PHYSICO-CHEMICAL PRETREATMENT OF CORN STALKS FOR BIOCONVERSION INTO PROTEIN-RICH MYCELIAL BIOMASS WITH *PLEUROTUS SAJOR-CAJU.*

A RESEARCH THESIS REPORT

BY

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Microbes of the future era will be called 'livestock' because in principle they are converters of organic matter, as are domestic animals.
- Borgstrom, G. (1976)

Dedication.

To my late mother, Bernadette Akatariba Awafo, whose sudden departure from this world coincided with the completion of this work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

RÉSUMÉ

Ce travail visait l'établissement et l'optimisation des conditions de prétraitement de paille de maïs avec l'irradiation gamma, pour leur bioconversion en biomasse mycéliale riche en protéine. Les paramètres évalués étaient; le contenu en protéines, l'efficacité de conversion du substrat en protéine ainsi que la productivité en protéine.

Pleurotus sajor-caju, un basidiomycète, fut choisi comme micro-organisme pour la bioconversion, parce qu'il est un champignon comestible et possède un système enzymatique lignocellulolytique. Le prétraitement à l'irradiation gamma fut comparé au prétraitement alcalin et aussi à des combinaisons des deux. Les pailles de maïs prétraitées à la vapeur et des substrats de glucose furent aussi utilisés afin de donner une meilleure perspective du rôle de l'irradiation comme prétraitement.

Les résultats expérimentaux générés dans cette étude ont fourni des informations sur la production de biomasse mycéliale de *Pleurotus sajor-caju* à partir de substrats de tiges de maïs prétraitées ou non et de glucose, desquelles les conclusions suivantes ont été formulées:

1. Les prétraitements simples des tiges de maïs avec l'irradiation gamma nécessite des doses d'au mois 1 MGy ou

plus pour une synthèse protéique efficace par *Pleurotus sajor-caju.*

2. Les prétraitements avec NaOH de 0,1 et 0,15 g/g de substrat sont supérieurs ($p \leq 0,05$) à la dose maximale d'irradiation de 1,7 MGy dans la production de protéines avec *Pleurotus sajor-caju.*

3. Un contenu en azote de 0,4 g L^{-1} fut la meilleure concentration pour la production de biomasse mycéliale de *Pleurotus sajor-caju* avec 1% de substrat glucose en milieu Mandel's.

4. Les prétraitements simples des tiges de maïs avec 0,15 g NaOH/g de substrat fut le meilleur traitement ($p \leq 0,05$) individuel pour la synthèse protéique avec *Pleurotus sajor-caju.*

5. Les prétraitments combinés de NaOH et l'irradiation gamma ont réduit à la fois la quantité de NaOH ainsi que les doses d'irradiation nécéssaires pour obtenir des rendements optimaux de protéines.

6. Les prétraitments combinés de 500 KGy et 0,1 g NaOH/g de substrat; 500 KGy et 0,05 g NaOH/g de substrat; 1 MGy et 0,05g NaOH/g de substrat; 8% sulfite de soude avec 0,5% NaOH et vapeur, ont donné des rendements protéiques comparables ($p \leq 0,05$) au meilleur prétraiment simple de 0,15 g NaOH/g de tiges de maïs.

7. L'holocellulose de tiges de maïs peut être aussi bonne ou meilleure que le glucose dans la production de biomasse mycéliale de *Pleurotus sajor-caju* riche en protéines lorsque les meilleurs conditions de prétraitements sélectifs mentionnés précédemment ont été utilisés.

8. Les fractions de cellulose et d'hémicellulose des tiges de maïs ont donné des rendements protéiques comparables ou supérieurs au glucose comme substrat pour la production de biomasse mycéliale riche en protéine de *Pleurotus sajor-caju*

9. Les rendements protéiques obtenus en flacons agités de 250 mL et en fermentations de 10 L ont été comparables, ce qui est une bonne indication pour une possibilité de production à grande échelle.

10. L'évaluation du coût de prétraitement a indiqué une préférence pour le traitement combiné de 0,1 g NaOH/g de substrat et 100 KGy d'irradiation gamma en se basant sur la productivité économique en protéines (g de protéines synthétisées h^{-1} $\$^{-1}$ de prétraitement) qui a donné un produit final contenant environ 40% de protéines (1,57 g protéines L^{-1}).

ABSTRACT

This work establishes a synergistic effect of pretreatment conditions of gamma irradiation and sodium hydroxide on corn stalk for the production of mycelial biomass of *Pleurotus sajor-caju* rich in protein. Comparisons between protein yields of substrate pretreated with sodium hydroxide and gamma irradiation separately and with their combinations; and steam and glucose have been discussed.

Combination treatments of NaOH and gamma irradiation reduced both the quantity of NaOH and doses of irradiation required to get optimum yields of protein. Combination treatments of 500 KGy and 0,1 g NaOH/g substrate; 500 KGy and 0,05 g NaOH/g substrate; 1 MGy and 0,05 g NaOH/g substrate gave protein yields comparable to or better than the best single pretreatment of 0,15 g NaOH/g substrate. The highest protein content of the final product (mycelial biomass) obtained from these combination treatments was 45% (2,02 g protein L^{-1}). This represented over 90% utilization of the corn stalk holocellulose for protein synthesis. It was further revealed that corn stalk holocellulose and its individual fractions of cellulose and hemicelluloses could be as good as or better than glucose as substrates for the production of protein-rich mycelial biomass of *Pleurotus sajor-caju.* The cellulose fraction produced a final product containing about 50% protein (2,73 g protein L^{-1}) which represented over 95% conversion efficiency into protein. Protein yields from both the 250-mL shake flasks and 10-L fermentor were comparable. The cost of pretreatment assessment indicated a preference for the combination treatment of 0,1 g NaOH/g substrate and 100 KGy gamma irradiation which gave a final product containing about 40% protein (1,57 g protein L^{-1}).

1.0 INTRODUCTION

The 1990 world population of 5.3 billion people (Piel, 1993) is projected to double to 10 billion people by the turn of the twenty-first century with a concomitant decrease in the production of food and feed.

On the other hand, there is also increasing demand for new foods, especially protein sources for human consumption in the developing countries as well as in the developed countries.

There is, therefore, the stimulus to explore further into the production of unconventional food/feed protein sources from the large base of agricultural and forestry residues which abound all over the world.

One of the ways in which unconventional protein sources could be easily accepted is to present them in familiar forms and to our advantage, the use of higher fungi (mushrooms) for human consumption precedes written history (Litchfield, 1983). The other advantage of fungal biomass of such higher fungi is that it may be processed to give a textured protein (Forage and Righelato, 1979).

Since the 1920's, filamentous fungi have been used for protein production (Thatcher,1954). Humfield and Sugihara (1949) reported the production of mushrooms mycelia of *Agaricus spp.* in submerged culture as a substitute of mushrooms for culinary purposes. During the 1960's and 70's, some researchers have used filamentous fungi for the production of protein from various substrates (Chahal et

al.,1977; Gray,1962; Gregory et al.,1976; Moo-Young et al.,1977; Peitersen, 1975; Bukhalo and Solomko,1978). In the 1980's, various researchers (Chahal,1984; Chahal et al.,1987; Friedrich et al.,1986; Hadar and Cohen-Arazi,1986; Manu-Tawiah and Martin,1987) have produced the mycelium of *Pleurotus spp.* in submerged culture on various substrates (beer wort, sulfite liquor, hemicellulose fraction, glucose, sugar beet, molasses, apple distillery wastes and peat extract).

Pleurotus sajor-caju is one of the known white-rot fungi which utilizes polysaccharides (cellulose and hemicellulose) from agricultural residues to produce mushrooms for human consumption (Chahal, 1989). Moreover, it can also degrade lignin to some extent (Nat. Acad. Sci., 1979 ; Bourbonnais and Paice, 1988; Chahal and Hachey, 1990).

The production of mushroom mycelium in submerged fermentation is one way to transform lignocelluloses into materials with good nutritive value (Block et al., 1958 ; Reusser et al., 1958) and has a potential use as a food, food additive or food supplement (Litchfield, 1968 ; Janardhanan et al., 1970). The submerged fermentation process has the advantage of being able to produce large amounts of mushroom mycelium in comparatively short periods of time throughout the year all over the world (Litchfield and Overbeck, 1965) as compared to the production of mushrooms in solid state fermentation (e.g straw). According to Taguchi et al. (1968), the growth rates of fungi on liquid media are often higher than on solid media. Also, factors such as pH,

nutrient concentration and aeration can be controlled to obtain a final product of uniform quality.

Corn stalks, with an estimated production figure of 449 000 metric tons, is the third most abundant agricultural residue in the world (Ishaque and Chahal, 1991). It has a structural polysaccharide or holocellulose (cellulose plus hemicelluloses) composition of about 60% which are sheathed in lignin (Barl et al., 1991).

In order to achieve successful bioconversion of polysaccharides from lignocelluloses, pretreatment becomes a pre-requisite for altering significantly the structural characteristics of the lignocellulosic matrix in order to increase the subsequent rate and extent of hydrolysis of their polysaccharides. The pretreatment should be capable of enhancing close contact between microbe and polysaccharides to provide an efficient enzyme action since enzymatic hydrolysis of native lignocellulosic material is prohibitively slow due to the compositional heterogeneity and structural complexity of the lignocellulose. Moreover, cellulose crystallinity, lignin content, associations between cellulose, hemicelluloses and lignin in the cell walls, and accessibility of surface area to enzymes are generally recognized as indicators of the extent of cell wall polysaccharide degradation by enzymes or rumen microorganisms (Cowling and Kirk,1976; Chesson,1981; Gharpuray et al.,1983).

Many physical and chemical pretreatments and biological processes, either singly or in combination have been reported to modify lignocelluloses for their use as animal feed or to be used as feedstock for production of single-cell protein, fuel ethanol and chemicals Fan et al. (1982) have listed 10 physical, 18 chemical and one biological treatments for lignocelluloses. However, the use of irradiation, a physical agent, as a tool for pretreatment remained largely unexplored for a long time.

Then, in 1962, Pritchard et al. indicated that gamma irradiation effectively disrupts lignocellulosic polymers in forages thereby making them more susceptible to microbial attack. Some research along these lines has also been reported by Han et al. (1981) which involved chemical pretreatment of lignocellulosics followed by high energy gamma irradiation using either a cobalt or caesium source. Dosages up to 500 KGy were required to effect solubilization of the substrate. According to Beardmore et al. (1980), the total absorbed dosage is important while the dose rate is not. Other researchers (Ibrahim and Pearce, 1980) have reported alterations in the physical properties of cellulose following irradiation; these include loss of mechanical strength and decreases in crystallinity. Irradiation of cellulose and hemicelluloses results in random cleavage of biopolymers giving a variety of products including xylose, arabinose, glucuronic acid, formic acid, malondialdehyde and low molecular weight products such as H_2O, CO, CO_2, and H_2 (Bludovsky and Duchacek, 1979).

Generally, radiolytic processes initiate oxidations or radical formations at regions within the internal structure of the irradiated material which cause bond scissions that can alter the structure sufficiently to aid hydrolysis. Such oxidations also directly depolymerize the lignocelluloses (Ehrlich and Han, 1991). The enhancement of enzymatic hydrolysis of cellulose by radiation pretreatment may result from increase in the number of chain termini available for digestion by exo-cellulase (1,4-ß-glucan cellobiohydrolase) which is the predominant component of the mixture of cellulase enzymes (Gritzali and Brown, 1979). Radiation could also increase the surface area available to the enzymes by oxidatively rendering the lignin more hydrophilic.

At present, the application of irradiation for food preservation (WHO 1988), for reducing the quantity of contaminating microorganisms in lignocellulosic materials (Kume et al., 1990), and for pretreatment of lignocelluloses for their hydrolysis into simple sugars to increase their digestibility for ruminant animals, and for their bioconversion into protein-rich animal feed (IAEA, 1983; Kumakura and Kaetsu, 1978) is gaining importance.

This study presents the effect of pretreatment of corn stalks singly with gamma irradiation and its comparison with other pretreatments (NaOH, steam), and finally with combination pretreatments of sodium hydroxide followed by gamma irradiation for their bioconversion into protein-rich mycelial biomass of *Pleurotus sajor-caju.*

2.0 LITERATURE REVIEW

2.1. PRODUCTION OF MYCELIAL BIOMASS PROTEIN FROM LIGNOCELLULOSES - A perspective.

Estimates on the production of cereal straws (approximately 1-2x grain yield), indicate that about 2246 - 3644 million tons of cereal crops are being produced annually in the world (Bano and Rajarathnam,1988 ; Ishaque and Chahal,1991). The production of these straws is relatively higher in the developing countries where there is less industrialization with a concomitant increase in the agricultural population. According to Penn (1976), 36% of the straw is burnt in England and Wales while in the Far Eastern countries and in most of the developing countries, the proportion is as high as 60%.

In Africa, straw is burnt either by hunters for game or in preparation of the land for the next crop. Bioconversion of lignocellulosic wastes into edible food and feed or chemicals will not only solve the pollution problem but will also help alleviate some of the food/feed shortages which have become a normal part of the daily realities of the developing countries.

Production of crude protein by fungi was studied both in submerged agitated cultures and in solid-state fermentations on various lignocellulosic materials (Hatakka and Pirhonen,1985). Crude protein concentrations as high as 29% could be obtained with certain waste materials in submerged agitated cultures. Recently, Chahal (1989)

reported a crude protein content of 45% in the production of mycelial biomass with *Pleurotus sajor-caju* in a submerged agitated culture with corn stover as carbon substrate.

2.2. FILAMENTOUS FUNGI IN SUBMERGED CULTURE FOR PROTEIN PRODUCTION - an overview.

Submerged or liquid state fermentation may be defined as the process in which fine particles of the substrate are fermented either solubilized or suspended in a large volume of water medium (Chahal, 1983, 1986).

In 1976, Balagopal and Maini found *Aspergillus niger NRRL 330* and *Rhizopus sp.* superior in terms of mycelial weight and protein production in a 25 g L^{-1} cassava starch waste liquid medium. Muindi and Hanssen (1981) grew *Trichoderma harzianum* in a slurry of cassava root meal. The crude protein content of the final product increased from 2.4% to 37.6% on dry basis with a resultant decrease in the final product weight from 100 g to an enriched 30 g product. Fermentation time was 60 h, 23^{0}C, pH 4.0-4.2 on a 4% slurry. Eklund et al.,1976 reported a yield of 0.63 for *Paecilomyces varioti* in the pentosan fraction of sunflower seed husk sulphuric acid hydrolysate and a yield of 0.94 from the hexose fraction. Worgan (1976) summarized data of the final biomass concentration obtained by *Fusarium semitectum*, 28.7, 22.8, 22.9 and 14.6 g L^{-1} in palm waste, citrus molasses, lucerne leaf liquor and maize leaf liquor

respectively, and by *Aspergillus oryzae*, 19.5 and 16.6 g L^{-1} in olive waste and lucerne leaf liquor respectively. Imrie and Righelato (1976) have reported that for *Fusarium sp. MR,* the growth rate decreased at acid pH values (around 3) and growth almost stopped at 40^{0}C on a sucrose medium. The oxygen demand for growing cultures at 10-20 g L^{-1} biomass concentration was in the range of 0.1-0.2 mol $L^{-1}h^{-1}$ and if this was not met the batch time to reach maximum biomass increased greatly but the biomass yield was not affected.*Aureobasidium pullulans* grew well in acid treated wheat straw increasing two fold the amount of protein (Israilides et al.;1982). Abraham and Srinivasan (1979) studied the growth of *Penicillium frequentans*, *Aspergillus nidulans* and *Fusarium sp.* in deproteinized whey in shake flasks at 28^{0}C for 4 days and found that the first two produced more protein and the third one more fat.

The rotating disc fermentor was suitable for growing filamentous fungi including representatives of the genera *Aspergillus, Rhizopus, Mucor* and *Penicillium.* The fermentor exploits their surface adherent properties and data have been presented which indicate the continued viability of the fungal cells in the innermost layers of the disc. Anderson et al. (1981) reported the growth of *A. niger* in a medium containing 2 g L^{-1} glucose with a resultant glucose uptake of 1 g $L^{-1}h^{-1}$ and complete glucose removal. Gibriel et al. (1981) grew *Aspergillus terreus* and *A. niger* in a medium containing 15 to 20 g L^{-1} of milled wheat bran and inorganic nitrogen supplementation in shake flasks for up to 15 days, obtaining a residual biomass with about 40% crude

protein. Garg and Neelakantan (1981) isolated *A. terreus GN 1* and grew it on 1% alkali-treated bagasse slurry in shake flasks for seven days. The highest protein content obtained of the biomass (mycelium plus unfermented bagasse) was around 20% at 30^{0}C and pH 4. In 1982, Garg and Neelakantan further reported that when *A. terreus GN 1* is grown on 1% alkali-treated bagasse slurry in a 10 L reactor for four days, a product with 10 times more protein was produced, utilising 73% of the initial cellulose present. Untreated bagasse was less degraded although a product with 7 times more protein was obtained after three days. Kim et al. (1981) studied the growth of *Aspergillus japonicus OM-4* both on the liquors obtained from hot water and steam treated wheat straw and in 5 g L^{-1} slurries of treated straw. Free reducing sugars in the liquor were depleted within a day, however phenolics and phenolic-carbohydrate complexes were more resistant. The fungus rapidly degraded lignin and cellulose from the slurries.

Labaneiah et al. (1979) reported the growth of *Agaricus bisporus* and *Morchella crassipes* on citrus peel extracts supplemented with glucose and inorganic nitrogen which gave biomass yields of 0.40 and 0.45 and productivities of 1.66 and 1.31 g biomass $L^{-1}h^{-1}$. Carroad and Wilke (1977) have shown that pellet forming lignin degrading *Polyporus versicolor ATCC 12679* and *Pleurotus ostreatus ATCC 9415* grew at a rate proportional to the two-thirds power of the cell mass, however it was not the best or simplest model as one based on a growth rate proportional to the cell mass.

