajor-caju* in Solid Fermentation

Incubation Period (Weeks)	E	RB	RW
		% of dry matter	
0	3.7 ± 0.1	4.3 ± 0.2	5.2 ± 0.1
1	3.8 ± 0.2	4.6 ± 0.3	5.6 ± 0.06
2	4.1 ± 0.0	4.6 ± 0.04	6.5 ± 0.1
3	4.3 ± 0.2	4.9 ± 0.2	6.8 ± 0.06
4	4.3 ± 0.06	5.1 ± 0.1	7.2 ± 0.09

100 g EFB with moisture content of 65 % was fermented by *P. sajor-caju* in a 300 ml conical flask and incubated at 30 °C.

E : EFB without additives

RB : EFB with 5 % rice bran

RW : EFB with 5 % rubber waste

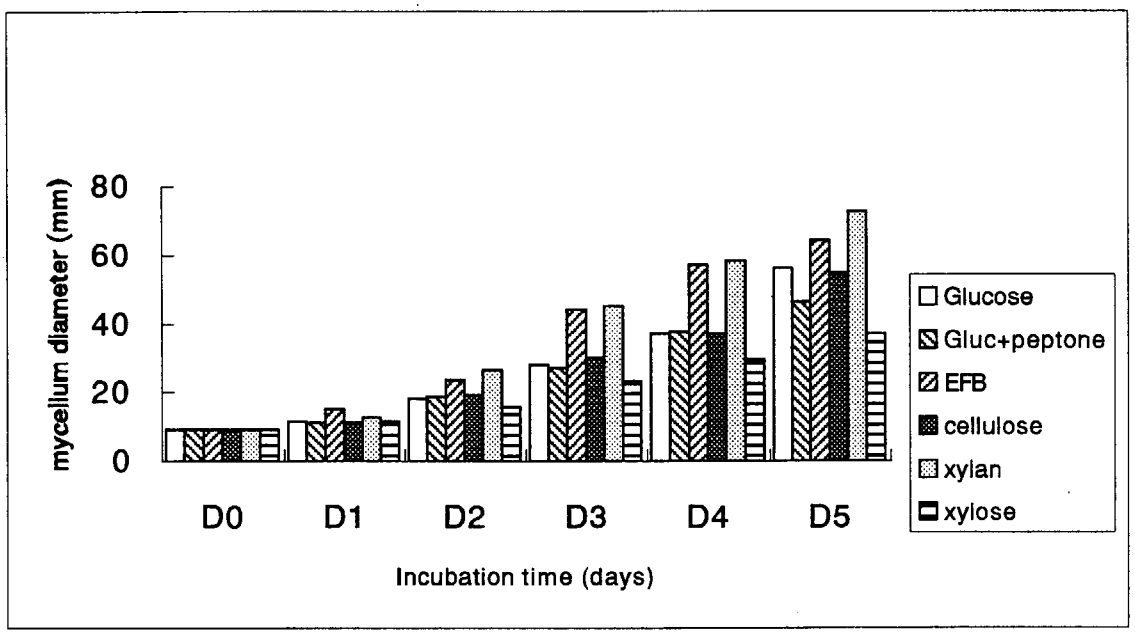

Fig. 8.3-1. Growth of *P. sajpr-caju* on various media wothout rubber waste

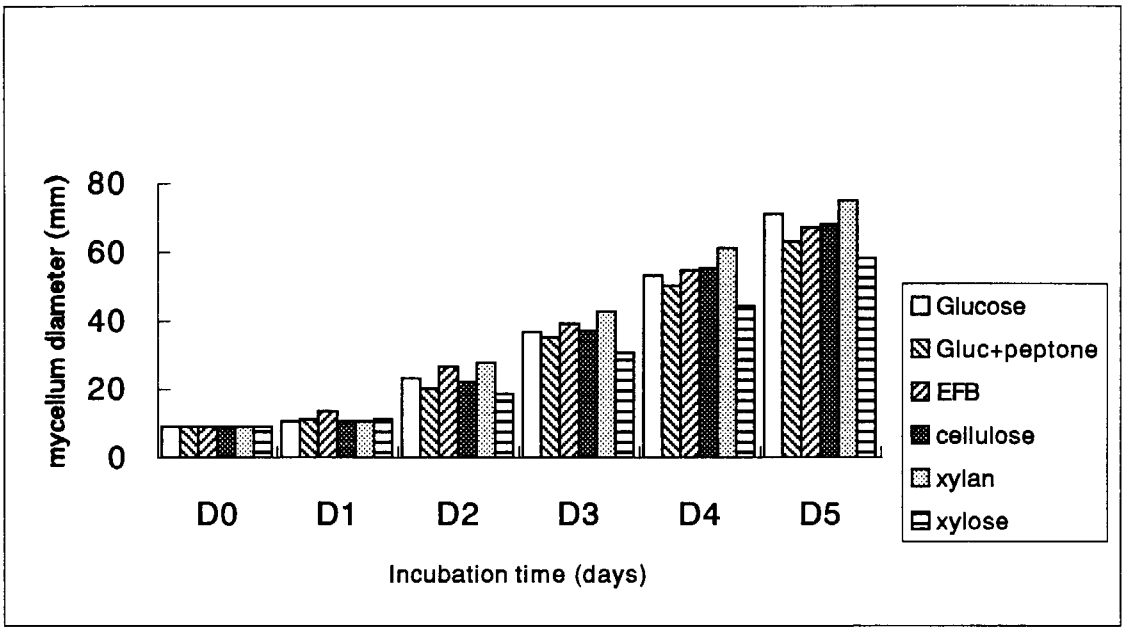

Fig. 8.3-2. Growth of *P. sajpr-caju* on various media containing 0.5% rubber waste