Research into the use of lignocellulose biomass slurries has also been explored. A review by Chahal and Moo-Young (1981) indicates that the earliest work on the production of single cell protein (SCP) was done by Pringsheim and Lichtenstein in 1920 when they fermented straws with *Aspergillus fumigatus* for animal feed. Then in 1972, Rogers et al. reported 13.3% DW crude protein after 4 days of growth of *Aspergillus fumigatus* on alkali-treated cellulose. Han and Callihan (1974) reported production of 1.76 g protein/L by growing a mixed culture of *Cellumonas* sp. and *Alcaligenes faecalis* for 5 days on 1-5% slurry of alkali-treated rice straw. Peitersen (1975) obtained 21-26% DW crude protein by growing *Trichoderma reesei* on alkali-treated barley straw for 2-4 days. Eriksson and Larsson (1975) obtained a product with 6% DW crude protein from powdered cellulose, 13.8% from wastes fibers, and 32% from highly amorphous cellulose by growing the lignocellulolytic organism, *Sporotrichum pulverulentum* for 6 days. Brown and Fitzpatrick (1976) grew *Trichoderma reesei* on shredded, heated and milled waste paper and obtained a solid material with about 11% protein on dry weight basis, a biomass yield of 0.5 and biomass productivity of 0.066 g $L^{-1}h^{-1}$. Chahal et al. (1979) found *Cochliobolus specifer* the most efficient fungus for protein synthesis from wheat straw. Rao et al. (1983) grew *Penicillium janthinellum* in alkali treated or in alkali treated plus acid neutralized wheat straw and obtained 90% cellulose consumption and 30% protein in the final product of the alkali treated wheat straw. The latter gave a protein content of 20%. Daugulus and Bone (1977) grew *Pleurotus sapidus A-241, S. pulverulentum A-387*, and *Polyporus*

anceps DAOM 21401 in suspensions of alkali treated and ball milled bark from cedar and silver maple, obtaining after four days at 30°C, 136 and 116 mg crude protein per g of bark for the last two fungi, respectively.

Chaetomium cellulolyticum is a thermotolerant fungus (Chahal and Hawskworth, 1976) which showed excellent growth rates and biomass-protein productivities when cultivated in acid and alkali pretreated sawdust of mixed hardwoods (Moo-Young et al.,1977).

The effect of substrate pretreatment is an important parameter in the fungus growth rate, substrate consumption and biomass-protein productivities. This has been shown for wheat straw. Chahal et al. (1977) reported a protein productivity of 69.7 mg $L^{-1}h^{-1}$ from alkali-treated wheat straw with the final product containing 40% crude protein. Most of the experiments have been done employing slurries at 10 g L^{-1}. Alkali pretreated wood residues gave a protein productivity of around 50 mg $L^{-1}h^{-1}$. It was increased to 75-78 mg when the substrate was steam pretreated using the Stake and Iotech processes. Cattle manure and mixtures of swine manure and straw (Moo-Young et al.,1981) have also been used as substrates for *C. cellulolyticum* growth in submerged culture.

Table 2-1 summarises a comparison of SCP production on various crop residues with various cellulolytic microorganisms.

TABLE 2-1: Comparison of SCP production on various crop residues with various cellulolytic microorganisms.

Microorganism	Substrate type	Substrate Concn. (% W/V)	Crude protein (DW)	Crude protein (g/L)	Culture time (h)	Protein productivity (mg/L/h)	Reference
Sporotrichum pulverulentum	Pure powdered cellulose	1	4.3	0.34	168	2.0	Eriksson and Larsson (1975)
Trichorderma reesei (QM 9123)	NaOH treated barley straw	1	23.8	1.84	48	38.3	Peitersen (1975)
Cellulomonas sp. + *A. faecalis*	4% NaOH treated rice straw	1 - 5	-	1.76	120	14.3	Han and Callihan (1974)
Aspergillus terreus	4% Solka floc	1	32.9	1.73	30	57.6	Miller and Srinivasan (1979)
C. cellulolyticum	Solka floc	1	45	1.8	24	80	Chahal and Wang (1978)
C. cellulolyticum	Untreated wheat straw	1	4.6	0.43	48	8.9	Chahal et al. (1977)
C. cellulolyticum	1% NaOH wheat straw	1	40.6	2.51	36	69.7	Chahal et al. (1977)
C. cellulolyticum	1% NaOH Corn stover	1	35.7	1.8	12	146.0	Chahal et al. (1980)
C. cellulolyticum	Cattle manure	1	37.0	1.52	11	108.2	Moo-Young and Chahal (1979)
C. cellulolyticum	1% NaOH wheat straw + swine manure	1	27.8	1.52	24	63.3	Chahal et al. (1980)

Reproduced in part from Chahal et al. (1981)

Some of the advantages associated with the use of filamentous fungi for protein production from various substrates include:

(i) Some filamentous fungi grow as fast as most single -cell organisms (Anderson et al.,1975).

(ii) The finished product is fibrous in nature.

(iii) They contain less nucleic acid with a higher digestibility and net protein utilization (NPU) than single-cell organisms.

2.3. COMPOSITION AND CHEMISTRY OF LIGNOCELLULOSES

2.3.1. Chemical composition.

Lignocellulosic materials represent the largest reservoir of potentially fermentable carbohydrates on earth. Lignocelluloses are composed principally of cellulose, hemicelluloses, lignin, protein and various extraneous materials. Table 2-2 shows the proximate chemical composition of the corn plant residues.

Table 2-2. Chemical composition of corn plant residues (g kg^{-1} Dm).

Constituent	Corn plant residue			
	stalk	cob	husk	leaves
Cellulose	345±5	391±2	382±4	302±4
Hemicelluloses	252±4	421±5	445±7	292±8
Lignin (Klason)	112±4	91±3	66±3	134±3
Protein (N × 6.25)	36±3	17±2	19±2	82±4
Ash	46±2	12±1	28±1	109±1

Source: Barl et al.,1991.

It is estimated that about 50 billion tons of cellulose are produced annually world wide by photosynthesis (Dale,1985) and at the same time, at least 12 billion tons of hemicelluloses are produced together with an equal amount of lignin (Lieth,1973).

Cellulose is the main constituent of wood and related lignocellulosic materials. Softwood species contain about 40% cellulose, 27-30% lignin and the rest are hemicelluloses. Hardwood species contain about 40% cellulose, 20-25% lignin and nearly 30% hemicelluloses (Sjöström,1981). In general, straws contain about 13-20% lignin (Hatakka et al.,1989).

2.3.1.1. Cellulose

Cellulose is the most abundant compound in the plant world and is defined in the 'wood' industry as a linear polymer of β-1,4 D-glucose units which form long fibrils when held together by intramolecular hydrogen bonds (Darvill et al.,1980). Attala (1983) describes the state of aggregation of cellulose as semi-crystalline solids and polymorphic crystalline solids.

According to Cowling (1975), the most important feature that influences the enzymatic hydrolysis is the accessibility of cellulose surface to the cellulolytic enzyme and direct physical contact between the enzyme molecules and the substrate cellulose is a pre-requisite to hydrolysis. Stone et al. (1969) reported a linear relationship between the initial hydrolysis rate and the surface area

that was accessible to molecules with a diameter of 40Å. This diameter is equal to that of cellulolytic enzyme molecules.

2.3.1.2. Hemicelluloses

The controversial history of the term hemicelluloses has been described by Thompson (1983). Hemicelluloses are low molecular weight generally amorphous polysaccharides strategically located in the cell wall fibre. They are water-insoluble but are easily solubilized by alkali. Covalent bonds are thought to bind hemicelluloses chemically to lignin, and some chemical delignification treatments may even bind residual lignin to hemicellulose and cellulose by transglycosidation reactions. For convenience, hemicelluloses may be emperically classified as glucans, xylans, mannans, galactans and galacturonans.

2.3.1.3. Lignin

Lignin gives the plant cell wall rigidity and renders the lignocellulose complex relatively intractable. It is a polyphenol formed by enzyme-initiated dehydrogenative polymerisation of phenylpropanoid precursors (Sarkanen and Lugwig,1971). Since the polymerisation mechanism is enzyme initiated and free radical propagated, the resulting molecule is complex and seemingly random (Brown,1964). The work of Morrison (1974); and Gaillard and Richards (1975) has shown alkali-labile bonding between lignin and the structural polysaccharides and an ester linkage appears to be the most likely type of bond (Morrison,1973).

The following properties are important in terms of microbial and enzymatic degradation of lignin:

(i) Lignin has a compact structure that is insoluble in water and difficult to be wetted and penetrated by microorganisms;

(ii) The intermonomer linkages that account for the structural rigidity of lignin comprise a variety of carbon-carbon and carbon-oxygen bonds, and

(iii) Lignins are, in essence a form of 'natural plastic' because the intermonomeric linkages are not directly hydrolyzable (Zeikus,1981).

Straw lignins contain ester-linked ferulic acid and para-coumaric acid which also occur linked to the polysaccharide (McCarthy et al.,1984). Lignin in the cell wall, therefore, not only encrusts the cellulose microfibrils in a sheath-like manner, but is also bonded physically and chemically to the plant ploysaccharide (Higuchi,1971).

2.4. ENZYMATIC HYDROLYSIS OF LIGNOCELLULOSES.

In the hydrolysis of natural lignocelluloses lignin is an important obstacle for enzymatic digestion. Physically, lignin forms a barrier, suppressing the penetration of polysaccharide-digesting enzymes (Kirk and Haskin,1973). Baker (1973) observed an inverse relationship between the lignin content and the digestiblilty of a wide variety of lignocelluloses. It has been reported by Gharpurray et al. (1983), that

the hydrolysis rate of wheat straw increased substantially with an increase in the extent of delignification, up to about 50% delignification. However, beyond this the hydrolysis rate increased only slightly. It has also been established that the lesser the lignin content of lignocelluloses, the higher the susceptibility to enzymatic hydrolysis and in vitro digestibility (Feist et al.,1970; Wilson and Pidgen,1964).

Phenolic acids may be produced during certain pretreatments of lignocelluloses and these could also limit the accessibility of cellulose and hemicelluloses to enzymatic attack (Hartley,1981).

Another obstacle in the hydrolysis of natural lignocelluloses is the crystallinity of the cellulose component. Crystalline material which consists almost wholly of true cellulose is concentrated near the lumen and diminishes towards the primary wall. The cellulose molecules are held together by very close packing and strong van der Waals forces of attraction. This makes the crystalline cellulose more resistant to enzymatic or acidic hydrolysis than that of amorphous region (Sultze, 1957).

Furthermore, in the native state, the available area of gross capillaries (cell lumen, pits, etc.) of lignocellulose cell walls are not enough to bring about considerable hydrolysis of cellulose. This is because a comparison of the sizes of capillaries and enzymes indicates that the cellulose enzyme molecules are quite big and will

not be able to diffuse in cotton and wood cell wall untill pretreated to increase the size of cell-wall capillaries.

2.5. PRETREATMENT OF LIGNOCELLULOSES.

The polysaccharides (cellulose and hemicelluloses) are held together by weak intermolecular hydrogen bonds while covalent bonds bind hemicellulose chemically to lignin.

Pretreatment of lignocelluloses is aimed at modifying the structural characteristics of the lignocellulosic matrix and disruption of the cellulose-hemicellulose-lignin seal for subsequent chemical or enzymatic hydrolysis. This is necessary because crystalline cellulose and encrustation of cellulose fibrils with lignin make the lignocellulose recalcitrant. Once lignin is depolymerised, solublized or removed, cellulose and hemicelluloses can be easily hydrolysed by cellulases and hemicellulases for their bioconversion into single-cell protein as food or feed and for production of ethanol and other products.

Several different pretreatment methods have been used to enhance the rate of hydrolytic degradation. Such methods include, physical (Chang et al.,1981, Gharpurray et al.,1983), chemical (Rolz et al.,1987) and biological (Hatakka,1983) treatments. Some of the physical or mechanical methods include; ball milling (Millett et al,1981); attrition milling (Ryu and Lee,1983), steam explosion (Wilke and Blanch,1981) and irradiation (Han and Ciegler,1982). Chemical pretreatments such as batch acid prehydrolysis (Wilke et al.,1981),

caustic treatment (Norvald et al,1977) and solvent delignification (Avgerionos et al, 1981) have been used to improve subsequent enzymatic hydrolysis.

This review details only attrition milling, steam explosion, irradiation and mild alkali treatments since they are the treatments of interest in this work.

2.5.1. Milling

Milling cellulosic material is a popular method for increasing cellulose digestiblilty. The shearing and compulsive forces generated by the mill for a period of time on the material brings about a reduction in crystallinity, increase in the fraction of the material that is water soluble and decrease in particle size (Lyons and Kelsall,1991). According to Mandels et al. (1974), mechanical milling is effective in disrupting crystalline cellulose. It also increases the susceptibility of both amorphous and crystalline components of cellulose. They found that the enzyme digestibillty of the crystalline component was enhanced to a greater extent by milling than that of the amorphous component. This led them to propose that the overall increase in the digestibililty is apparently a result of decreased particle size and increased available surface area rather than a result of reduced crystallinity.

2.5.2. Steam treatment

Steam treatment was originally developed in 1925 and has been extensively used in the manufacture of hardboard by the Masonite process employing wood. In the late 70s, Iotech Corp. Ltd and Stake Technology Ltd in Canada started using this process for the production of feed for ruminants.

Studies of steam pretreatment have shown that the lignocellulosic matrix of wood is modified drastically and the remaining solids are more susceptible to enzymatic hydrolysis. The structural changes and the chemical reactions taking place seem to be a function of temperature and time of pretreatment. The residence time at higher temperatures is kept low to minimise reactions which produce inhibitory byproducts (Neese et al.,1977).

Steam explosion is a thermomechanical process in which the lignocellulose can be separated into three component fractions by rapidly heating wood chips in a sealed vessel and then explosively releasing the fibre steam mixture through a nozzle. Steam treatment under high pressure (autohydrolysis) makes the lignocelluloses easily accesible to hydrolytic enzymes (Noble,1980).

According to MacDonald and Mathews (1979), steaming biomass leads to increased enzymatic digestibility and the increased pore size, autohydrolysis and wetting allow the enzymes to penetrate further and increase bioconversion yields substantially over unpretreated material.

2.5.3. Types and sources of high energy radiation

High energy radiation is the term applied to beams of accelerated particles and to high frequency electromagnetic radiation. Radiation effects are produced when energy is absorbed from the beams by the constituents of the irradiated materials. High energy may be supplied by particle accelerators which produce accelerated electrons or by nuclear reactors which produce neutrons. Interaction of the neutrons with reactor materials yields beams of photons or gamma (γ) rays, electrons and a variety of charged particles with higher masses. Materials under study may be irradiated simultaneously by neutrons and their interaction products.

A widely used gamma source is ^{60}Co which is formed by the capture of a neutron by the ^{59}Co nucleus. In this isotope, half of the atoms decay to non-radioactive nickel within 5.3 years by the emission of gamma and beta rays. Another source of gamma rays is ^{137}Cs, produced as a by-product from nuclear fission. It has a half life of 30 years.

The energy emitted by either of these isotopes is high but is not sufficient to make irradiated materials radioactive. Threshold energy levels required to make materials radioactive are about 5-10 MeV. Gamma rays from ^{60}Co and ^{137}Cs are 1.32 MeV and 0.66 MeV respectively. Therefore radiation hazard from treated material is not a concern.

The absorbed dose of any ionising radiation is the energy absorbed per unit mass of irradiated material. The 'Système International' (SI)

derived unit of dose is the gray (Gy), equal to 1 joule per Kg International Atomic Energy Agency (IAEA, 1977). The traditional special unit of absorbed dose is the rad (1 rad = 0.01 Gy), which is being phased out. The absorbed dose rate is the absorbed dose per unit time, the SI derived unit is GyS^{-1}.

2.5.4. Effect of gamma radiation on lignocelluloses.

The main effect of radiation on lignocelluloses include, depolymerization, solubilization and decrease in cellulose crystallinity (Ehrlich and Han, 1991).

2.5.5. Depolymerization

Depolymerization of lignocelluloses arises mainly from random formation of radicals within the cellulose glycosyl moieties. These radicals cause cleavage of weakest bonds, the β-1,4-glucose linkage. In the presence of water and oxygen, γ-irradiation initiates oxidation of sugars from lignocelluloses to aldehydes, ketones and carboxylic acids which result in the breakdown of the cellulose polymeric chain. Depolymerization may be accompanied by breakage of hydrogen bonds between parallel cellulose chains in the lignocelluolse complex. According to Lawton et al. (1951), lignin has a protective effect and the degree of polymerization (DP) is greater in wood than in purified cellulose. The ionisation induced in the cellulose is not hindered by the crystalline-amorphous nature of cellulose structure (Charlsby,1955)

2.5.6. Solubilization

In 1981, Han et al. reported that irradiation caused solubilization of a considerable portion of cellulosic dry matter with a more pronounced effect when the substrate was suspended in liquid media. Gamma irradiation doses over 500 KGy cause a considerable portion of lignocelluloses to become water soluble (Charlsby,1955; Han and Ciegler,1982). Complete solubilization occurs at doses greater than 3 MGy or at lower (~ 1 MGy) when irradiation was carried out in the presence of sodium hydroxide or chlorine (Kumakura and Kaetsu, 1979).

The concentration of soluble substances formed during irradiation of cellulose in 0.1N NaOH; 0.1N H_2SO_4 and neutral water was higher (about 50%, 45% and 5%, respectively) than during irradiation of a dried sample (Duchacek and Bludusky,1979)

Purified cellulose is more sensitive than lignocelluloses and becomes completely soluble after a sodium hydroxide treatment and irradiation at 500 KGy. The increase in soluble matter in irradiated lignocelluloses may be as a result of hemicelluloses breakdown. In the presence of NaOH, gamma ray initiated radical formation of water molecules bound with lignocellulosic matrix is enhanced (Ehrlich and Han,1991)

2.5.7. Radiolysis of cellulose

Fig. 2-1 shows a section of a polysaccharide, cellulose, which consists of glucose units. The glucose units are combined by the β-glycosidic linkages from C-1 to C-4 of the neighbouring unit.