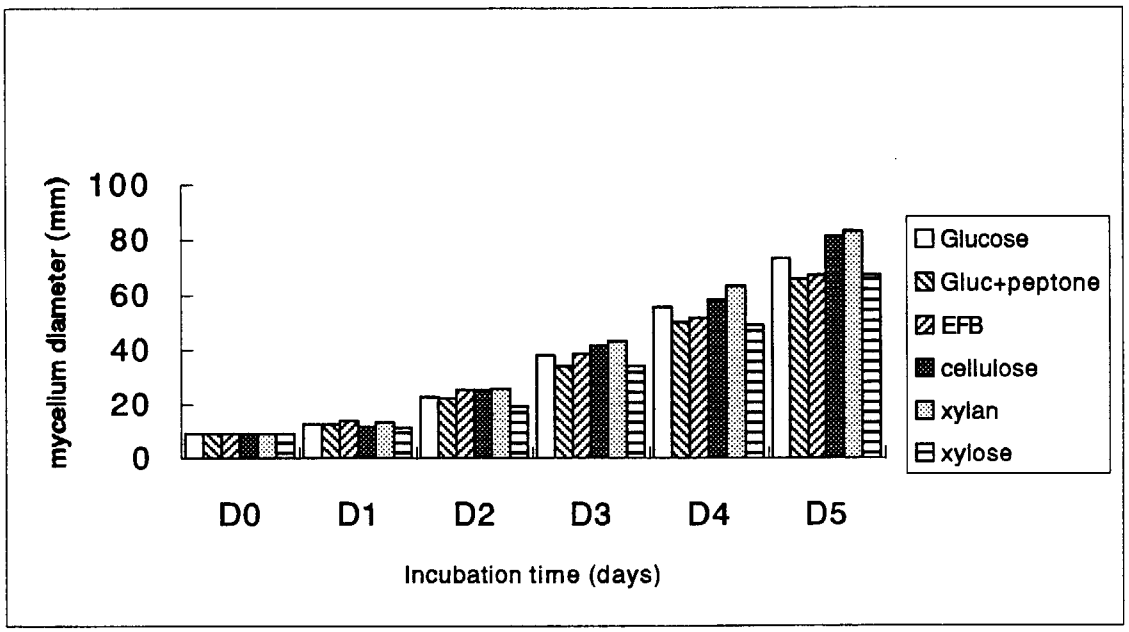

Fig. 8.3-3. Growth of *P. sajpr-caju* on various media containing 1.0% rubber waste

8.4 Growth inhibition of Fungi by EFB Extract

8.4.1 Introduction

Empty Fruit Bunch is consisting of 40 - 60% cellulose with hemicellulose, lignin and other materials. The lignocellulosic waste can be used as substrate for the growth of various type of fungi but the substrates often contain phenolic monomers (Cherney et al, 1989) that are released during biological attack on the lignin component (Cai et al, 1993). Several of these monomeric phenols inhibit both fungal growth (Akin and Rigsby, 1985) and the hydrolytic enzymes those catalyze the plant breakdown of the cellulolytic and hemicellulolytic constituents of plant cell walls. Cai et al (1993) reported that different mushrooms were exhibited with different sensitivity profiles to lignin-related phenols and tannin derivatives. This may be linked to the ability of some mushroom species to produce lignolytic and other enzyme which degrade and/or detoxify inhibitory substances.

In this section, the inhibitors from EFB extracts and the effect of sterilization with autoclave and radiation were investigated.

8.4.2 Materials and methods

(1) Preparation of 1% EFB extract

About 10 g ground EFB, size less than 2 mm was placed into a 1000 ml volumetric flask and filled with distilled water up to 1000 ml. The solution was stirred for about 1 hr to dissolve the soluble materials in EFB. The solution was strained through a nylon net to separate solid residue and liquid solution. The liquid solution collected as EFB extract was then centrifuged at 10,000 rpm for 20 min to obtain clear supernatant.

(2) Inoculation of fungi on the plate media

The petri dishes with potato dextrose agar and 1% EFB extract were inoculated with five strains of fungi. The fungi used were *Pleurotus sajor-caju, Favolus arcularias, Coprinus cinereus, Hevelom vinosophyllum* and *Coprinus phlyctidosporus*. The inoculum of each fungi was placed at the center of the agar media. The petri dish were incubated at 30℃. The mycelium diameter was measured daily for one week.

8.4.3 Results and discussion

(1) Growth of fungi on solid media

Figures 8.4-1～8.4-5 show that the effect of 1% EFB extract on the growth of fungi, *P. sajor-caju, C. phlyctidosporus, C. cinereus, F. arcularius* and *H. vinosophyllum*. EFB extract suppress the growth of some fungi especially *C. cinereus* whereas no inhibition was observed on the growth of *P. sajor-caju*. The growth suppression might be due to the presence of inhibitors in the extracts and the growth inhibitors were low molecular weight (less than 1000) fraction of this extracts. The low molecular weight components separated using ultra-membrane filter suppressed the growth of *C. cinereus* (Fig. 8.4-6). The inhibitory activity was higher for the lower molecular weight below 1000 as compared with the higher molecular weight EFB extract. The inhibitor might be from the phenolic compound that have molecular weight below 1000. On the other hand, the low molecular weight fraction of EFB extract enhanced the growth of *P. sajor-caju* (Fig. 8.4-7). The results suggest that *P. sajor-caju* possibly produced phenol-oxidases, which consequently digested the inhibitor in EFB extract at the lower concentration or the presence of other growth factors in EFB extracts.

(2) Growth of fungi in liquid media

The optimum dry weight content in the final product was obtained between 2 to 4 days of fermentation. Figures 8.4-8 and 8.4-9 show that *P. sajor-caju* grew well in 1% EFB extract and 1% glucose as compared to 1% glucose only. This may due to *P. sajor-caju* can utilize EFB extract as carbon source and consumed the extract to produce microbial biomass. There was no significant effect using autoclave or 30 kGy gamma-irradiation for sterilization of substrates.

8.4.4 Conclusion

EFB extracts contain the inhibitor that can suppress the growth of some fungi at 1% concentration. The low molecular weight fraction of EFB extract (less than 1000) suppressed the growth of *C. cinereus* but accelerated the growth of *P. sajor-caju*. The result suggests the possibility to omit the soaking treatment of EFB before the fermentation of *P. sajor-caju*.

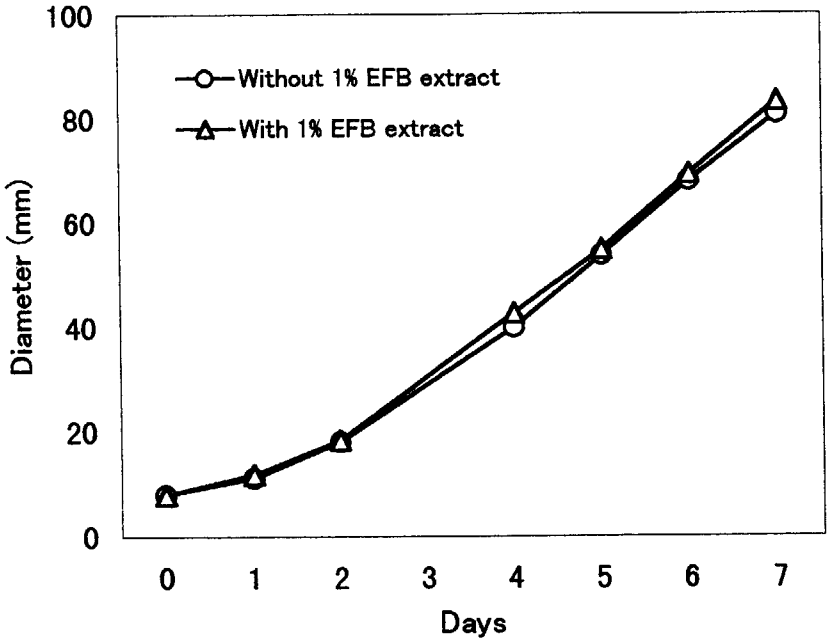

Fig. 8.4-1. Growth curve of *P. sajpr-caju* on PDA with and without 1% EFB extract

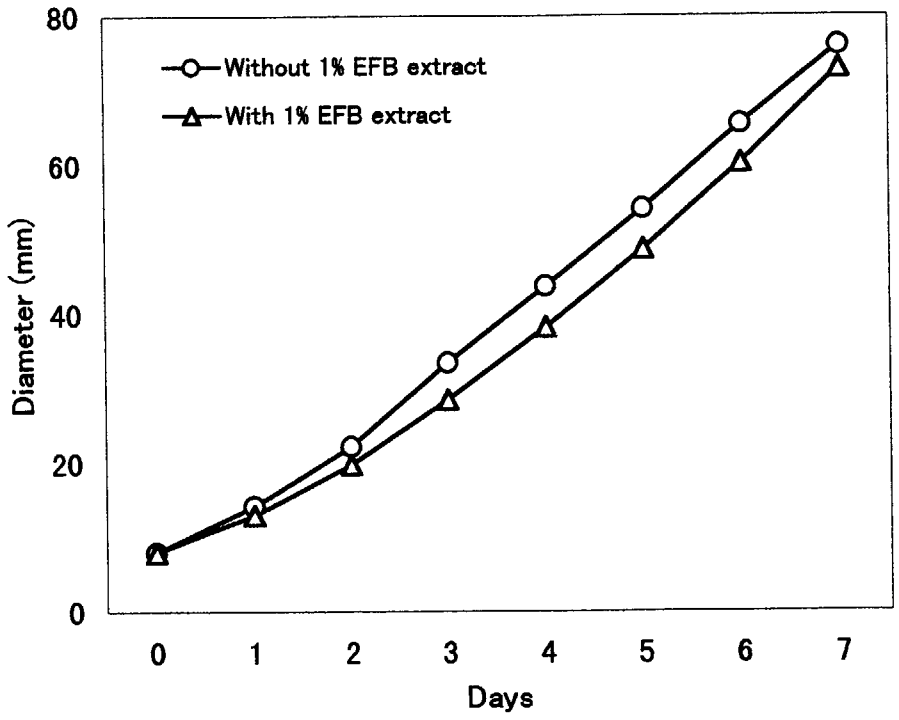

Fig. 8.4-2. Growth curve of *C. phlyctidosporus* on PDA with and without 1% EFB extract