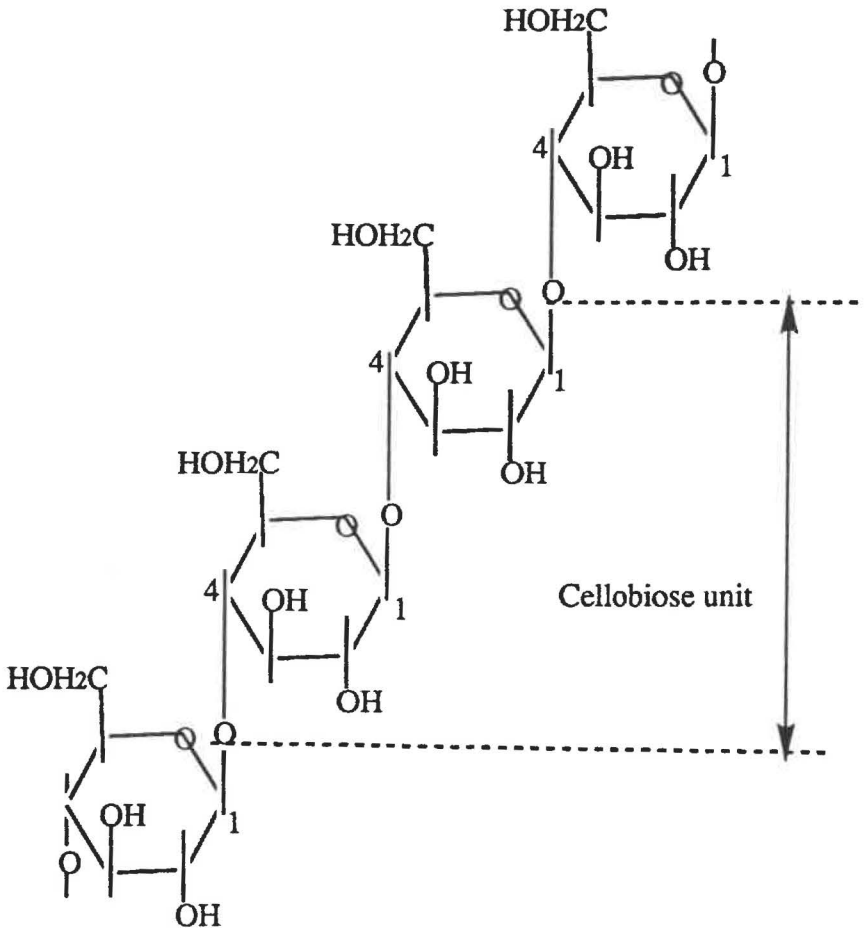


Fig. 2-1 Sketch of cellulose chain made up from glucose molecules linked through C-1 and C-4 positions. Subunits of two glucose molecules are considered as cellobiose units. (Reproduced from Adam (1983) I.A.E.A. Panel Proceedings Series).

During lignocellulose irradiation, OH radicals are generated by radiolysis of water (Scherz,1970). The radicals then react with glucose moieties in cellulose to produce free radicals at all possible carbons. Radicals at carbons 1 and 4 would lead directly to chain cleavage while those at other positions could lead to a variety of oxidation products. Products formed include hexoses, pentoses, saccharide acids, deoxysaccharides, malondialdehyde, dihydroxyacetone, and low molecular weight products such as water, carbon monoxide, CO_2, H_2, HCHO and HCOOH. Malondialdehyde is one of the major products formed when irradiation is carried out under alkaline or neutral conditions (Bludovsky and Duchacek,1979; Scherz,1970). There is no substantial degradation of glucose or cellobiose under 10 KGy but at 1 MGy almost complete oxidation of these products was found (Han et al.,1981)

2.5.8. Crystallinity and physical properties.

The effect of gamma irradiation on the crystallinity of cellulose is a subject of controversy. Some workers reported that gamma irradiation has no effect on the crystallinity of cellulose (Beardmore et al.,1980; Murayama,1963), while others reported that there was a significant change in crystallinity of cellulose by gamma irradiation (Aoki et al. 1977; Han and Ciegler,1982). According to Aoki et al. (1977), radiation at doses greater than 500 KGy causes a decrease in crystallinity in the cellulose molecules of lignocelluloses. Han and

Ciegler (1982) also reported a decrease in crystallinity in sugar cane bagasse at radiation doses more than 100 KGy.

Han and Ciegler (1982) reported that the fibre strength of sugar cane bagasse decreases with increase in radiation dose while Beardmore et al. (1980) noted that gamma irradiation at or above 100 KGy drastically increased the specific surface area of cellulosic samples and consequently increased susceptibililty to enzymatic hydrolysis.

When lignocellulosic materials were irradiated (1 KGy-100 KGy) prior to acid or enzymatic hydrolysis, the rate of enzymatic hydrolysis was increased but sugar yield upon acid hydrolysis decreased (Kumakura and Kaetsu,1978).

2.7. Alkali pretreatment

Dilute alkali increases the fibre saturation point and the swelling capacity of lignocelluloses. The increase in swelling capacity results from the saponification of esters of 4-0-methylglucuronic acid attached to xylan chains. In the native state the esters act as cross-links, limiting the swelling or dispersion of polymer segments in water. Such treatments increase the accessibility of plant polysaccharides to cellulolytic microorganisms (Tarkow and Feist,1969).

Both alkali-labile and alkali-resistant lignin-carbohydrate bonds exist and their ratio in a particular plant material governs the decision to use alkali as a pretreatment to increase its digestibililty.

The alkali-labile bonds include the hemicellulose-phenolic acid and acetyl constituents of the cell walls (Hartley, 1981) both of which affect holocellulose hydrolysis (Jung and Fahey, 1983).

Alkali concentration of 0.1 g/g substrate has been reported by Choudhurry et al. (1984) and Gray et al. (1978), to be optimum for significant increases in the rate and extent of hydrolysis of lignocelluloses.

Moo-Young et al. (1981) reported a protein content of 35.7% in the final product after 12h fermentation on 1% NaOH treated corn stover with *Chaetomium cellulolyticum*. A productivity of 146 mg $L^{-1}h^{-1}$ was also reported. *Chaetomium cellulolyticum* asporogenous mutant (Chahal,1985) gave a protein content of 35% in 1% NaOH and protein productivity of 0.066 g $L^{-1}h^{-1}$. It was further reported by Chahal (1985) that when sodium hydroxide pretreatment on corn stover was decreased from 1.5% to 1% there was a corresponding decrease of percent protein in the final product from 45% to 36% on *Pleurotus sajor-caju*. Then in 1989, Chahal observed that when corn stover is pretreated with 1.5% NaOH solution and fermented with *Pleurotus sajor-caju*, the protein content in the final product was 40-49% depending on the the fermentation medium. The highest percent protein was obtained with Mandels and Weber medium (1969). There are many more chemical and physical treatment methods available in the literature. These methods have been listed in Table 2-3 (see page 32).

Table 2-3. Other pretreatment methods for lignocellulosic materials.

Chemical methods	Physical methods
Ammonia	mechanical-
Ammonium bisulfite	ball milling
Chemical pulping	hammer milling
(sodium benzoate, nitric acid etc.)	extrusion
Sodium chlorite	grinding
Gases (SO_2, NO_2 etc.)	soaking in water
Acids (HCl, H_2SO_4 etc.)	non mechanical-
Oxidizing agent (peracetic acid,	uv-radiation
hydrogen peroxide etc.)	pyrolysis
Organic solvents (ethanol,acetone etc.)	freezing
Cellulose solvents (CMCS, cadoxen)	

Source: I. Goldberg (1985)

2.5.10. Problems associated with pretreatments and technical difficulties with lignocellulose conversion into food/feed.

Problems associated with pretreatments of lignocelluloses include:

(i) They require energy, equipment and often chemicals.

(ii) They result in an irretrievable loss of sugar, undesirable side-reactions and by-product formation.

(iii) If acids, alkali, or organic chemicals are used, they must be recycled to minimize cost, or disposed of to prevent pollution.

Technical difficulties for lignocellulose conversion into food and feed by wood-rotting fungi are:

(i) Scaling up with appropriate control of humidity, aeration and temperature

(ii) Preventing contamination, and

(iii) Slowness of fungal treatment action.

2.5.11. Biological delignification of lignocelluloses by white-rot fungi.

Lignin-utilizing organisms have been used for biological pretreatment of lignocellulosic material. Detroy et al. (1980) used *Pleurotus ostreatus* to partially delignify wheat straw and to increase enzymatic saccharification after 50 days of fermentation. The lignolytic white-rot fungus *Phanaerochaete chrysosporium* has been used to pretreat wood chips to make them more amenable to pulping and saccharification (Eriksson et al.,1980). The in vitro digestibility of wheat straw is increased during fermentation by, for example, *Pleurotus ostreatus* (Kaneshiro,1977; Detroy et al.,1980), *Pleurotus spp.* and *Stropharia rugosoannulata* (Zadrazil,1977).

The lignolytic organisms need easily metabolizable carbon substrates like sugars, hemicelluloses or cellulose to be able to grow and degrade lignin (Ander and Ericksson,1978).

However, different white-rot fungi vary considerably in the relative rates in which they attack lignin and carbohydrates in woody tissues. Some remove lignin much more than carbohydrates and on the basis of the action in wood, white-rot fungi can be separated into two groups (Ljungdahl and Eriksson,1985):

(i) Fungi which degrade the different wood components at approximately the same rate are called simultaneous rot fungi, and

(ii) Fungi which preferentially degrade lignin, leaving considerable amounts of cellulose are called white-pocket and white-mottled rot fungi.

2.6. FUNGAL BIOMASS PRODUCTION FOR FOOD AND FEED

2.6.1. An overview

Microorganisms have been a component part of human foods since ancient times. Examples include yeast in bread-making; lactic acid bacteria in making fermented milks, cheeses, and sausages; and moulds in making a variety of oriental fermented foods (Litchfield,1983).

The first effort to grow microbes for food was in Germany during World War II when *Candida utilis* (Torula yeast) was cultivated on sulfite waste liquor and wood sugar as a protein source for humans and animals (Wiley et al.,1950). During the period between the two world wars, production of microbial food reached a level of over 15 000 tons per year (Laskin,1977).

In the late 50s, commercial production of food on hydrocarbons was started (Champagnant et al.,1963) while in the 70s, methanol was the substrate of choice for microbial food production. The use of methane and ethanol followed for microbial food production. Most of these processes did not reach commercialisation levels because of the cost of the substrates. Cost analysis indicated that carbohydrate substrates (sugars from molasses or crop residues) were the cheapest

source of carbon except methane (Tsao et al.,1978). Moreover, some of the carbohydrates (agricultural and forestry residues, wastes from food industries etc) are abundantly available at lower costs compared to that of hydrocarbons, methanol and ethanol.

2.6.2. Biomass resource conversion into protein-rich food/feed.

Various carbon substrate sources (hydrocarbons, alcohols, organic acids, oils and lignocelluloses have been used for the production of single-cell or mycelial biomass protein. For instance, lignocellulosic wastes can be transformed into mycelial biomass protein (MBP) or single-cell protein (SCP) by three processes:

(i) MBP or SCP can be obtained by the direct growth of microorganisms on pretreated lignocellulosic materials,

(ii) MBP or SCP is produced by the growth of microorganisms, especially yeasts, on a sugar solution obtained by the enzymatic or acid hydrolysis of lignocellulosic materials.

(iii) MBP or SCP may be produced by a mixed culture of microorganisms where lignocellulose is hydrolysed by one intact organism and the hydrolysed product converted into MBP or SCP by another organism.

2.6.3. The choice of *Pleurotus sajor-caju* for MBP production and its growth requirements.

Pleurotus sajor-caju is a basidiomycete which in nature grows well on living or dead parts of plants, which are generally poor in nutrients and vitamins. It has been adapted over the years for mycelial biomass production in submerged culture under well defined synthetic nutrient media. For lignocelluloses such as corn stover, all grain straws, paper, wood-shavings, sawdust, nutshells and vegetable wastes (eg food industry wastes), a C : N relationship of 1:50 is sufficient for mycelium growth in nature.

For the production of mycelial biomass, *Pleurotus sajor-caju* has an optimum growth temperature of 30^0C, pH 5-6 under aerobic conditions.

Pleurotus sajor-caju is a good choice in the production of mycelial biomass as food/feed from lignocelluloses because, among others, it confers the following advantages;

(i) It has the lignocellulolytic enzyme system (i.e cellulases, hemicellulases and ligninases).

(ii) The finished product is fibrous in nature.

(iii) It is an edible mushroom.

The type of process (batch or continuous), growth rate, sterility requirements, type of fermentor or bioreactor, extent of feedstock

utilization and yield, temperature, pH and methods used for product recovery, are all important in determing the economic viablility of MBP or SCP processes. Non-photosynthetic organisms like molds, yeasts, and higher fungi all require aerobic conditions for growth in MBP or SCP processes.

2.6.4. Selected fungal biomass processes.

Several processes are now available for the bioconversion of lignocelluloses into food/feed rich in protein. The underlisted processes are based on the direct and indirect utilization of lignocelluloses.

The direct processes include;

(i) The Louisiana State University process (Callihan and Clemmer,1979)

(ii) The waterloo process (Moo-Young et al., 1979).

(iii) The Institut Armand-Frappier (IAF) process (Chahal D.S, 1991).

Indirect proceses for the bioconversion of lignocelluloses into food/feed include;

(i) The Scholler process (Callihan and Clemmer, 1979).

(ii) The Pekilo process (Romantschuk and Lehtomaki, 1978).

(iii) The Rank Hovis McDougall process (Anderson and Solomon, 1983).

(iii) The Tate and Lyle process (Imrie and Vlitos, 1975).

(iv) The Indian Institute of Technology process (Ghose and Kostick,1970).

(vi) The University of California process (Wilke et al., 1976).

(vii) The Kyoto University process (Tanaka and Matsuno, 1985).

A brief description of some of these processes is given.

2.6.4.1. The Institut Armand-Frappier process (Chahal, 1991b)

The Institut Armand-Frappier (IAF) process was developed at the IAF, Canada as an integrated process for the production of food, feed and fuel from biomass for complete utilization of polysaccharides from lignocelluloses. Fig. 2-2 illustrates the IAF process.

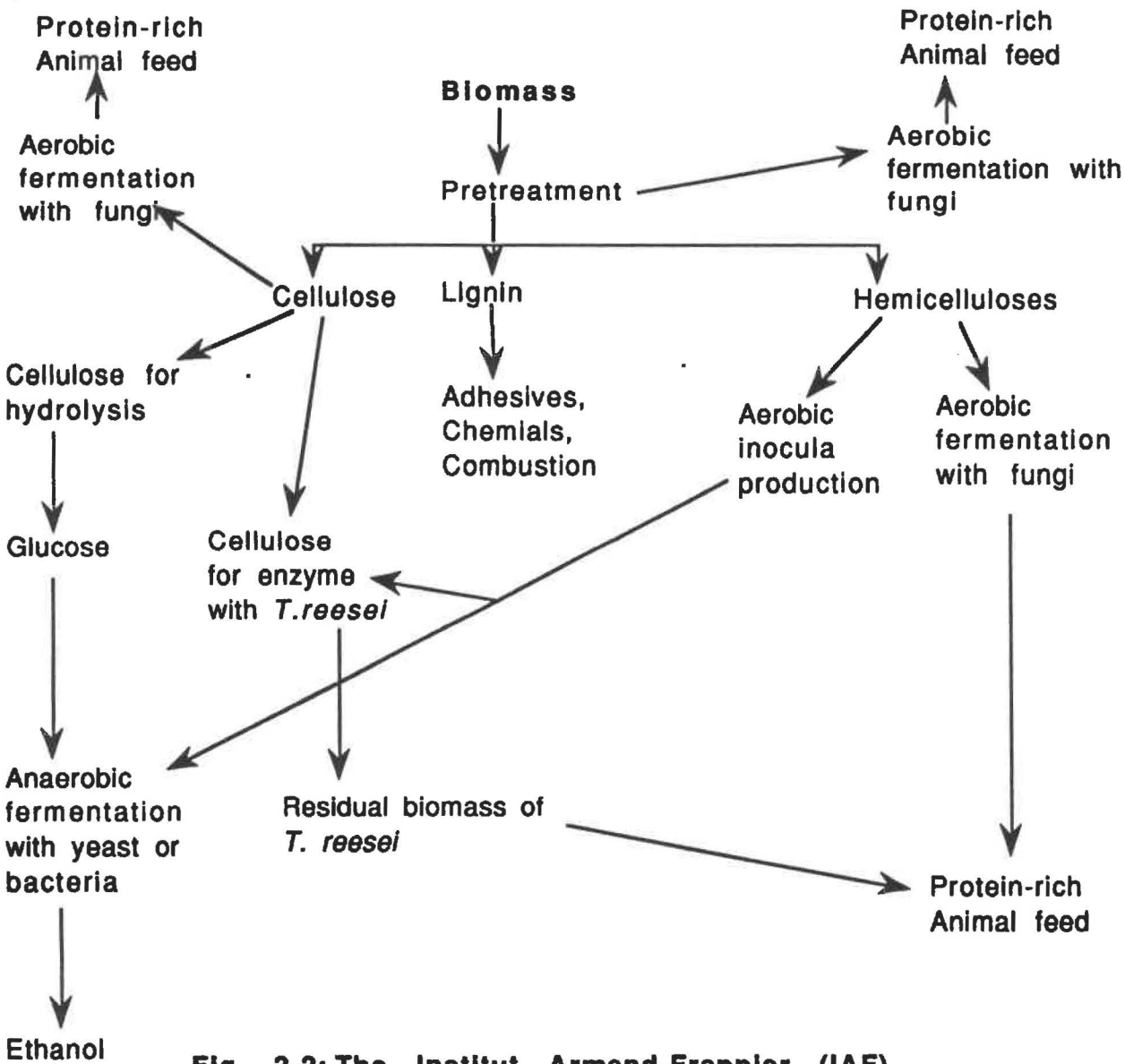


Fig. 2-2: The Institut Armand-Frappier (IAF) Process (Chahal, D. S. 1991)

In this process, pretreated lignocellulose is fermented with suitable fungi which can produce enzymes to hydrolyse the holocellulose (cellulose and hemicelluloses) into monomeric sugars in situ and simultaneously convert these monomeric sugars into mycelial biomass rich in protein. The pretreated lignocellulose could also be fractionated into cellulose, hemicelluloses and lignin. The cellulose or the mixture of hemicelluloses and lignin could also be fermented with suitable fungi for the production of mycelial biomass rich in protein. The mycelial biomass obtained from the fractionated cellulose substrate could find end use as food while the unfractionated and fractionated hemicelluloses and lignin substrates could produce mycelial biomass rich in protein for animal nutrition.

Chaetomium cellulolyticum asporogenous mutant (Chahal, 1985) and *Pleurotus sajor-caju* are the fungi of choice in the IAF process because *Chaetomium cellulolyticum* asporogenous mutant has a rapid turnover rate and is not toxic while *Pleurotus sajor-caju* is an edible mushroom accepted the world over.

This process produces a final product containing 40-45% crude protein with *Pleurotus sajor-caju* on corn stover pretreated with NaOH as substrate.