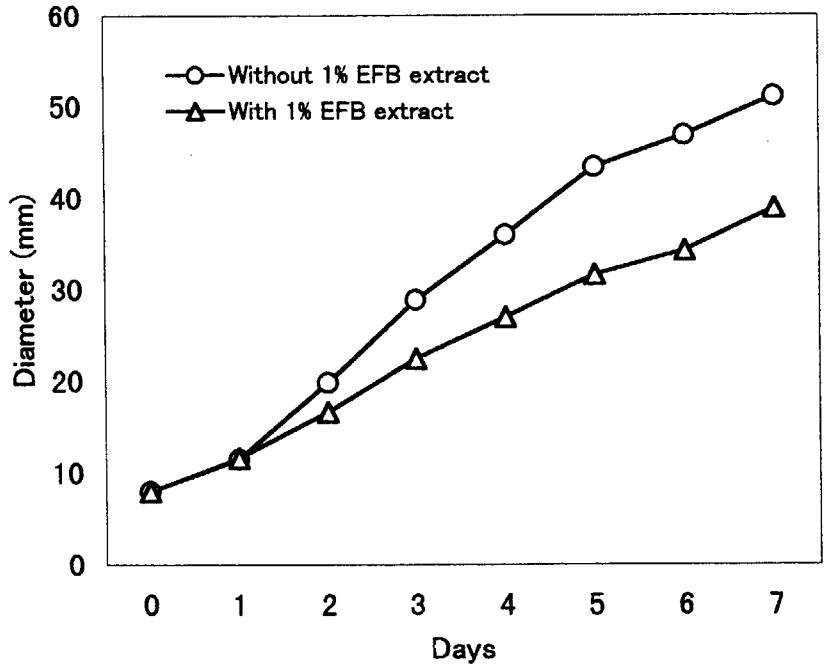

Fig. 8.4-3. Growth curve of *C. cinereus* on PDA with and without 1% EFB extract

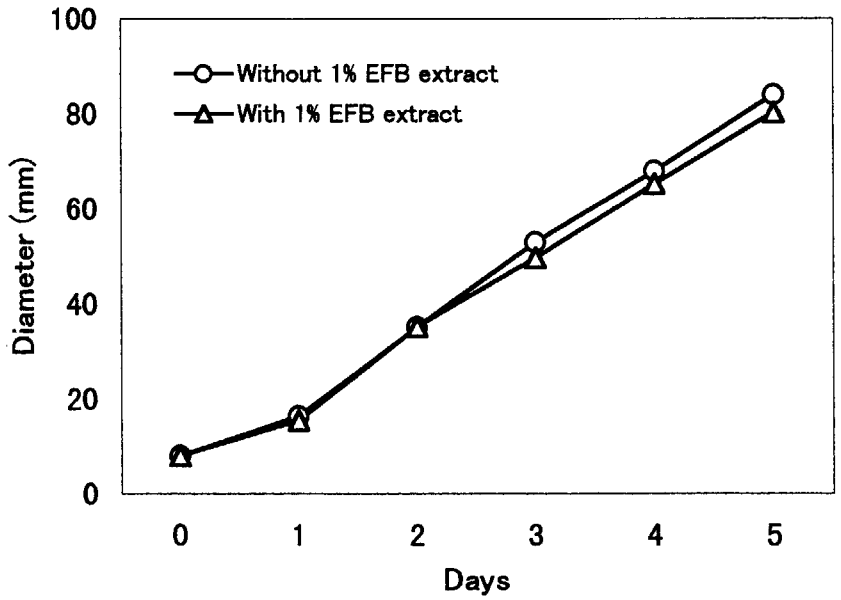

Fig. 8.4-4. Growth curve of *F. arcularius* on PDA with and without 1% EFB extract

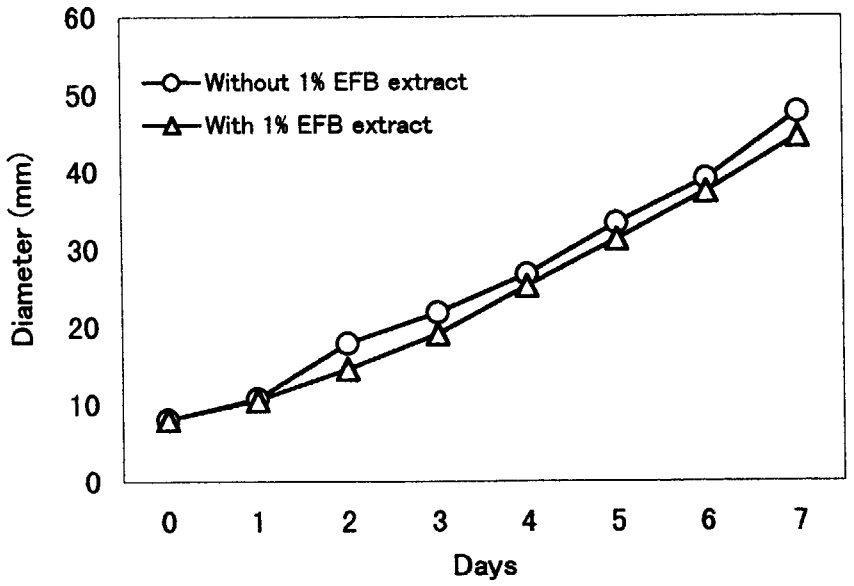

Fig. 8.4-5. Growth curve of *H. vinosophyllum* on PDA with and without 1% EFB extract

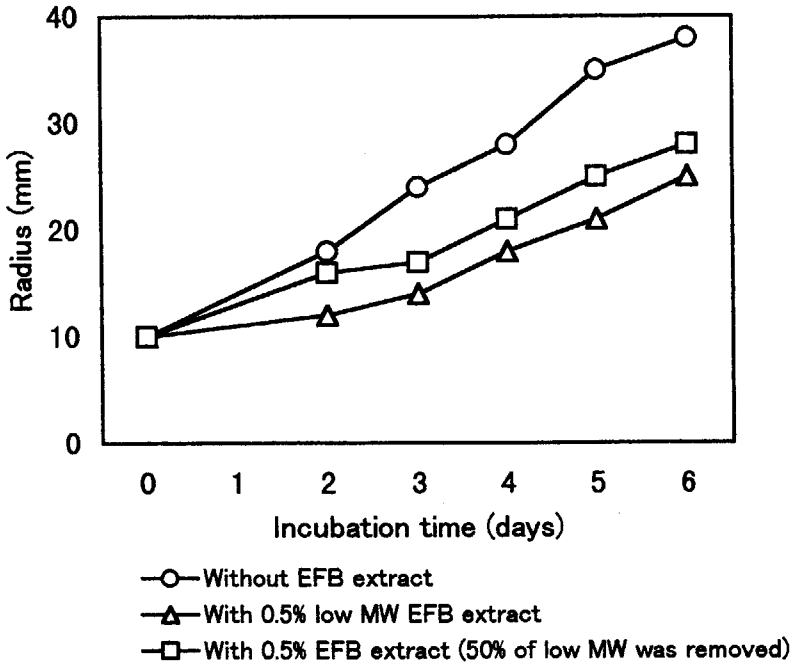

Fig. 8.4-6. Effect of low molecular weight fraction in EFB extract on growth of *C. cinereus*

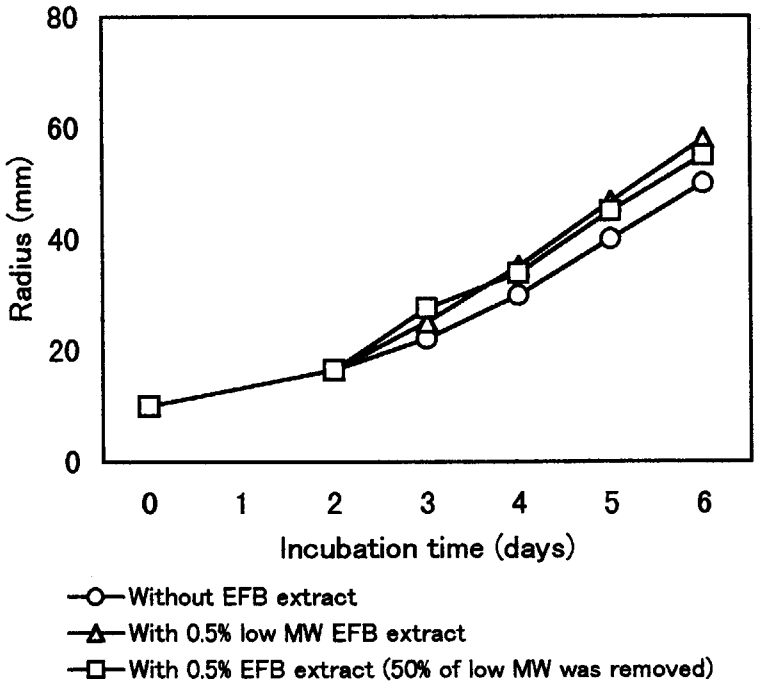

Fig. 8.4-7. Effect of low molecular weight fraction in EFB extract on growth of *P. sajpr-caju*

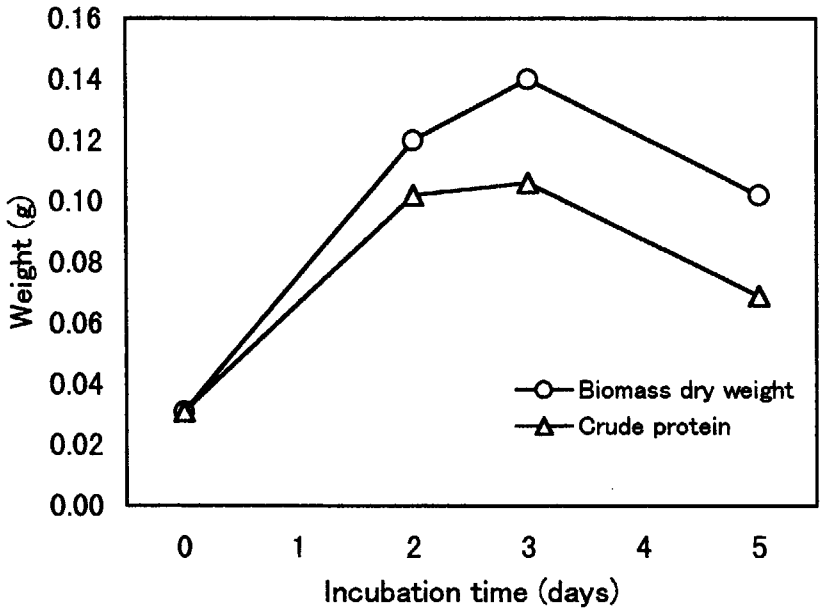

Fig. 8.4-8. Growth of *P. sajpr-caju* in liquid substrate with 1% glucose

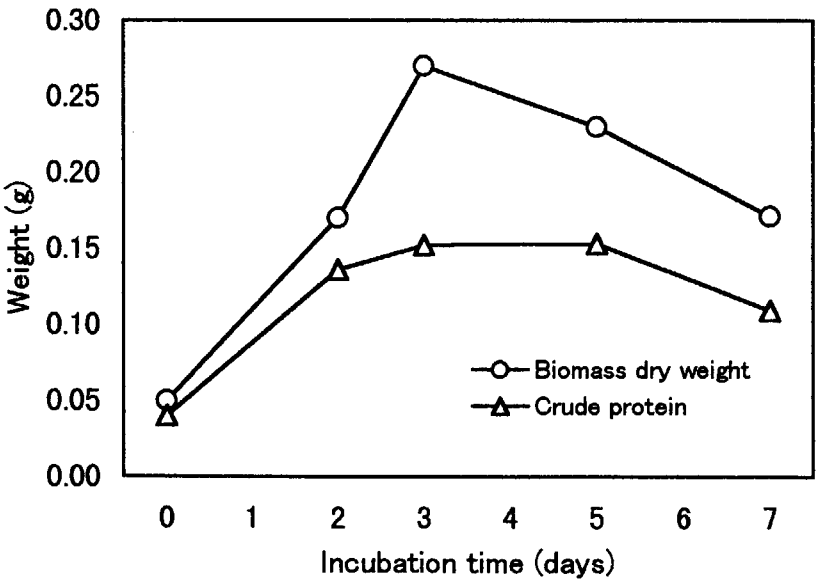

Fig. 8.4-9. Growth of *P. sajpr-caju* in liquid substrate with 1% EFB extract and 1% glucose

9. CONCLUSION

The project for "Upgrading of Oil Palm Wastes" has been jointly carried out under Implementing Arrangements between the Japan Atomic Energy Research Institute (JAERI) and the Government of Malaysia on bilateral research co-operation in the field of Radiation Processing. The bilateral research co-operation of JAERI- MINT has been implemented in two phases, Annex 1 (1987 - 1992) and 2 (1992 - 1997), and the results obtained during 10 years are summarized as follows:

9.1 Distribution, identification and radiation decontamination of microorganisms

The distribution of microorganisms in EFB and PPF was enumerated using the samples collected from 15 palm oil mills in peninsular Malaysia. All samples were highly contaminated with bacteria (10^8 - 10^{10} cells/g) and fungi (10^4 - 10^8 cells/g). The dominant species in EFB and PPF were identified and it was found that some strains of fungi produce mycotoxin. From the results of the radiosensitivity obtained by survival curves of contaminating microorganisms, it was concluded that the necessary doses were 10 kGy for pasteurization and 30 kGy for sterilization.

9.2 Physico-chemical properties of irradiated wastes

EFB are composed of 54 - 60% holocellulose (36 - 40% of cellulose), 22 - 27% lignin and other materials. Soluble fraction of EFB was slightly increased by irradiation up to 50 kGy, whereas holocellulose and α-cellulose were slightly decreased at a high dose of 50 kGy and lignin content did not change. Similar results were obtained in the case of PPF. From these results, it can be concluded that slight degree of degradation in the chemical components of EFB and PPF occurred by irradiation up to 50 kGy but these changes were not significant.