2.6.4.2. The Waterloo process (Moo-Young et al., 1979)

The Waterloo process, developed at the University of Waterloo in Canada is based on the celluloytic fungus *Chaetomium cellulolyticum* ATCC 32319 and can be operated either in a conventional aerated

liquid fermentation system or in a solid substrate aerated fermentation system. In the solid substrate process, materials such as corn stover or kraft paper mill clarifier sludge are subjected to an initial thermal or chemical treatment followed by aerobic fermentation, separation of the fungal mycelium and drying. The final product contains up to 45% protein. This process has run into difficulties because of possible production of toxin in the parent *Chaetomium cellulolyticum* ATCC 32319 (Sekita et al., 1981).

2.6.4.3. The Pekilo process (Romantschuk and Lehtomaki, 1978).

The largest mold-SCP process now being operated is the Pekilo Process developed in Finland at the Finish Pulp and Paper Institute. This process is operated continuously. Residual SO_2 is stripped from the sulfite liquor feedstock. Sterile air, ammonia and feedstock are supplied to two baffled, agitated-aerated fermentors to produce 15-16.5 tons (dry wt.) of *Paecilomyces varioti* mycelium in 24h, and protein content of 55%.

2.6.4.4. Rank Hovis Macdougall process (Anderson and Solomon,1983).

In the United Kingdom, Rank Hovis MacDougall have grown *Fusarium graminearum* continuously in 1300 L fermentors, with glucose as substrate and ammonia as nitrogen source. The specific growth rate is approximately $0.18h^{-1}$ and the cells contain 45% protein and has been approved for human food uses.

2.6.4.5. Tate and Lyle process (Imrie and Vlitos, 1975).

Tate and Lyle in the United Kingdom have investigated the use of *Aspergillus niger* in solid substrate systems for recovering fruit and vegetable processing wastes. The yield of the fungal product on carrot wastes was 0.11 g/g substrate and the crude protein content was increased from 9% in the starting material to 29% in the finished product.

2.6.5. Fermentation media optimization for production of mycelial biomass of *Pleurotus sajor-caju.*

Nutritional requirements for fungi have been reviewed by several authors (Litchifield,1983; Goldberg,1985). Reid (1979) has studied the effects of concentrations of nitrogen, sulfate and phosphate on the degradation of lignin by *Phanaerochaete chrysporium* while Leblanc (1988) optimized the different elemental and growth conditions for the production of mycelial biomass by *Chaetomium cellulolyticum* asporogenous mutant.

In the optimization of fermentation media, two nutritional factors are essential to microbial activity:

(i) a source of energy for cell metabolic processes

(ii) a source of materials from which cellular matter and products can be synthesized.

Generally, the microorganisms of greatest commercial importance are the heterotrophs which require carbon in more complicated molecular forms as energy sources. The second nutritional factor is the requirement of all the elements including carbon, nitrogen, phosphorus, sulfur, potassium etc that will be combined in various ways to form cellular material or products. A synthetic or defined medium could then be prepared using pure compounds in precisely defined proportions.

Carbon sources like carbohydrates, hydrocarbons, alcohols, organic acids and oils are versatile substrates that form sources of energy for any bioconversion process. Following carbon and oxygen, nitrogen is the next most abundant element in cellular material on a dry weight basis. It is therefore the next most plentiful substrate in fermentation media after the carbon source. Nitrogen sources are used metabolically to supply this element for the anabolic synthesis of nitrogen containing cellular substances such as amino acids, purines, pyrimidines, and their polymers- protein, DNA and RNA. Like carbon sources, nitrogen sources can also be used by some microorganisms to generate metabolic energy.

In the production of microbial biomass, the product is produced at the same time as the cell (it may sometimes be the cell). Media conditions which enhance cell growth then normally enhance product formation.

In most microbial biomass processes, the carbon substrate is present in relatively low concentration either because of its solubilility or because the amount that can be tolerated by a given microorganism is limited. For non-photosynthetic microorganisms grown in batch culture, carbon and energy source concentration generally range from 1 to 5% when soluble carbohydrates are used (Litchfield,1983)

In addition to the carbon and energy source, microorganisms require supplemental nutrients such as vitamins. Suitable nitrogen sources for microbial biomass production are ammonia, ammonium salts, nitrates, urea and organic nitrogen sources such as protein hydrolysates (Litchfield,1983). Many species are able to assimilate ammonium salts, ammonium sulphate being the least expensive and most commonly used (Solomon, 1969).

It is important to adjust the supply of nitrogen source so that the ratio of 10 : 1 or less for carbon to nitrogen can be maintained in the medium during growth to minimize the accumulation of lipids or storage substances such as poly-ß-hyroxybutyrate and to favour high protein contents in the cell (Litchfield, 1983).

2.6.6. Product quality, safety and role of mycelial biomass as food/feed.

Microbial biomass protein can be used as

(i) Protein supplements in human foods e.g., mycoprotein

(ii) Functional food ingredients

(iii) Protein supplements for livestock feeding.

If the SCP is to be used as a major part of the product, and not as a minor food or feed additive, e.g., as a source of flavours or vitamins, it should have the the following properties: dry powder form, no colour or odor, low viable microbial count, no pathogens, high biological value, low RNA or toxic factors, and functionality and taste.

Data on crude protein determined by multiplying nitrogen contents by the factor of 6.25 do not reflect the true value of these products in human and animal nutrition since amino acid profiles may vary widely (Lewis,1976) and non-protein nitrogen substances such as nucleic acids are included. It should also be commented that when dealing with mushroom protein sources, the factor 6.25 is in itself controversial. The conversion factor of 6.25 is based on the presumption that most of the proteins contain 16% nitrogen and taking into account that they are 100% digestible and that negligible amounts of non-protein nitrogen are present. Studies of crude protein, however, suggest that only 34 to 89% of the protein (N $\times$ 6.25) is digestible (Bano and Rajarathnam,1988). The reduced coefficient of digestibililty is partially explained by the fact that mushrooms contain a significant amount of non-protein nitrogen in the form of glucoseamine in the chitinous cell walls. A closer approximation of mushroom protein content can be obtained by using a conversion

factor equal to (70% N × 6.25) i.e (N × 4.38) (Bano and Rajarathnam,1988). To compound the confusion concerning mushroom protein, Fitzpatrick et al.(1946) found that purified mushroom protein isolate contained 11.79% nitrogen rather than the expected 16%. Based on their data, a conversion factor of (N × 8.48) would be more appropriate for estimating mushroom protein if the nitrogen content of the chitin and free amino acids could be ignored.

The Protein Caloric Advisory Group of the United Nations System (SCP Guideline, no. 4, 1970) has published recommendations for the maximum acceptable nucleic acid content. A daily intake of 4 g of nucleic acid, 2 g of which are obtained from microbial protein, can avoid kidney stone formation or gout. Compared to the nucleic acid content of algae (Viikari and Linko, 1977) yeast and other microbes (Kihlberg,1972) which have values ranging from 5-10%, the nucleic acid content of *Pleurotus* is quite low 2.46-2.91% (Bano and Rajarathnam,1988).

3.0 MATERIALS AND METHODS.

3.1 Substrate.

Corn stalk samples were collected from a farm near the Institut Armand-Frappier, Laval. They were dried to a constant weight in an oven at 60°C and were ground in a Wiley mill with a mesh screen of 20. The ground corn stalks were packed into a number of 500 mL jars and the moisture content was determined.

3.2. Moisture content of the substrate (Adapted from A.O.A.C,1984)

The moisture content of the substrate was determined by weighing accurately approximately 1 g substrate into overnight pre-dried and weighed alluminium dishes. These were dried overnight at 105°C, cooled in a dessicator and re-weighed. This was done in triplicate and the difference in dry weight was expressed as the amount of moisture/g substrate.

3.3. Substrate pretreatments

The main focus was to determine the effect of gamma irradiation treatment on the production of protein-rich mycelial biomass either as a single treatment or in combination with sodium hydroxide. For the purposes of comparison, single pretreatments of sodium hydroxide and steam explosion were also considered. Thus, four sample lots were subjected to the following pretreatments:

Lot No.1 - was stored at room temperature (20°C) to serve as standard untreated sample.

Lot No.2 - was submitted to gamma radiation doses of 100 KGy, 500 KGy, 1 MGy and 1,7 MGy.

Lot No.3 - was treated with sodium hydroxide at 0,025 g/g, 0,05 g/g, 0,10 g/g and 0,15 g/g corn stalk.

Lot No.4 - was pretreated with NaOH as in lot No.3 and then irradiated as in lot No.2

3.3.1. Single pretreatment of gamma irradiation

A 100 g sample each of the substrates was subjected to γ-irradiation doses of 100 KGy, 500 KGy, 1 MGy and 1,7 MGy. The irradiation was carried out in air in a ^{60}Co Underwater calibrator (UC-15 Nordion International) situated at the Institut Armand-Frappier Campus, Laval. The dose rate of the gamma source was 21,7 KGy/h as measured by Fricke dosimetry. Irradiated samples were stored at 4°C.

3.3.2. Single pretreatment of mild sodium hydroxide (NaOH)

Sodium hydroxide was added at 0,025 g/g, 0,05 g/g, 0,10 g/g and 0,15 g/g dry weight of substrate for 100 g samples. The required quantity of NaOH was dissolved in distilled water to obtain moisture contents of 75% based on the initial moisture level of the substrate. The

samples were then thoroughly mixed and left overnight at room temperature for the NaOH to be imbibed by substrate particles. They were further autoclaved at 121°C and at 80 Psig for 1 hour.

3.3.3. Steam pretreatment

A sample of 100 g each of substrate were subjected to the following:

i. steam only

ii. 8% Na_2SO_3 added in a ratio of 6:1 (substrate : steam)

iii. 8% Na_2SO_3 + 0.5% NaOH added in a ratio of 6:1 (substrate : steam)

The steam explosion of the substrate was carried out by Dr. B.V. Kokta of the Pulp and Paper Research Centre, Université du Québec, Trois-rivières, Québec. The procedure consisted of an exposure to steam at 190°C for 4 minutes. About 10% of the substrate soluble matter was lost during washing after the steaming process.

3.3.4. Combination pretreatment of gamma irradiation and NaOH.

Samples of 100 g each of substrate were treated with Sodium hydroxide as in section 2.4.2 and then subjected to γ-irradiation as in section 2.4.1.

3.4. Fractionation of pretreated corn stalk.

Only substrate pretreated with NaOH was fractionated as outlined in Figure 3-1. Substrate treated with γ-irradiation was not fractionated because there was almost no solublization of any fraction of the substrate.

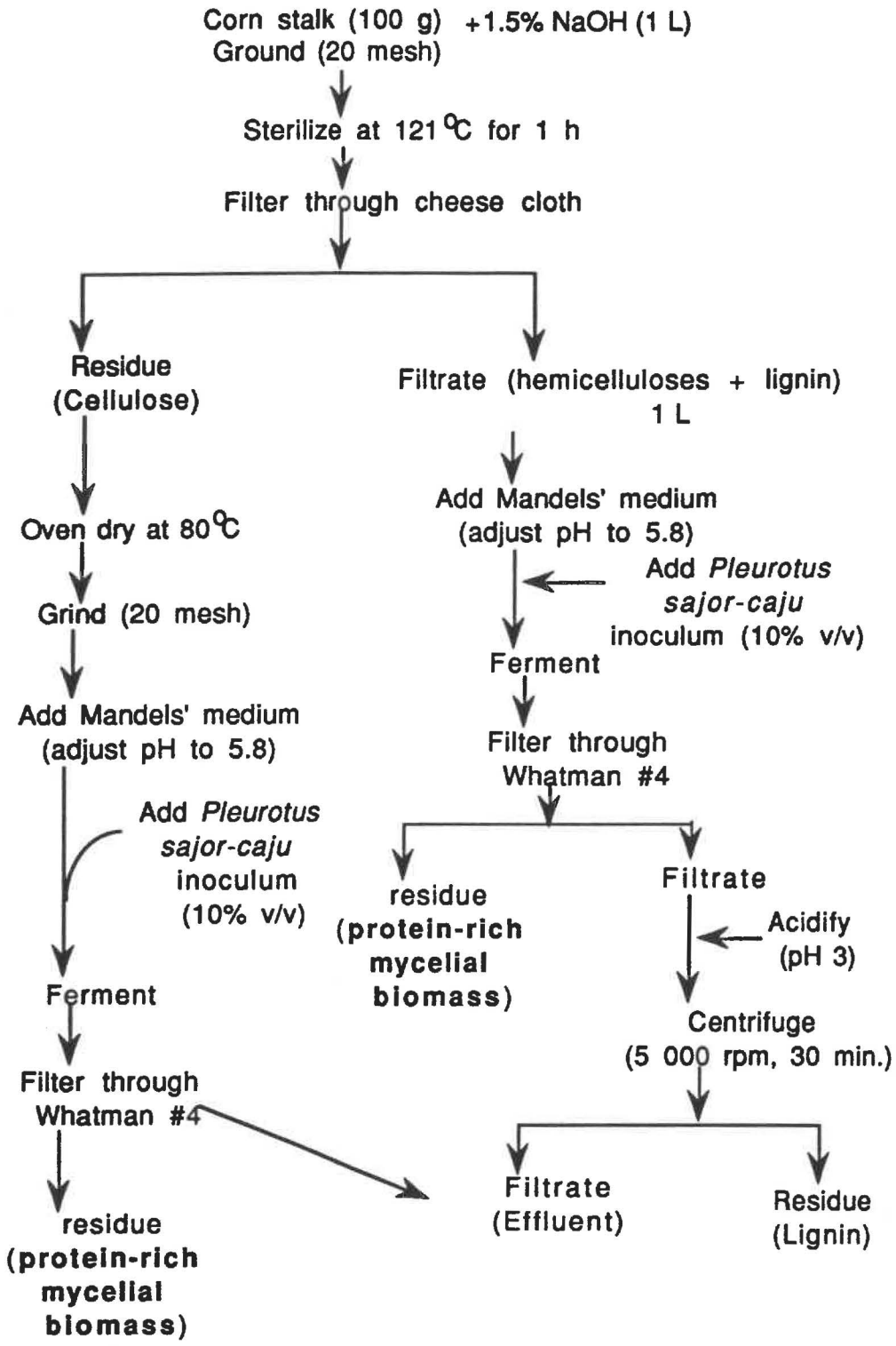


FIGURE 3-1 FRACTIONATION SCHEME
(Adapted from Chahal D.S, 1993,
Personal Communication).

3.5. Microorganism

Pleurotus sajor-caju NRRL 18757 was grown in petri dishes of Mandels and Weber standard agar medium (1969) with 1% delignified wheat straw. After one week of growth, the cultures were stored at 4 °C.

3.6. Cultivation medium

Mandels and Weber (1969) liquid medium was used in the study. Some modifications were made to the media and have been indicated at their respective places in the text. For the purposes of this work, this medium shall subsequently be referred to as Mandels' medium.

Table 3-1 shows the composition of Mandels' medium for 10 g glucose or substrate in one litre water.

Table 3-1: Mandels' medium

$(NH_4)_2SO_4$	1.4 g
KH_2PO_4	2.0 g
Urea	0.3 g
$CaCl_2$	0.3 g
$MgSO_4.7H_2O$	0.3 g
Yeast extract	0.5 g
$FeSO_4.7H_2O$	5.0 mg
$MnSO_4.7H_2O$	1.6 mg
$ZnSO_4.7H_2O$	1.4 mg
$CoCl_2$	2.0 mg
Substrate (Corn stalk or glucose)	10 g

Distilled water to make up to one litre.

pH	5.8

3.7. Pre-inoculum and inoculum preparation

The pre-inoculum of *Pleurotus sajor-caju* was started by inoculating 250 mL shake flasks containing 100 mL of complete standard Mandels' medium with 3-5 tiny pieces of agar having actively growing mycelia of the microorganism. The pieces were cut from the cultures growing on petri plates of Mandels' medium with delignified wheat straw. The shake flasks were placed in an incubator shaker (New Brunswick Scientific, Edison, N.J.) with the temperature and agitation controlled at 30°C and 200 rpm, respectively. The cultures were allowed to grow for 5-7 days to serve as the pre-inoculum. More flasks with the same medium and same volume were inoculated with 10% (V/V) macerated mycelial broth of pre-inoculum. These flasks were incubated under similar conditions for 3-4 days to be used as an inoculum. The pH of the inoculum was manually regulated with a Corning model 220 digital pH/ion meter using either 6% NaOH or 6% H_2SO4 to maintain pH around 5.8.

3.8. Fermentation

The fermentations were carried out both in 250 mL Erlenmeyer flasks and in a 16-L fermenter. The substrates used for the fermentations were the corn stalk samples and glucose (dextrose).

3.8.1. Shake-flask and scale-up fermentation

For the shake flask and 16-L fermenters, 1% of the substrates was used. In the shake flask experiments, 10% (v/v) of the inoculum were transferred into 250mL erlenmeryer flasks containing 90 mL medium, having nutrients for 100 mL and 1 g of substrate. The final volume after inoculation was 100 mL. They were incubated as before in incubator shakers. A similar transfer was made into a 16-L fermenter (New Brunswick Scientific, Edison, N.J.) with a working volume of 10 L. The fermenter was filled with 9 L of medium (containing nutrients of 10 L) and autoclaved at 121°C for 30 minutes. After cooling, the vessel was inoculated aseptically by pouring a 10% inoculum (1L) grown in shake flasks. The final volume after inoculation was 10 L. The following fermentation conditions were maintained:

Temperature: 30°C

Air flow rate: 1vol/vol/min. (VVM)

Agitation speed: 200 rpm

The dissolved oxygen was not monitored and the pH was automatically controlled at 5.8 with sterilized 20% NaOH or H_2SO_4. Sterile samples were obtained through a steam sterilizable sampling port.

3.9. Mycelial biomass harvest and analysis.

Samples from both the shake flasks and scale-up fermentation were taken at various time intervals and the final product (mycelial

biomass) was obtained by filtering the mycelial broth through dry pre-weighed Whatman filter paper # 4 and dried overnight at 80^0C. The dry weight was expressed as g/L.The final product contained the newly synthesized mycelial biomass and any unutilized substrate. All determinations were done in duplicate and the mean dry weight reported.

3.9.1. Crude protein determination

The crude protein was determined in yield as grams per litre and as a percent of the final product. The percent crude protein content was determined indirectly by the measurement of liberated nitrogen of dry samples (200-700 mg) in a Nitrogen analyser (Leco Company, USA). A schematic representation of the analyser is shown in Appendix 1. The weighed sample is subjected to combustion in a furnace at 925^oC. The combusted air which comprise mainly, CO_2, N_2, O_2 and water vapour is carried by helium gas through O_2, CO_2 and water vapour absorbers. The final gas left is N_2 which passes through a thermoconductivity cell and the thermal conductivity difference between the N_2 and Helium (He) is recorded and expressed as percent nitrogen on dry weight basis and by multiplying with a conversion factor of 6.25 to obtain per cent protein (Bano and Rajarathnam, 1988). The protein content in g/L was obtained by multiplying the per cent protein by the dry weight of the final product in one litre. All determinations were done in triplicate and mean values reported.