9.3 Survey of useful microorganisms and fermentation conditions

Several fungi and mushroom strains were used for fermentation. The strains tested were *Coprinus cinereus*, *Verticillium sp.*, *Aspergilus niger*, *Trichoderma viride*, *T. koningi*, *Pleurotus cystidiosus*, *P. sajor-caju* and others. *C. cinereus* and *P. sajor-caju* were the most suitable microorganisms for the fermentation of EFB based on their ability to degrade fiber and increase protein content. The optimum pH and temperature for the fermentation of EFB were 7 - 9 and 30 - 40℃, respectively. Finaly, *P. sajor-caju* was selected for the seed strain on upgrading of oil palm waste

because of the ability to decrease the lignin content, produce edible mushroom and easy cultivation than *C. cinereus*.

9.4 Evaluation of nutritional values of products

The quality of EFB for animal feeds was improved by fermentation with *P. sajor-caju* for 30 days at 30℃. The protein content increased from 3-4% to 7-11%, crude fiber content decreased from 43-47% to 22-34% and lignin content decreased from 18% to 11-12%. Digestibility of the fermented EFB *in vitro* and *in vivo* was increased and the product was palatable for cattle. Further studies on feeding test need to clarify the long term effect on livestock.

9.5 Techno-economic feasibility study

Large scale fermentation was examined by performing fermentation using various sizes of fermentation bags, ranging from 1- 20 kg / bag. Among those sizes that have been successfully accomplished were the 1 kg / bag size. Others need further detailed observations. Based on this result, the large scale fermentation could be produced by increasing the numbers of bags (1 kg / bags), piled up on incubation rack.

EFB is a good substrate for mushroom production and the residue after cultivation of mushroom contains high protein for animal feed. The process to produce both mushrooms and animal feeds is effective and economic. Electron beam is better for the irradiation process of huge amount of samples more than 30,000 ton/year. EB machine at MINT (30 MeV, 30 mA) is capable of treating 100,000 ton/year EFB.

9.6 Pilot plant study

The pilot plant for upgrading of EFB to animal feed named "Sterifeed" was built at MINT in 1996. This pilot plant consists of cultivation building (substrate preparation room, inoculation room, cultivation room, pelleting room) with various facilities such as cutter, mixer, seed auto-inoculator, pelleting machine, etc. The EFB substrate in a bag (1 kg / bag) is pasteurized by ^{60}Co γ-irradiation facility (3 MCi) or Electron Beam facility (3 MeV) and the throughput capacity of the plant is 30 ton/month. The pilot plant has been operating to produce the samples for animal feeding test at the rate of 10 ton/month.

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Dr. Daud Mohamad (DDG)
Ir. Kasubun Kamat (DDG)
Ir. Fatimah Mohd. Amin (Acting DDG)
Mr. Adnan Hj. Khalid (Director of Planning & International Affair Division)
Dr. Khairul Zaman Hj. Dahlan (Director of Radiation Processing Technology Division)
Dr. Norimah Yusoff (Director of Agro & Bio Science Division)
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Mr. Abdul Aziz Raja Adnan (Planning & International Affair Division)
Dr. Ainul Hayati Hj. Daud (Planning & International Affair Division)
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Dr. Isao Ishigaki (DG of TRCRE)
Dr. Miyuki Hagiwara (DG of TRCRE)
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Mr. Masahiro Nakayama (General Manager, Dept. Radiati. Res. Environ. Resources)
Mr. Yuichi Takayanagi (General Manager, Dept. Radiati. Res. Environ. Resources)

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Appendix 1. Steering Committee Meetings and Participants

1. First Steering Committee Meeting

(9 - 11 December, 1987 at MINT, Bangi)

JAERI Dr. Isao Ishigakli
Head, Radiation Processing Development Laboratory, Department of Development

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Principal Scientist, Radiation Processing Development Laboratory, Department of Development

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Acting Deputy Director General

Mr. Adnan Hj. Khalid
Acting Director of Planning

Observers

Dr. Razley Mohd. Nordin
Mr. Daud Mohamad
Dr. Mat Rasol Awang
Mr. Dahlan Mohamad
Mr. Mohamad LebaiJuri

2. Second Steering Committee Meeting

(2 - 4 June, 1988 at MINT, Bangi)

JAERI Dr. Isao Ishigakli
Head, Radiation Processing Development Laboratory, Department of Development

Dr. Hitoshi Ito
Principal Scientist, Radiation Processing Development Laboratory, Department of Development

MINT Dr. Ahmad Sobri Hj. Hashim
Acting Deputy Director General

Dr. Mat Rasol Awang
Research Officer, Isotope and Radiation in Industry Program

Observers

Mr. Adnan Hj. Khalid
Dr. Razley Mohd. Nordin

Mr. Daud Mohamad
Mr. Dahlan Hj. Mohd
Mr. Mohamad LebaiJuri
Ms. Zainon Othman
Mr. Hassan Hamdani Hassaan Mutaat
Ms. Foziah Ali

3. Third Steering Committee Meeting

(24 - 25 July, 1989 at JAERI, Takasaki)

JAERI Dr. Shoichi Sato
Director, Department of Research
Dr. Tamikazu Kume
Senior Scientist, Department of Research

MINT Dr. Ahmad Sobri Hj. Hashim
Acting Deputy Director General
Dr. Mat Rasol Awang
Research Officer, Isotope and Radiation in Industry Program

Observers

Dr. Sueo Machi
Dr. Takeshi Tsujino
Dr. Isao Ishigaki
Dr. Hitoshi Ito
Dr. Shoji Hashimoto

4. Fourth Steering Committee Meeting

(16 - 18 July, 1990 at MINT, Bangi)

JAERI Dr. Sueo Machi
Director General, TRCRE
Dr. Tamikazu Kume
Principal Scientist, Department of Research

MINT Mr. Kasbun Kamat
Deputy Director General
Dr. Mat Rasol Awang
Research Officer, Department of Research and Development

Observers

Dr. Norimah Yusoff

Dr. Khairulzaman Hj. Mohamad Dahlan
Dr. Razley Mohd. Nordin
Ms. Zainon Othman
Ms. Foziah Ali
Mr. Hassan Hamdani Hassaan Mutaat
Dr. Kenzo Yoshida

5. Fifth Steering Committee Meeting

(19 - 21 August, 1991 at JAERI, Takasaki)

JAERI Dr. Shoichi Sato
Director General
Dr. Waichiro Kawakami
Director, Department of Radiation Research for Environment and Resources

MINT Ms. Fatimah Mohd. Amin
Acting Deputy Director General
Dr. Mat Rasol Awang
Research Officer, Department of Research and Development

Observers
Mr. Akio Toraishi
Mr. Hideo Osono
Dr. Shoji Hashimoto
Dr. Hitoshi Ito
Dr. Tamikazu Kume
Dr. Shinpei Matsuhashi

6. Sixth Steering Committee Meeting

(23 - 24 October, 1992 at MINT, Bangi)

JAERI Dr. Dr. Shoichi Sato
Director General, TRCRE
Dr. Tamikazu Kume
Principal Scientist, Resources Utilization Technology Laboratory, Department of Radiation Research for Environment and Resources

MINT Datuk Dr. Mohd. Ghazali Hj. Abdul Rahman
Director General
Ms. Fatimah Mohd. Amin

Acting Deputy Director General

Observers

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Dr. Khairul Zaman Hj. Mohamad Dahlan
Dr. Mat Rasol Awang
Mr. Ishak Manaf
Mr. Mohamad LebaiJuri
Mr. Raja Abdul Aziz Raja Adnan
Mr. Hassan Hamdani Hassaan Mutaat

7. Seventh Steering Committee Meeting

(20 - 21 September, 1993 at JAERI, Takasaki)

JAERI Dr. Shoichi Sato
Director General
Dr. Shin-ichi Ohno
Director, Department of Radiation Research for Environment and Resources

MINT Dr. Nahrul Khair Alang Md Rashid
Deputy Director General
Dr. Mat Rasol Awang
Research Officer, Department of Research and Development

Observers

Dr. Okihiro Tokunaga
Mr. Yoichi Suto
Dr. Shoji Hashimoto
Dr. Tamikazu Kume
Dr. Shinpei Matsuhashi
Mr. Yusri bin Atan

8. Eighth Steering Committee Meeting

(5 - 6 August, 1994 at MINT, Bangi)

JAERI Dr. Waichiro Kawakami
Director, Department of Material Development
Dr. Tamikazu Kume
Principal Scientist, Resources Utilization Technology Laboratory, Department of Radiation Research for Environment and Resources

MINT Dr. Nahrul Khair Alang Md Rashid
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Head, Radiation Processing of Agro-industrial Waste Group, Radiation Processing Programme, Division of Research

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Mr. Adnan Hj. Khalid
Dr. Khairul Zaman Hj. Mohamad Dahlan
Mr. Raja Abdul Aziz Raja Adnan
Mr. Yusri bin Atan
Ms. Zal'Uyum Wan Mahmud
Mr. Mohd. Sukri Mahmood
Mr. Wan Badrin Wan Husain
Mr. Tajudin Osman
Ms. Norihan Zainal
Mr. Hassan Hamdani Hassaan Mutaat

9. Ninth Steering Committee Meeting

(11 - 12 September, 1995 at JAERI, Takasaki)

JAERI Dr. Okihiro Tokunaga
Director, Department of Radiation Research for Environment and Resources
Dr. Tamikazu Kume
Principal Scientist, Resources Utilization Technology Laboratory, Department of Radiation Research for Environment and Resources

MINT Dr. Nahrul Khair Alang Md Rashid
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Observers

Dr. Isao Ishigaki
Dr. Hitoshi Ito
Mr. Masahiro Nakayama
Dr. Shinpei Matsuhashi

Ms. Machiko Takigami
Dr. Masamitsu Shimazu
Mr. Tajudin bin Osman

10. Tenth Steering Committee Meeting

(12 - 14 June, 1996 at MINT, Bangi)

JAERI Dr. Okihiro Tokunaga
Director, Department of Radiation Research for Environment and Resources
Dr. Tamikazu Kume
Principal Scientist, Resources Utilization Technology Laboratory, Department of Radiation Research for Environment and Resources

MINT Dr. Nahrul Khair Alang Md Rashid
Deputy Director General
Dr. Mat Rasol Awang
Head, Radiation Processing of Agro-industrial Waste Group, Radiation Processing Programme, Division of Research

Observers

Dr. Khairul Zaman Hj. Mohamad Dahlan
Dr. Ainul Hayati Hj. Daud
Mr. Jamal Khaer bin Ibrahim
Mr. Mohd. Kamaruddin bin Omar
Mr. Hassan Hamdani Hassaan Mutaat

11. Eleventh Steering Committee Meeting

(14 - 15 July, 1997 at JAERI, Takasaki)

JAERI Dr. Okihiro Tokunaga
Director, Department of Radiation Research for Environment and Resources
Dr. Tamikazu Kume
Principal Scientist, Resources Utilization Technology Laboratory, Department of Radiation Research for Environment and Resources

MINT Dr. Nahrul Khair Alang Md Rashid
Deputy Director General
Dr. Mat Rasol Awang
Head, Radiation Processing of Agro-industrial Waste Group,

Radiation Processing Programme, Division of Research

Observers

Dr. Miyuki Hagiwara
Dr. Keizo Makuuchi
Dr. Hitoshi Ito
Mr. Yuichi Takayanagi
Dr. Shinpei Matsuhashi

Appendix 2: List of Abbreviations

ADF: Acid Detergent Fiber
ADL: Acid Detergent Lignin
AOAC: Association of Official Analytical Chemists
BCR (B/C): Benefit Cost Ratio
CF: Crude Fiber
CP: Crude Protein
DCF: Discounted Cash Flow
DMD: Dry Mater Digestibility
EFB: Empty Fruit Bunch
IRR: Internal Rate of Return
IVDMD: *in vitro* Dry Matter Digestibility
ME: Metabolic Energy
MINT: Malaysian Institute for Nuclear Technology Research
RM (M$): Malaysian Dollar (Ringid)
NDF: Neutral Detergent Fiber
NPV: Net Present Value
OPF: Oil Palm Frond
OPT: Oil Palm Trunk
PBP: Pay Back Period
PDA: Potato Dextrose Agar
PKC: Pal Kernel Cake
POME: Palm Oil Mill Effluent
PPF: palm press fiber
TAPPI: Technical Association of Pulp and Paper Industry Standard Method (American National Standard)
UPC: Unit Production Cost
WHC: Water holding capacity