The factor 6.25 used is based on the presumption that most of the proteins contain 16% nitrogen. Some studies of crude protein, however, suggest that a closer approximation of mushroom protein content can be obtained by using a conversion factor equal to 70 % of N × 6.25 i.e N × 4.38 (Bano and Rajarathnam, 1988). Based on studies with purifed mushroom protein isolate, another conversion factor of (N × 8.48) would be more appropriate for estimating mushroom protein if the nitrogen content of the chitin and free amino acids could be ignored (Fitzpatrich et al.,1946). However for this work, the conventional conversion factor of N × 6.25 was used to determine the protein content of the product.

3.9.2. Growth rate, efficiency and productivity measurements. (Adapted from Quierzy et al.,1979)

The growth rate was obtained by the gradient of the change in protein content per time of fermentation and expressed as h^{-1}. The efficiency of bioconversion was calculated as a function of final protein synthesized and total amount of polysaccharide available from corn stalks for conversion into protein. For instance, 1 g of glucose would produce about 0,5 g of mycelial biomass containing about 50% protein, under optimal conditions. Therefore from 1 g of glucose about 0,25 g protein could be produced. In 1 g corn stalk, 0.6 g are the polysaccharides available which can be converted into about 0.15 g of protein according to the above factor. Based on these assumptions, the productivity in g/L was calculated as the amount of protein

synthesized per unit time of fermentation. The following formulae have been used for the calculation of the growth rate, efficiency and productivity.

Growth rate = dC/Cdt, where dC is change in protein concentration, and Cdt is protein concentration × change in fermentation time.

Efficiency = C - Co/So, where C is protein concentration (g L^{-1}), Co is initial protein concentration (g L^{-1}), and So is initial available substrate (g). For glucose, So is 2,5 g and for corn stalk, So is 1,5 g.

Productivity = C - Co/t - to, where t - to is change in fermentation time from time zero.

Statistical analysis- Comparisons between treatments and adjusting for fermentation time were made using the method of analysis of covariance (Berry and Armitag, 1987).

3.10. Schematic presentation of the procedure for the production of mycelial biomass protein.

A general scheme of the procedure for the production of mycelial biomass protein of *Pleurotus sajor-caju* from corn stalks is shown in Figure 3-2. The procedure consisted of grinding the corn stalks and subjecting them to single treatments of γ-irradiation and sodium hydroxide or to the combination treatments of γ-irradiation followed by sodium hydroxide treatment. In another development, small pieces of corn stalk were directly subjected to steam explosion without grinding.

The pretreated substrate samples were fermented fractionated or as such in Mandels' medium with *Pleurotus sajor-caju.* The final downstream product was harvested at various time intervals and analyzed. The final product is the mycelial biomass rich in protein. The effluent does not contain any toxic material and could therefore be safely used for irrigating crops (Chahal, 1993)

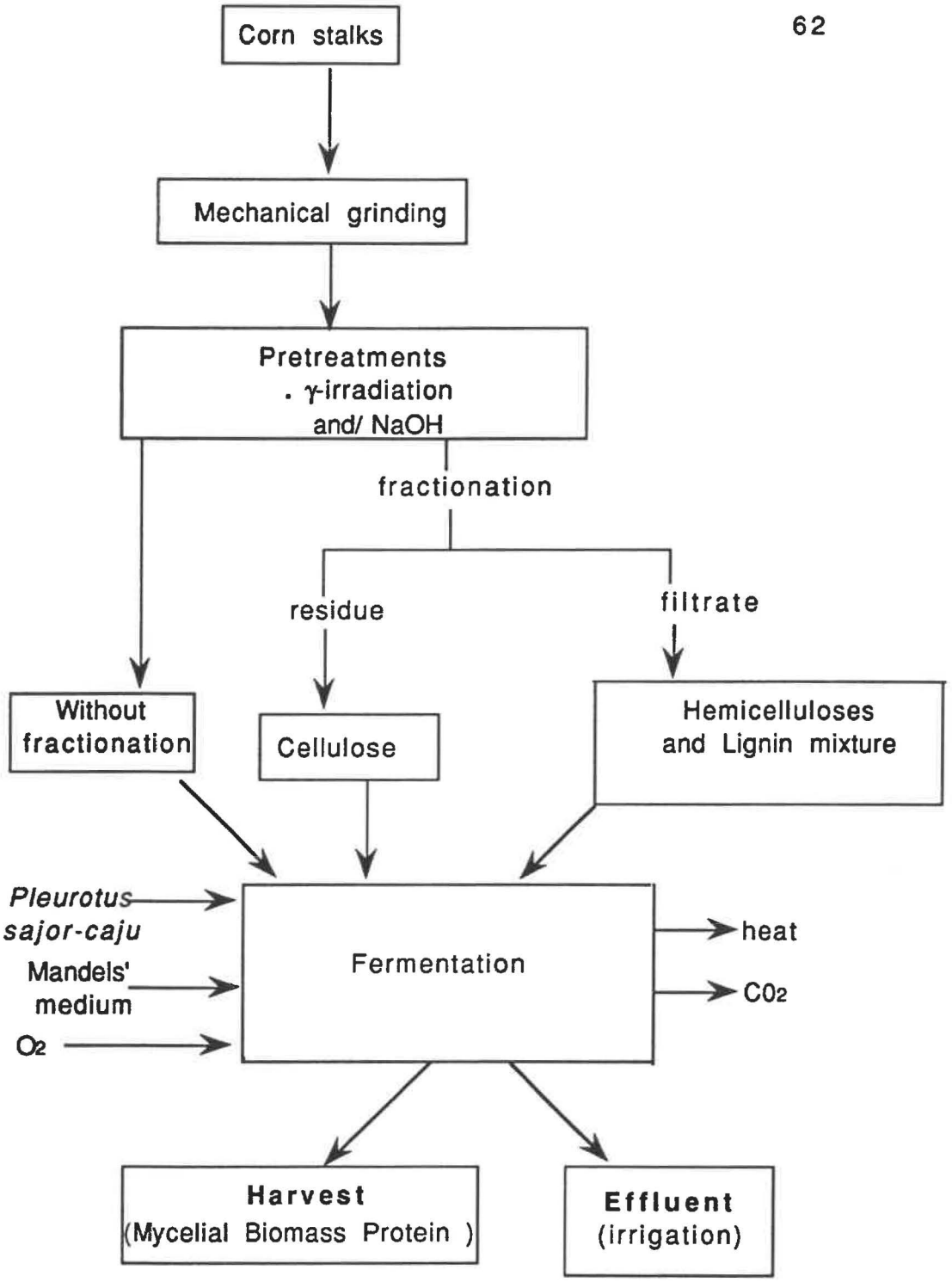


FIG. 3-2 SCHEME OF PROCESS
(Adapted from IAF Process,
Courtesy of Chahal, D.S (1991)

4.0. RESULTS AND DISCUSSION

4.1. Growth characteristics of Pleurotus sajor-caju on glucose and corn stalk.

Typically, there are four distinct phases in the growth of a microorganism, namely, the lag phase, the exponential or log phase, the stationary phase and decline phase. These phases were observed when synthesis of mycelial biomass and protein by *Pleurotus sajor-caju* on glucose and untreated corn stalk were determined (Fig.4-1 and 4-2). In batch fermentations, the type of substrate, end product, substrate concentration, extent of substrate utilization, age of inoculum, growth medium and production of autotoxic substances all influence the presence and magnitude of all these phases of growth.

The results obtained from the production of mycelial biomass of *Pleurotus sajor-caju* on glucose and corn stalk include ; the per cent protein in the final product, the growth rate, the efficiency of conversion of substrate into protein and protein productivity.

4.1.1 Effect of glucose on the production of mycelial biomass and protein by *Pleurotus sajor-caju.*

Glucose is a monomer that is readily utilized by microorganisms as carbon and energy sources. Table 4-1 and Figure 4-1 indicate that the dry weight of mycelial biomass of *Pleurotus sajor-caju* increases with the increase in the time of fermentation. The per cent protein in mycelial biomass increased up to 70 h of fermentation and then

started to decrease. The increase in protein is accounted for by the fact that a part of the glucose is utilized for the cell metabolic processes of *P. sajor-caju.* The rest of it is used along with nitrogen and other nutritional sources for anabolic synthesis into cellular substances and their polymers like protein, nucleic acids etc.

Normally, one gram glucose provides about 0,5 g each for anabolic and catabolic processes during microbial metabolism. Out of the 0,5 g utilized for anabolic processes, the amount of protein that can be synthesized is about 50% of the cell (mycelial) biomass i.e. 0,25 g. It is, therefore, expected that during the metabolism of 1 g glucose into mycelial biomass protein, a conversion efficiency of 100% will be considered when 0,25 g of protein is synthesized.

From Table 4-1, it is observed that the final product in the bioconversion of 10 g L^{-1} glucose into 5,4 g L^{-1} mycelial biomass of *P. sajor-caju* containing about 42% protein gave a yield of 1,95 g protein L^{-1} representing a 78% efficiency of conversion according to the above factor. A protein productivity of 27,8 mg $L^{-1}h^{-1}$ was recorded. Chahal (1989) obtained a protein content of 49% with a yield of 2,12 g protein L^{-1} representing an 85% efficiency and 37 mg $L^{-1}h^{-1}$ protein productivity under similar experimental conditions. However, during the optimisation of the carbon - nitrogen (C : N) ratio, protein contents up to 51% with yield of 2,49 g protein L^{-1}, conversion efficiency of 100% and protein productivity of 30 mg $L^{-1}h^{-1}$ were obtained under the optimum C: N ratio when only ammonium sulphate was used as nitrogen source. Urea as another source of

nitrogen is also used along with ammonium sulphate in Mandels medium and may, therefore, have some implications in the low protein value reported in Table 4-1.

Table 4-1: Production of mycelial biomass and protein by *P. sajor-caju* on 1% glucose.

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		GROWTH RATE dC/Cdt (h-1)	EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
		(%)	(g/L)			
0	1,250	25,062	0,313			
18	2,560	26,494	0,678			
24	3,480	31,994	1,113			
40	4,870	37,656	1,834			
52	5,010	39,162	1,962			
64	5,430	37,431	2,033			
70	5,390	41,956	2,261	0,045	77,926	27,830
76	4,880	38,119	1,860			
88	4,370	34,462	1,506			
112	3,330	26,987	0,899			
136	2,260	31,912	0,721			

Figs. 4-2 and 4-3 show the relationship of protein synthesis and biomass production of untreated corn stalk and glucose substrates. In Figure 4-2 the growth pattern for the glucose substrate showed an early surge in protein synthesis. This indicates the ease of accessibility of the glucose from the growth medium. As the time of fermentation increases, a fast exponential phase of protein synthesis is observed which starts declining after 70h. The decline in protein synthesis also corresponded to a decrease in mycelial biomass (Fig. 4-1). The decrease in mycelial biomass reported in Fig. 4-1 is due to the depletion of glucose which had a concomitant effect on protein synthesis hence the observed decline in protein synthesis depicted in Fig.4-2. An additional factor which could have contributed to the decrease in protein synthesis is that with prolonged fermentation in the absence of any sustainable energy, proteolytic enzymes extracellularly secreted by *P. sajor-caju* could also act on the available protein and start a degradation process aimed at providing the organism sustainable energy. Autolysis of older mycelium is a common phenomenon in fungi (Cochrane,1958) and this could have additionally contributed to a decline in mycelial biomass.

This pattern of growth behaviour is similar in many aspects when corn stalk is substituted for glucose in the production of mycelial biomass of *P.sajor-caju*. It is in the light of this and in the mainstream objective of assessing the efficacy of pretreated corn stalk for protein synthesis, that growth patterns observed in both

glucose and untreated corn stalk will be compared with irradiated corn stalk substrates.

FIGURE 4-1 Production of final product (mycelial biomass) by *Pleurotus sajor-caju* on 1% glucose and 1% untreated corn stalk.

FIGURE 4-2 Crude protein content of final product (mycelial biomass) by *Pleurotus sajor-caju* on 1% glucose and 1% untreated corn stalk.

Fig. 4-1

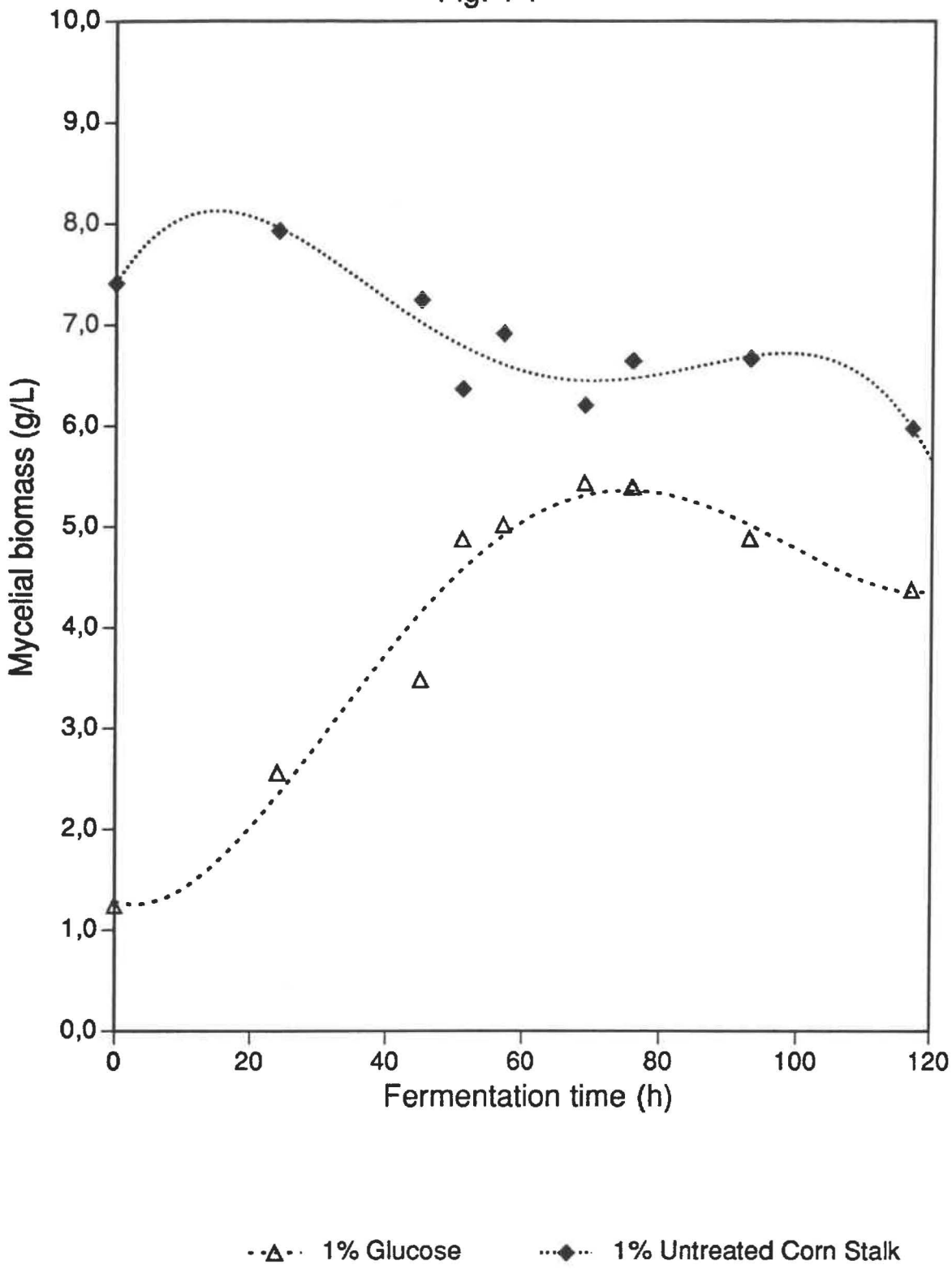
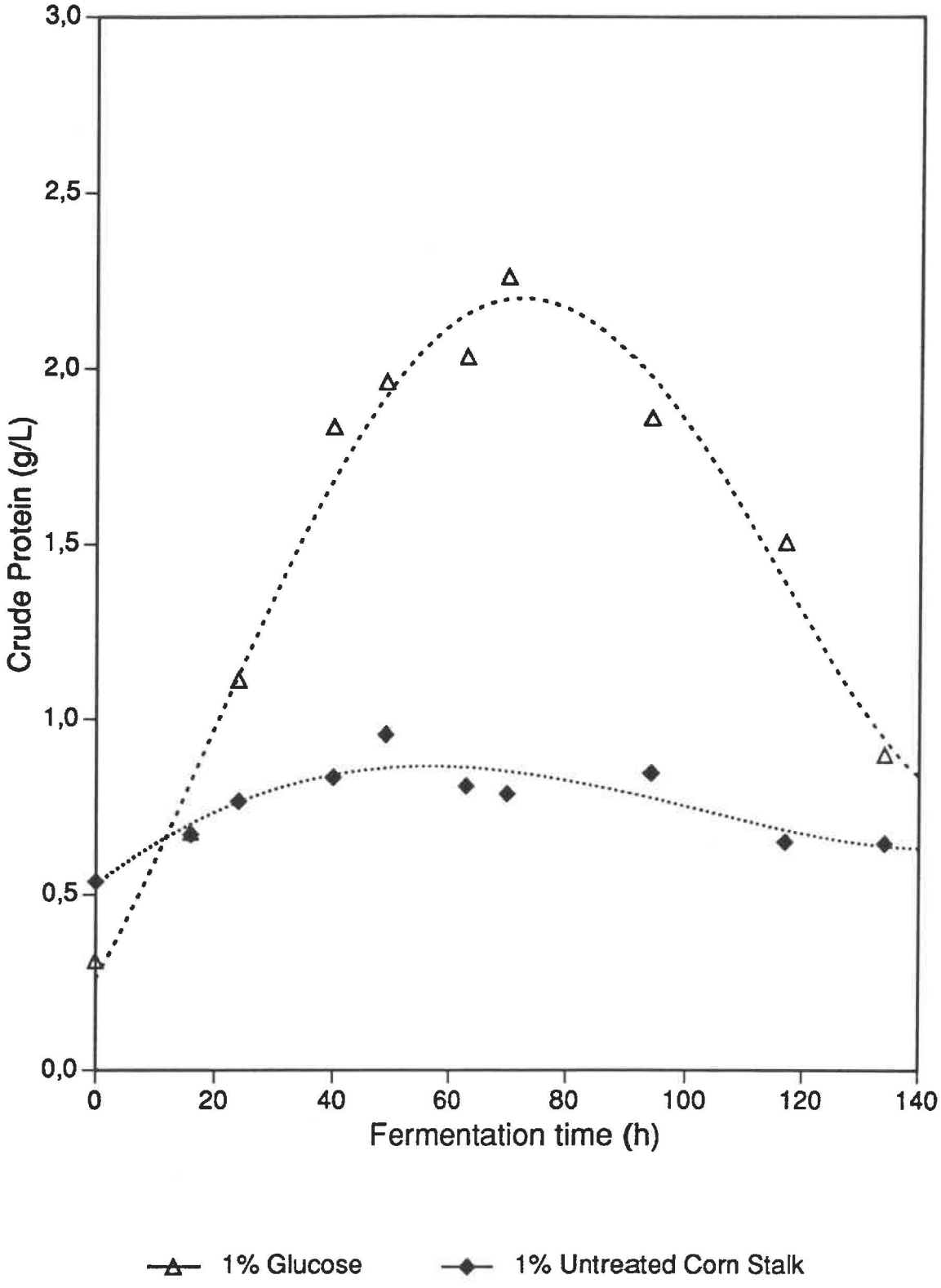

10,0
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
Mycelial biomass (g/L)
0
20
40
60
80
100
120
Fermentation time (h)
1% Glucose
1% Untreated Corn Stalk

Fig. 4-2


3,0
2,5
2,0
1,5
1,0
0,5
0,0
Crude Protein (g/L)
0
20
40
60
80
100
120
140
Fermentation time (h)
1% Glucose
1% Untreated Corn Stalk

4.1.2 Effect of untreated corn stalk on the production of mycelial biomass and protein by *Pleurotus sajor-caju.*

Table 4-2 represents the results obtained from the production of final product (mycelial biomass) of *Pleurotus sajor-caju* on 1% corn stalk in shake flasks.