国際単位系（SI）と換算表

表1　SI基本単位および補助単位

量	名称	記号
長さ	メートル	m
質量	キログラム	kg
時間	秒	s
電流	アンペア	A
熱力学温度	ケルビン	K
物質量	モル	mol
光度	カンデラ	cd
平面角	ラジアン	rad
立体角	ステラジアン	sr

表2　SIと併用される単位

名称	記号
分，時，日	min，h，d
度，分，秒	°，′，″
リットル	l，L
トン	t
電子ボルト	eV
原子質量単位	u

1 eV=1.60218×10^{-19}J

1 u=1.66054×10^{-27}kg

表3　固有の名称をもつSI組立単位

量	名称	記号	他のSI単位による表現
周波数	ヘルツ	Hz	s^{-1}
力	ニュートン	N	m·kg/s^2
圧力，応力	パスカル	Pa	N/m^2
エネルギー，仕事，熱量	ジュール	J	N·m
工率，放射束	ワット	W	J/s
電気量，電荷	クーロン	C	A·s
電位，電圧，起電力	ボルト	V	W/A
静電容量	ファラド	F	C/V
電気抵抗	オーム	Ω	V/A
コンダクタンス	ジーメンス	S	A/V
磁束	ウェーバ	Wb	V·s
磁束密度	テスラ	T	Wb/m^2
インダクタンス	ヘンリー	H	Wb/A
セルシウス温度	セルシウス度	℃	
光束	ルーメン	lm	cd·sr
照度	ルクス	lx	lm/m^2
放射能	ベクレル	Bq	s^{-1}
吸収線量	グレイ	Gy	J/kg
線量等量	シーベルト	Sv	J/kg

表4　SIと共に暫定的に維持される単位

名称	記号
オングストローム	Å
バーン	b
バール	bar
ガル	Gal
キュリー	Ci
レントゲン	R
ラド	rad
レム	rem

1 Å=0.1nm=10^{-10}m

1 b=100fm^2=10^{-28}m^2

1 bar=0.1MPa=10^{5}Pa

1 Gal=1cm/s^2=10^{-2}m/s^2

1 Ci=3.7×10^{10}Bq

1 R=2.58×10^{-4}C/kg

1 rad=1cGy=10^{-2}Gy

1 rem=1cSv=10^{-2}Sv

表5　SI接頭語

倍数	接頭語	記号
10^{18}	エクサ	E
10^{15}	ペタ	P
10^{12}	テラ	T
10^9	ギガ	G
10^6	メガ	M
10^3	キロ	k
10^2	ヘクト	h
10^1	デカ	da
10^{-1}	デシ	d
10^{-2}	センチ	c
10^{-3}	ミリ	m
10^{-6}	マイクロ	μ
10^{-9}	ナノ	n
10^{-12}	ピコ	p
10^{-15}	フェムト	f
10^{-18}	アト	a

（注）

1．表1－5は「国際単位系」第5版，国際度量衡局1985年刊行による。ただし，1 eVおよび1 uの値はCODATAの1986年推奨値によった。

2．表4には海里，ノット，アール，ヘクタールも含まれているが日常の単位なのでここでは省略した。

3．barは，JISでは流体の圧力を表わす場合に限り表2のカテゴリーに分類されている。

4．EC閣僚理事会指令ではbar，barnおよび「血圧の単位」mmHgを表2のカテゴリーに入れている。

換算表

力	N(=10^{5}dyn)	kgf	lbf
	1	0.101972	0.224809
	9.80665	1	2.20462
	4.44822	0.453592	1

粘度　1 Pa·s(N·s/m^2)=10 P(ポアズ)(g/(cm·s))

動粘度　1m^2/s=10^{4}St(ストークス)(cm^2/s)

圧力	MPa(=10bar)	kgf/cm^2	atm	mmHg(Torr)	lbf/in^2(psi)
	1	10.1972	9.86923	7.50062×10^3	145.038
	0.0980665	1	0.967841	735.559	14.2233
	0.101325	1.03323	1	760	14.6959
	1.33322×10^{-4}	1.35951×10^{-3}	1.31579×10^{-3}	1	1.93368×10^{-2}
	6.89476×10^{-3}	7.03070×10^{-2}	6.80460×10^{-2}	51.7149	1

エネルギー・仕事・熱量	J(=10^7 erg)	kgf·m	kW·h	cal(計量法)	Btu	ft·lbf	eV
	1	0.101972	2.77778×10^{-7}	0.238889	9.47813×10^{-4}	0.737562	6.24150×10^{18}
	9.80665	1	2.72407×10^{-6}	2.34270	9.29487×10^{-3}	7.23301	6.12082×10^{19}
	3.6×10^6	3.67098×10^5	1	8.59999×10^5	3412.13	2.65522×10^6	2.24694×10^{25}
	4.18605	0.426858	1.16279×10^{-6}	1	3.96759×10^{-3}	3.08747	2.61272×10^{19}
	1055.06	107.586	2.93072×10^{-4}	252.042	1	778.172	6.58515×10^{21}
	1.35582	0.138255	3.76616×10^{-7}	0.323890	1.28506×10^{-3}	1	8.46233×10^{18}
	1.60218×10^{-19}	1.63377×10^{-20}	4.45050×10^{-26}	3.82743×10^{-20}	1.51857×10^{-22}	1.18171×10^{-19}	1

1 cal= 4.18605J（計量法）

= 4.184J（熱化学）

= 4.1855J（15℃）

= 4.1868J（国際蒸気表）

仕事率　1 PS(仏馬力)

= 75 kgf·m/s

= 735.499W

放射能	Bq	Ci
	1	2.70270×10^{-11}
	3.7×10^{10}	1

吸収線量	Gy	rad
	1	100
	0.01	1

照射線量	C/kg	R
	1	3876
	2.58×10^{-4}	1

線量当量	Sv	rem
	1	100
	0.01	1

（86年12月26日現在）

STUDY ON UPGRADING OF OIL PALM WASTES TO ANIMAL FEEDS BY RADIATION AND FERMENTATION PROCESSING