From Table 4-2, the optimum protein content of 14% in the final product was obtained after 49h of fermentation. The corresponding amount of protein synthesized was 0,96 g L^{-1} which represented a conversion efficiency of 28% of the polysaccharide content of the untreated corn stalk to yield a protein productivity of 8,6 mg $L^{-1}h^{-1}$. The average growth rate was 0,009 h^{-1} while the growth rate in the glucose substrate was 0,045 h^{-1} (Table 4-1). The higher growth rate from the glucose substrate is yet another reflection of the ease of utilization of glucose compared to the corn stalk.

The chemical composition of corn stalks indicates that the holocellulose or polysaccharide portion is about 60% of its organic matter (Barl et al.,1991). This means that for every gram of corn stalk, 0,6 g is the optimum quantity of polysaccharides which could be utilized for bioconversion into protein-rich mycelial biomass. Whether or not all this polysaccharide portion is actually available for bioconversion depends on the efficiency of pretreatment in depolymerizing, solublizing or removing the lignin in addition to reducing the crystallinity of the cellulose.

Table 4-2: Production of mycelial biomass and protein by *Pleurotus sajor-caju* on 1% untreated corn stalk.

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (%)	PROTEIN CONTENT OF FINAL PRODUCT (C) (g/L)	GROWTH RATE dC/Cdt (1/h)	EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
0	7,410	7,244	0,537			
16	7,930	8,478	0,672			
24	7,240	10,569	0,765			
40	6,360	13,107	0,834			
49	6,910	13,837	0,956	0,009	27,933	8,551
63	6,200	13,032	0,808			
70	6,640	11,850	0,787			
94	6,660	12,700	0,846			
117	5,970	10,881	0,650			
134	6,060	10,657	0,646			

*FINAL PRODUCT: The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass in the final product increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *P. sajor-caju*.

Theoretically, we are talking of only 0,3 g corn stalk polysaccharide that would be available for anabolic processes from every gram of corn stalk compared to 0,5 g from glucose. Moreover, the corresponding optimum protein obtainable from the corn stalk would be 0,15 g compared to 0,25 g from glucose. Thus a protein content of 1,5 g L^{-1} from 1% corn stalk medium would represent 100% efficiency of bioconversion into mycelial biomass protein. All the values reported in this work are based either on the extent of bioconversion of 0,6% carbohydrates from 1% corn stalk or 1% glucose in the fermentation medium. The initial substrate concentrations (So) used in the calculations of the efficiencies and productivities of the corn stalk substrates and glucose are respectively, 1,5 g L^{-1} and 2,5 g L^{-1} based on total substrate provided that could be used for protein synthesis. To obtain a realistic comparison between glucose and corn stalk substrates, it may be more useful to calculate values obtained from corn stalk on the basis of 1% carbohydrate. In subsequent discussions, reference to this would help put in a better perspective our expectations of the performance of corn stalk in protein synthesis.

In the production of biomass like Microbial Biomass Protein (MBP), cultural conditions supporting the maximum cell population are used. It is in this vein that cultural conditions were optimized with emphasis on the carbon- nitrogen ratio.

4.2. Optimization of the carbon : nitrogen (C : N) ratio for the synthesis of mycelial biomass and protein by *Pleurotus sajor-caju.*

Glucose was used as the carbon source while ammonium sulphate was the sole nitrogen source. Tables 4-3 to 4-7 show the effect of varying the concentration of ammonium sulphate on the synthesis of mycelial biomass and protein by *P. sajor-caju* while maintaining the glucose content constant at 10 g L^{-1} (4 g Carbon L^{-1}) in 100 mL batch fermentations in 250 Erlenmeyer flasks.

When the nitrogen content was zero (Table 4-3), there was a slight increase in the synthesis of mycelial biomass and protein possibly as a result of the presence of traces of nitrogen introduced from the inoculum. The general trend indicates that by increasing the concentration of nitrogen, there were corresponding increases in the amounts mycelial biomass and protein synthesized as the fermentation progressed. Also, the efficiency of conversion of glucose into protein increased at higher concentrations of nitrogen. It was, however, noted that at nitrogen concentrations more than 0,4 g L^{-1} the amount of protein synthesized and efficiency of conversion of glucose into protein appeared to decrease. In the production of mycelial biomass, Fig. 4-3 shows that there was increase in mycelial biomass production up to 82 h of fermentation for all the concentrations of nitrogen. This trend was directly reflected in the crude protein content (Fig. 4-4). Thus, *P. sajor-caju* could support optimum protein synthesis at the nitrogen concentration of 0,4 g L^{-1}.

The importance of nitrogen in protein synthesis can not be over emphasized as it is needed at the cellular level for RNA, DNA and their macromolecules, protein. Nitrogen could also serve as a source of energy. According to Litchfield (1983), it is important to adjust the supply of nitrogen source so that the ratio of 0,1 or less for carbon to nitrogen can be maintained during growth to favour high protein synthesis. The C : N ratio that gave optimum protein synthesis by *P. sajor-caju* came out to be exactly 10:1 (4 C : 0,4 N) which is in obvious agreement with the above statement. The C : N ratio of 0,1 ($p \leq 0,05$) was significantly superior to the other C : N ratios (appendix 2a).

When the results from Table 4-5 are compared with those obtained from Mandels medium (Table 4-1), where the C : N ratio is about 10 : 1, it is clear that the former were better. This lends direct support to the earlier suggestion that urea, as the other nitrogen source, in Mandel's medium may have played a role in the lower results obtained.

Table 4-3 : Effect of carbon : nitrogen ratio of zero (4 g/L carbon : 0 g/L nitrogen) on the synthesis of mycelium biomass and protein by *Pleurotus sajor-caju.*

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
		(%)	(g/L)		
0	0,865	34,634	0,300		
22	1,795	42,752	0,767	18,713	21,264
34	2,180	38,598	0,841		
46	2,840	29,187	0,829		
58	3,055	25,974	0,793		
70	3,550	24,369	0,865		
82	3,560	24,279	0,864		
95	4,000	24,741	0,990		
106	4,650	19,742	0,918		
118	4,630	19,269	0,892		

Table 4-4 : Effect of carbon : nitrogen ratio of 0,05 (4 g/L carbon : 0,2 g/L nitrogen) on the synthesis of mycelium biomass and protein by *Pleurotus sajor-caju.*

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		EFFICIENCY E = C-Co/So	PRODUCTIVITY P = C-Co/t-to
		(%)	(g/L)	(%)	(mg/L.h)
0	0,910	48,192	0,439		
22	1,870	48,120	0,900		
34	1,965	51,446	1,011		
46	2,605	50,510	1,316		
58	3,260	45,950	1,498		
70	4,835	42,253	2,043		
82	5,765	39,040	2,251		
95	4,875	42,191	2,057		
106	4,795	44,259	2,122	67,347	15,884
118	4,380	43,686	1,913		

Table 4-5 : Effect of carbon : nitrogen ratio of 0,1 (4 g/L carbon : 0,4 g/L nitrogen) on the synthesis of mycelium biomass and protein by *Pleurotus sajor-caju.*

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
		(%)	(g/L)		
0	0,780	47,133	0,368		
22	2,060	50,822	1,047		
34	2,080	53,066	1,104		
46	3,630	47,243	1,715		
58	4,200	45,339	1,904		
70	5,570	45,100	2,512		
82	5,630	50,749	2,857	99,580	30,360
95	4,910	50,495	2,479		
106	4,900	47,416	2,323		

Table 4-6 : Effect of carbon : nitrogen ratio of 0,125 (4 g/L carbon : 0,5 g/L nitrogen) on the synthesis of mycelium biomass and protein by *Pleurotus sajor-caju.*

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
		(%)	(g/L)		
0	1,080	46,003	0,497		
24	2,090	49,442	1,033		
40	2,780	48,190	1,340		
49	3,670	46,608	1,711		
65	4,650	46,581	2,166		
74	4,670	45,374	2,119		
87	5,280	52,500	2,772	91,006	26,151
108	4,040	46,735	1,888		
116	3,760	41,955	1,578		

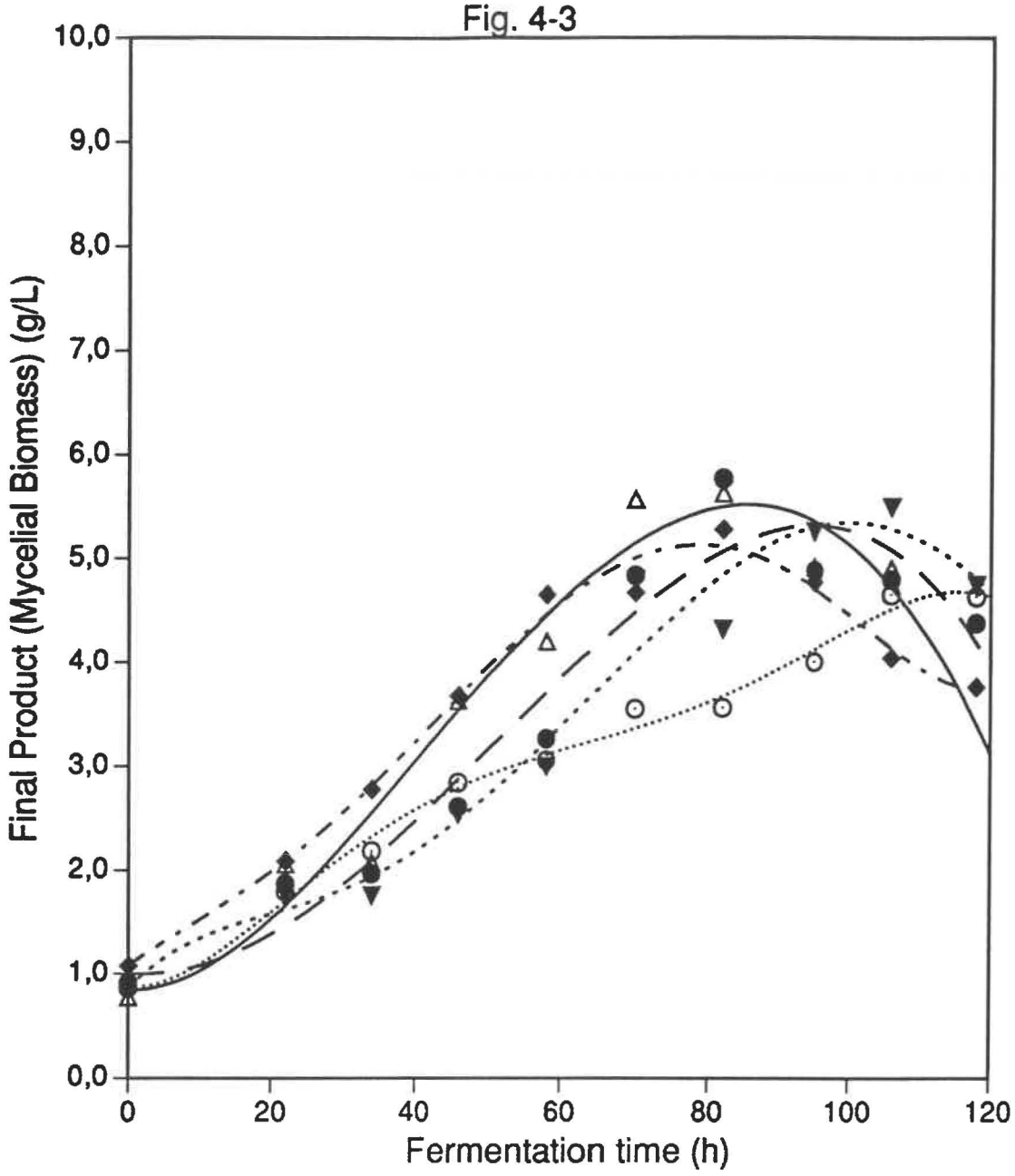
Table 4-7 : Effect of carbon : nitrogen ratio of 0,15 (4 g/L carbon : 0,6 g/L nitrogen) on the synthesis of mycelium biomass and protein by *Pleurotus sajor-caju.*

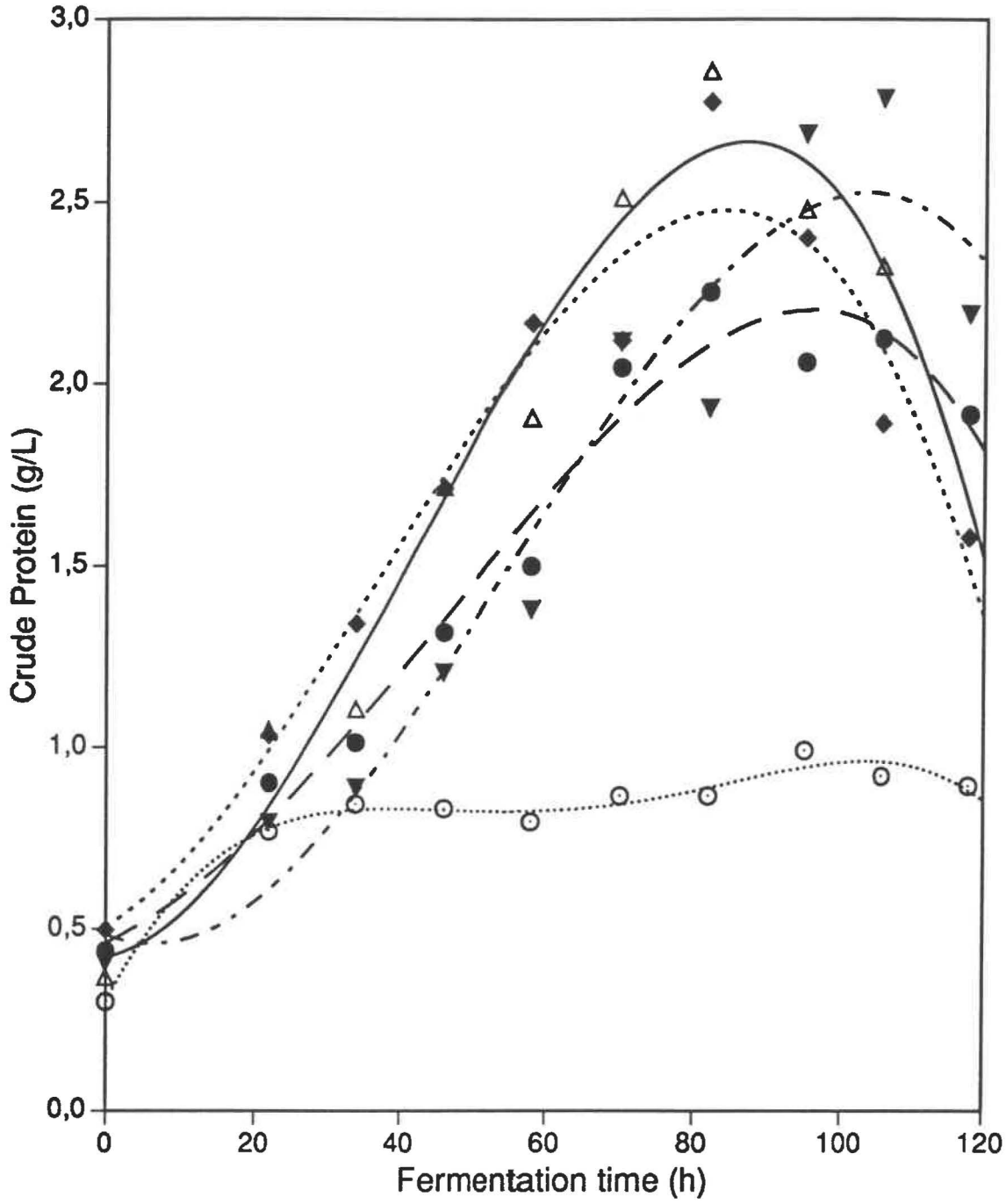
TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
		(%)	(g/L)		
0	0,840	48,252	0,405		
22	1,760	44,900	0,790		
34	1,750	50,659	0,887		
46	2,530	47,597	1,204		
58	2,990	46,078	1,378		
70	4,790	44,136	2,114		
82	4,310	44,760	1,929		
95	5,250	51,078	2,682		
106	5,490	50,624	2,779	94,958	22,396
118	4,740	46,181	2,189		

FIGURE 4-3: Effect of the carbon : nitrogen ratio on the production of mycelial biomass by *Pleurotus sajor-caju.*

FIGURE 4-4: Effect of the carbon : nitrogen ratio on the crude protein content of the mycelial biomass by *Pleurotus sajor-caju.*

Fig. 4-3


10,0
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
Final Product (Mycelial Biomass) (g/L)
0
20
40
60
80
100
120
Fermentation time (h)
C : N = 0
C : N = 0,05
C : N = 0,1
C : N = 0,125
C : N = 0,15


3,0
2,5
2,0
1,5
1,0
0,5
0,0
Crude Protein (g/L)
0
20
40
60
80
100
120
Fermentation time (h)
C : N = 0
C : N = 0,05
C : N = 0,1
C : N = 0,125
C : N = 0,15

4.3. Effect of γ-irradiation (100 KGy - 1,7 MGy) pretreatment of corn stalks on final product (mycelial biomass) and protein synthesis by *Pleurotus sajor-caju.*

Tables 4-8 to 4-11 and corresponding Figures 4-5 to 4-8 illustrate the results on final product (mycelial biomass) and protein synthesis by *P. sajor-caju* on 1% corn stalk pretreated with γ-irradiation at dose levels of 100 KGy, 500KGy, 1 MGy and 1,7 MGy. Corn stalk samples were irradiated at a moisture content of 75% because in the presence of water and oxygen, γ-irradiation easily initiates oxidation of sugars from lignocelluloses which results in the breakdown of the cellulose polymeric chain.

At the absorbed radiation dose of 100 KGy (Table 4-8), corn stalk could produce a maximum of only 11,6% protein in the final product (0,77 g protein L^{-1}) with a conversion efficiency of 24,2% and protein productivity of 10 mg L^{-1} h^{-1}. When the dose was increased to 500 KGy (Table 4-9), there was a slight increase in the protein content to 15,6% in the final product (0,86 g protein L^{-1}) . There was, however, a decrease in the conversion efficiency to 13,8% and decrease in productivity to 3,4 mg $L^{-1}h^{-1}$. The slight increase in protein content may have been a result of slight structural modifications in the structure of the corn stalk cell wall enabling relative ease of accessibility of the corn stalk holocellulose. However, production of inhibitory substances may have contributed to the low efficiency of

conversion and productivity since the fermentation time was much more prolonged from 36h in the 100 KGy substrate to 60h in the 500 KGy substrate. When the results were compared as in Figures 4-5 and 4-6, the amount of final product (mycelial biomass) produced decreased with both increase in radiation dose and fermentation time. This decrease was reflected in the protein curve as increase in protein synthesis. The protein synthesis profile indicates that at 500 KGy, protein synthesis was only slightly above untreated corn stalk. The lower dose of 100 KGy even seemed to produce less protein than the untreated corn stalk. It is not exactly clear if doses lower than 500 KGy seem to produce toxic substances or rather strengthen the polymeric chains of lignocelluloses. The production of a harmful substance like malondialdehyde when irradiation is carried out under neutral or alkaline conditions has been reported by some workers (Bludovsky and Duchacek, 1979; Scherz,1970). It is, however, apparently clear that doses of 500 KGy up to 1,7 MGy have a more positive effect on protein synthesis as observed in Tables 4-10 and 4-11.

At 1 MGy, the optimum protein content obtained was 16% in the final product, (0,83g protein L^{-1}) which represented a conversion efficiency of 34% and protein productivity of 14 mg $L^{-1}h^{-1}$. When the dose was increased to 1,7 MGy, the protein content in the final product was increased to 28%, (0,9 g protein L^{-1}) but the conversion efficiency and protein productivity decreased to 36% and 4,5 mg $L^{-1}h^{-1}$ respectively. These production trends when depicted in Figures

4-7 and 4-8 indicate that corn stalk irradiated at 1 MGy, had an earlier rate of decline in final product (mycelial biomass) than at 1,7 MGy. This resulted in higher protein synthesis at early stages of fermentation for the 1 MGy samples as compared to the 1,7 MGy samples. However, at the tail end of the fermentation, there was a rapid decline in the final product (mycelial biomass) of the 1,7 MGy sample which resulted in the surge in protein synthesis as depicted in Fig. 4-8. Statistical analysis showed that the protein content of the 1,7 MGy sample was superior to the rest of the irradiation samples at $p \leq 0,05$ (appendix 2b).

The results presented here seem to suggest that gamma irradiation at or below 500 KGy does not increase susceptiblilty of corn stalk polysaccharide for bioconversion by the enzyme system of *P. sajor-caju*. The possible effects of irradiation on lignocellulosic material include depolymerization, solublization and decrease in crystallinity. Gamma irrdiation doses lower than 500 KGy did not seem to manifest any of these on corn stalk. However, it has been reported by Han and Ciegler (1982) that gamma irradiation at or above 100 KGy was sufficient to drastically increase the specific surface area of sugarcane bagasse for enzymatic hydrolysis.

The increase in protein synthesis with increase in irradiation as compared to the untreated substrate may suggest that depolymerization of corn stalk is radiation dependent and supports the view that radiation doses greater than 500 KGy can cause a decrease in crystallinity (Aoki et al.,1977). Several workers have also

noted that gamma irradiation doses over 500 KGy cause a considerable portion of lignocelluloses to become water soluble. This trend was not observed here even at the 75% substrate moisture level used.

Table 4-8: Production of mycelial biomass and protein with *Pleurotus sajor-caju* on 1% corn stalks irradiated at 100 KGy.

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		GROWTH RATE dC/Cdt (1/h)	EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
		(%)	(g/L)			
0	6,020	6,787	0,409			
12	6,670	8,656	0,577			
24	6,680	10,625	0,710			
36	6,640	11,612	0,771	0,012	24,164	10,070
48	6,550	10,819	0,709			
60	6,280	11,350	0,713			
72	6,510	10,862	0,707			
96	6,450	10,837	0,699			
120	5,930	10,825	0,642			

*FINAL PRODUCT: The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *Pleurotus sajor-caju.*

Table 4-9: Production of mycelial biomass and protein with *Pleurotus sajor-caju* on 1% corn stalks irradiated at 500 KGy.

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		GROWTH RATE dC/Cdt (1/h)	EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
		(%)	(g/L)			
0	6,660	9,775	0,651			
12	6,780	11,725	0,795			
24	6,830	10,694	0,730			
36	6,870	12,581	0,864			
48	5,850	13,169	0,770			
60	5,510	15,569	0,858	0,003	13,789	3,447
72	5,680	12,409	0,705			
96	5,710	13,519	0,772			
120	5,650	12,806	0,724			

*FINAL PRODUCT: The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *Pleurotus sajor-caju*.

Table 4-10: Production of mycelial biomass and protein with *Pleurotus sajor-caju* on 1% corn stalks irradiated at 1 MGy.

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		GROWTH RATE dC/Cdt (1/h)	EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
		(%)	(g/L)			
0	5,380	5,962	0,321			
12	5,270	6,906	0,364			
24	5,250	12,881	0,676			
36	5,110	16,200	0,828	0,019	33,804	14,090
48	4,850	13,925	0,675			
60	4,590	14,387	0,660			
72	4,370	16,212	0,708			
96	4,840	15,762	0,763			
120	3,910	16,325	0,638			

*FINAL PRODUCT: The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *Pleurotus sajor-caju.*

Table 4-11: Production of mycelial biomass and protein with *Pleurotus sajor-caju* on 1% corn stalks irradiated at 1,7 MGy.

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (%)	PROTEIN CONTENT OF FINAL PRODUCT (C) (g/L)	GROWTH RATE dC/Cdt (1/h)	EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
0	4,670	7,700	0,360			
12	4,650	7,850	0,365			
24	4,700	11,644	0,547			
36	4,710	11,325	0,533			
48	3,870	12,862	0,498			
60	4,540	12,775	0,580			
72	4,520	16,994	0,768			
96	4,400	18,469	0,813			
120	3,210	28,037	0,900	0,019	36,027	4,500
148	3,197	13,462	0,430			

*FINAL PRODUCT: The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *Pleurotus sajor-caju*.

FIGURE 4-5: Production of final product (mycelial biomass) by *Pleurotus sajor-caju* on 1% corn stalks irradiated at 100 and 500 KGy.

FIGURE 4-6: Crude protein content of final product (mycelial biomass) by *Pleurotus sajor-caju* on on 1% corn stalks irradiated at 100 and 500 KGy.

Fig. 4-5

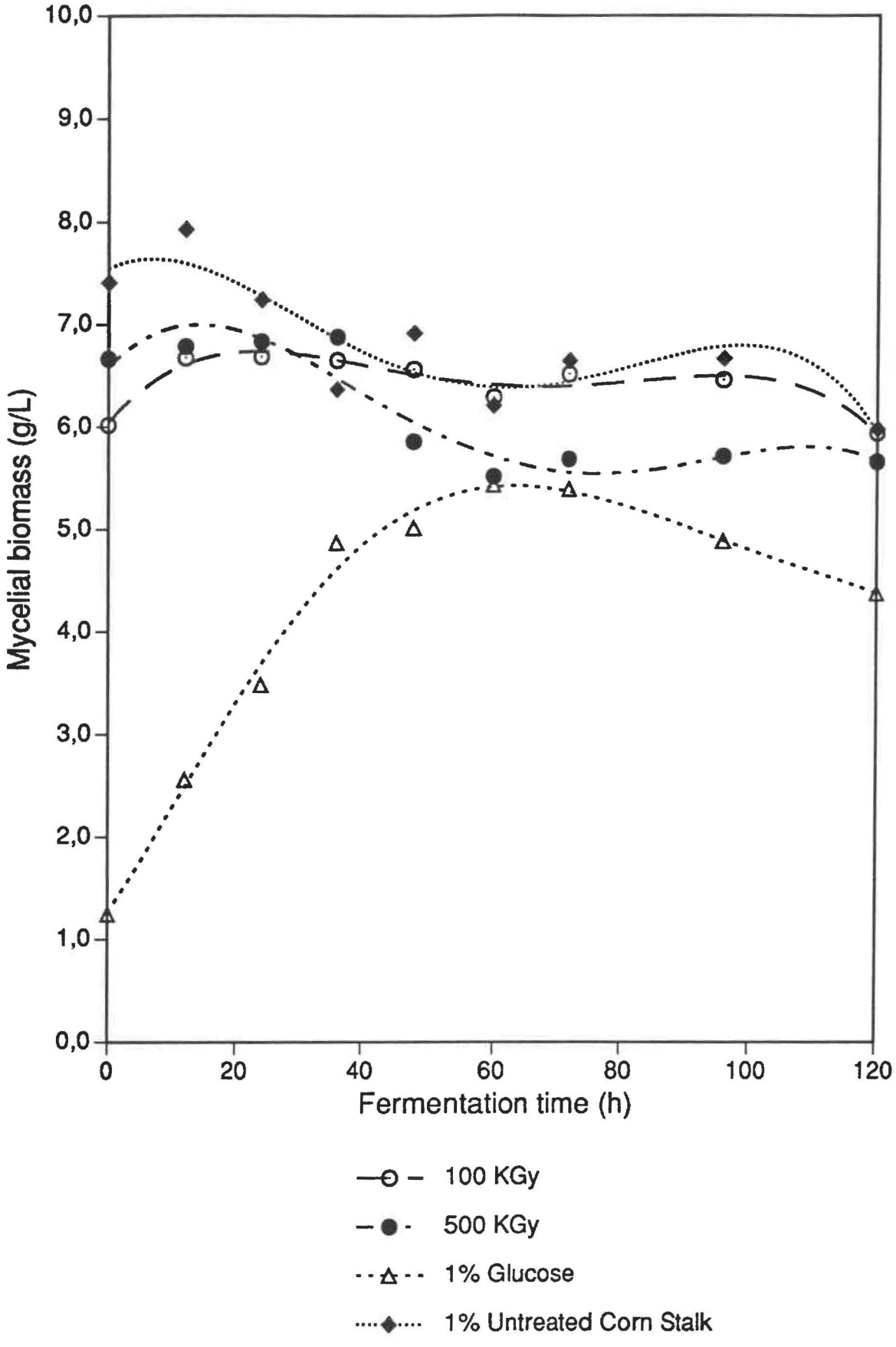

10,0
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
Mycelial biomass (g/L)
0
20
40
60
80
100
120
Fermentation time (h)
100 KGy
500 KGy
1% Glucose
1% Untreated Corn Stalk

Fig. 4-6

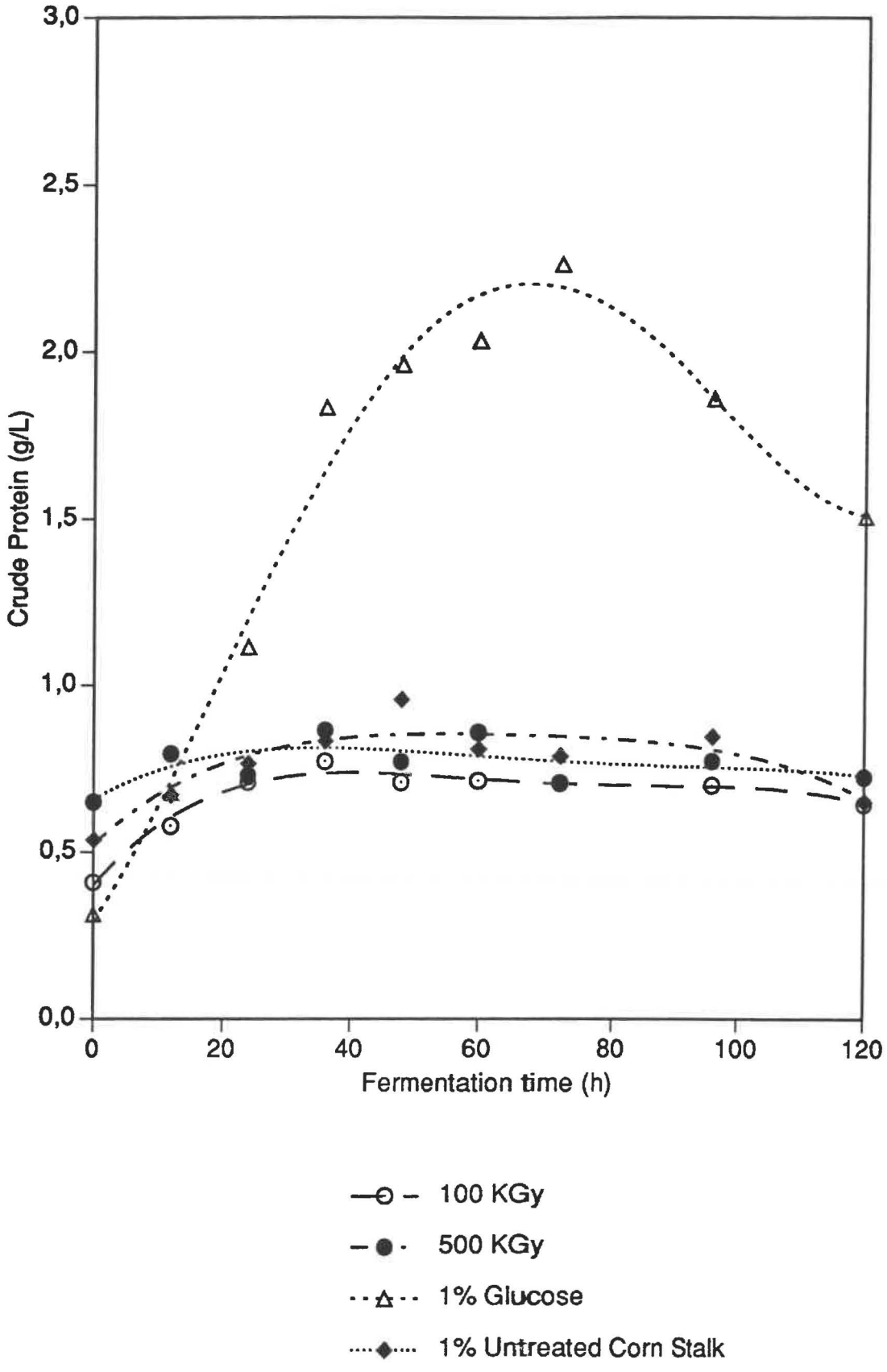

3,0
2,5
2,0
1,5
1,0
0,5
0,0
Crude Protein (g/L)
0
20
40
60
80
100
120
Fermentation time (h)
100 KGy
500 KGy
1% Glucose
1% Untreated Corn Stalk

FIGURE 4-7: Production of final product (mycelial biomass) by *Pleurotus sajor-caju* on 1% corn stalks irradiated at 1 and 1,7 MGy.

FIGURE 4-8: Crude protein content of final product (mycelial biomass) by *Pleurotus sajor-caju* on on 1% corn stalks irradiated at 1 MGy and 1,7 MGy.

Fig. 4-7

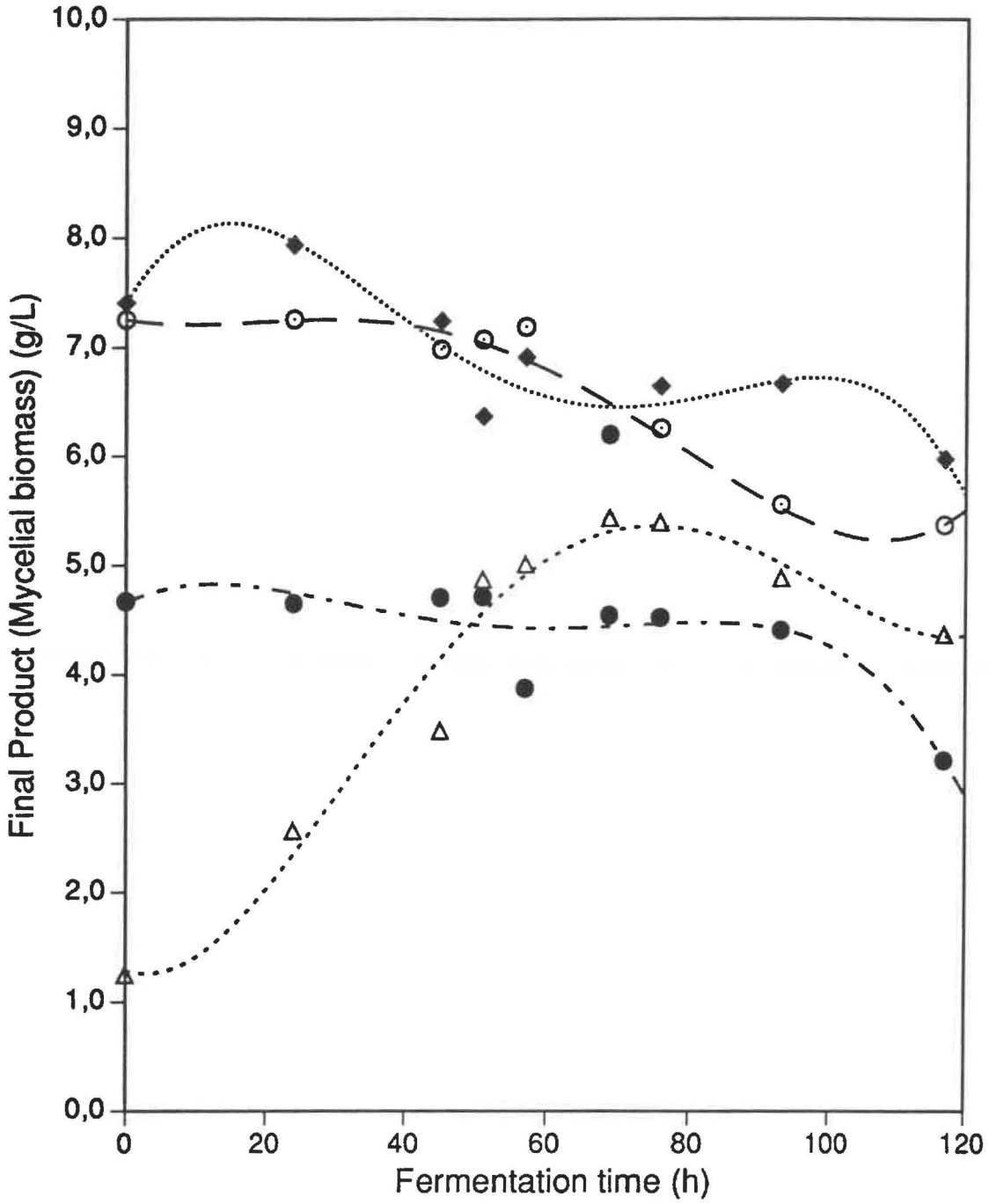
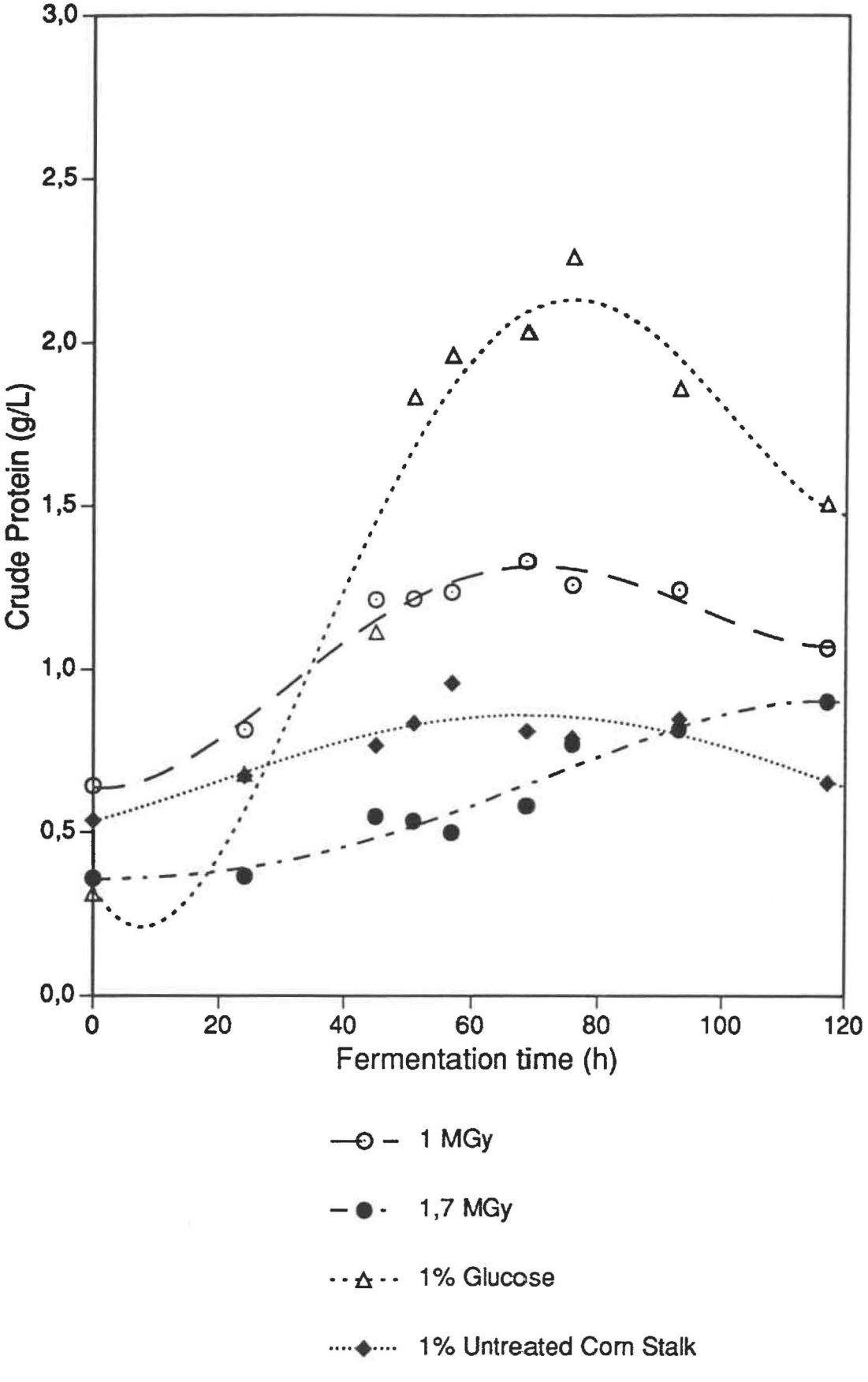

10,0
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
Final Product (Mycelial biomass) (g/L)
0
20
40
60
80
100
120
Fermentation time (h)
1 MGy
1,7 MGy
1% Glucose
1% Untreated Corn Stalk

Fig. 4-8


3,0
2,5
2,0
1,5
1,0
0,5
0,0
Crude Protein (g/L)
0
20
40
60
80
100
120
Fermentation time (h)
1 MGy
1,7 MGy
1% Glucose
1% Untreated Corn Stalk

4.4. Effect of NaOH (0,025-0,15 g/g substrate, 75% moisture) pretreatments of corn stalks on mycelial biomass and protein synthesis by *Pleurotus sajor-caju.*

Traditionally, sodium hydroxide (NaOH) has been widely studied and used as a pretreatment agent for lignocelluloses and optimum levels have been cited in certain cases for significant hydrolysis of lignocelluloses (Choudhury et al., 1984; Gray et al., 1978). It would, therefore, be normal to assess our technique of irradiation against the use of NaOH. Moreover, it would also be appropriate to determine optimum concentration levels of NaOH in the pretreatment of corn stalk for protein synthesis by *P. sajor-caju.*

The results obtained from the production of mycelial biomass of *P.sajor-caju* on 1% corn stalk, pretreated with different concentrations of NaOH, are presented in Tables 4-12 to 4-15 and corresponding Figures 4-9 to 4-12. Use of 0,025 g NaOH/g corn stalk yielded a protein content of 13,8% in the final product (0,9 g protein L^{-1}) with a conversion efficiency of 21,5% and productivity of 6 mg $L^{-1}h^{-1}$ (Table 4-12). These values were correspondingly increased to 19,9%, (1,2 g protein L^{-1}), 41,8% and 9,5 mg $L^{-1}h^{-1}$ when the NaOH concentration was increased to 0,05 g/g corn stalk (Table 4-13)

When the NaOH concentration was further increased to 0,1 g/g substrate, the protein content increased to 31% in the final product (1,4 g protein L^{-1}) with efficiency of 61% and 11,8 mg $L^{-1}h^{-1}$ productivity. These values further shot up to 49%, (1,5 g protein L^{-1}), 82,5% and 15,5 mg $L^{-1}h^{-1}$ respectively for substrate treatment at 0,15 g NaOH/g . Under shake flask conditions, Chahal (1985) reported protein contents of 36% and 45%, in the final product on corn stover treated with 10% and15% NaOH, respectively, when fermented with *P. sajor-caju.*

One significant effect of mild alkali treatment is to increase the swelling capacity and pore size of substrates. It is, therefore, clear that increasing NaOH concentration per gram of corn stalk resulted in increased pore size and swelling capacity. The advantage of increasing the pore size is to attain a diameter of 40Å which is equal to the diameter of cellulolytic enzyme molecules (Gharpuray et al., 1983) like those of *P.sajo-caju*. Stone et al.(1969) has already reported a linear relationship between the initial hydrolysis rate and the surface area that was accessible to cellulolytic enzyme molecules. Datta (1981) has also reported that dilute alkali causes saponification of esters of uronic acid associated with hemicelluloses which can result in the breaking of polymeric unit cross-links. All these factors, therefore, explain the trend of results as depicted in Tables 4-12 to 4-15. A look at Figures 4-9 to 4-12 would reveal that when the NaOH concentration increased to 1,5 g/g

substrate, the protein profiles were similar to those obtained from glucose.

For a realistic comparison of these results with 1% glucose in the medium, recalculation on the basis of 1% polysaccharide in the medium available from corn stalk of each NaOH treatment will yield 1,4 g L^{-1} protein for 0,05 g NaOH/g corn stalk, 1,6 g L^{-1} for 0,1 g NaOH/g corn stalk and 2,6 g L^{-1} for 0,15 g NaOH/g corn stalk. When these results are compared to the values of 2,26 g protein L^{-1} obtained for 1% glucose, it becomes clear that the 0,15 g NaOH/g substrate treatment gave protein yield superior to glucose. A possible explaination is that at the NaOH concentration of 0,15 g/g substrate, the alkali-labile hemicellulose-phenolic acid and acetyl constituents of the corn stalk cell walls were sufficiently broken to result in marked increase in soluble matter of hemicelluloses and increased pore size for direct contact by the enzyme system of *P. sajor-caju.* This observation may be supported by the fact that at lower NaOH concentration of 0,025 g/g the protein content was almost the same as that of the untreated substrate. This study also indicated that the optimum level of NaOH concentration for protein synthesis with *P. sajor-caju* on corn stalk is 0,15 g/g and was significantly superior at $p \leq 0,05$ (appendix 2c). This value is slightly higher than the observation by Choudhury et al. (1984) and Gray et al. (1978) that alkali concentration of 0,1 g/g substrate is optimum for significant increases in rate and extent of hydrolysis of lignocelluloses.

It may also be explained that there exist in plant material both alkali-labile and alkali-resistant lignin-carbohydrate portions and that their ratio may govern the decision on the quantity of alkali to be used in pretreatment. The empirical reference from these results suggest that alkali concentration threshold values may be another important factor to consider when alkali is considered as a pretreatment tool.

It may also be noted that when corn stalk is used as substrate, there is an inverse relationship between the dry weight of the final product and the protein content such that a decrease in final product weight gave rise to an increase in protein content. These trends are clearly observed at the higher NaOH concentration levels. This supports the theoretical base that on complete utilization of a carbon substrate by fungi, about one-half of the weight of the substrate is obtained as its mycelial biomass. In 1991, Chahal and Khan reported a similar trend when they used 15% NaOH pretreated rice straw to produce mycelial biomass of *P. sajor-caju*.

In overview, the protein content of 1,56 g L^{-1} reported in this work for corn stalk treated with 0,15 g/g substrate represents more than 100% utilization of the corn stalk polysaccharide for bioconversion into mycelial biomass. This work and the cited references further suggest that 0,15 g NaOH/g corn stalk is the optimum NaOH required for pretreatment of corn stalk for mycelial biomass and protein production with *P. sajor-caju*.

Table 4-12 : Effect of NaOH treatment(0,025 g/g substrate, 75% moisture) on corn stalks on the production of mycelial biomass by *Pleurotus sajor-caju*

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (%)	PROTEIN CONTENT OF FINAL PRODUCT (C) (g/L)	GROWTH RATE dC/Cdt (1/h)	EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
0	7,365	7,769	0,572			
24	8,005	10,068	0,806			
30	6,170	10,162	0,627			
42	6,595	12,096	0,798			
54	6,495	13,782	0,895	0,008	21,530	5,981
66	6,045	12,966	0,784			
78	6,230	12,858	0,801			
90	5,900	11,996	0,708			
100	5,730	12,233	0,701			

*FINAL PRODUCT: The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *Pleurotus sajor-caju*.

Table 4-13 : Effect of NaOH treatment(0,05 g/g substrate, 75% moisture) on corn stalks on the production of mycelial biomass by *Pleurotus sajor-caju*

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		GROWTH RATE dC/Cdt (1/h)	EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
		(%)	(g/L)			
0	7,215	7,771	0,561			
24	7,205	11,935	0,860			
30	6,795	11,802	0,802			
42	7,205	16,603	1,196			
54	6,360	16,181	1,029			
66	5,980	19,859	1,188	0,011	41,793	9,498
78	6,455	16,368	1,057			
90	6,280	15,880	0,997			
100	6,180	14,046	0,868			
126	5,550	12,530	0,695			

*FINAL PRODUCT: The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *Pleurotus sajor-caju*.

Table 4-14 : Effect of NaOH treatment(0,1 g/g substrate, 75% moisture) on corn stalks on the production of mycelial biomass by *Pleurotus sajor-caju*

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		GROWTH RATE dC/Cdt (1/h)	EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
		(%)	(g/L)			
0	6,580	8,031	0,528			
24	6,285	10,940	0,688			
30	5,945	11,799	0,701			
42	5,115	19,833	1,014			
54	4,470	24,600	1,100			
66	4,340	31,345	1,360			
78	4,635	31,231	1,448	0,014	61,274	11,784
90	3,985	27,782	1,107			
100	4,035	27,540	1,111			
126	3,810	24,117	0,919			

*FINAL PRODUCT: The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *Pleurotus sajor-caju.*

Table 4-15 : Effect of NaOH treatment(0,15 g/g substrate, 75% moisture) on corn stalks on the production of mycelial biomass by *Pleurotus sajor-caju*

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		GROWTH RATE dC/Cdt (1/h)	EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
		(%)	(g/L)			
0	4,380	5,494	0,241			
22	4,550	7,681	0,349			
31	4,430	6,981	0,309			
44	5,090	8,169	0,416			
57	4,150	15,844	0,658			
69	2,840	46,319	1,315			
80	3,010	49,106	1,478	0,023	82,498	15,470
100	3,160	42,306	1,337			
130	3,060	40,206	1,230			

*FINAL PRODUCT: The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of*Pleurotus sajor-caju.*

FIGURE 4-9: Production of mycelial biomass by *Pleurotus sajor-caju* on 1% corn stalks treated with NaOH at 0,025 and 0,05 g/g substrate.

FIGURE 4-10: Crude protein content of mycelial biomass by *Pleurotus sajor-caju* on 1% corn stalks treated with NaOH at 0,025 and 0,05 g/g substrate.

Fig. 4-9

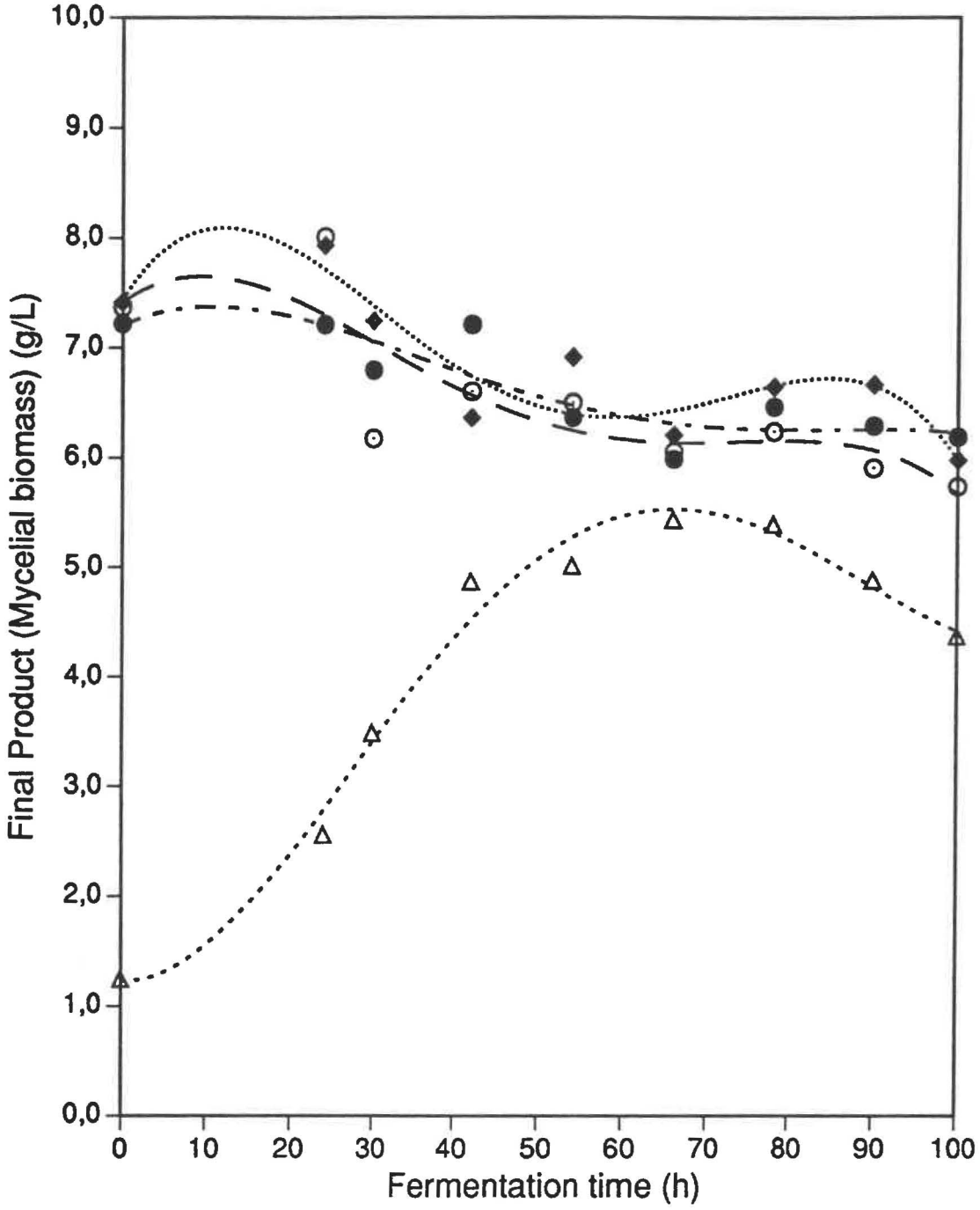

Final Product (Mycelial biomass) (g/L)
10,0
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
0
10
20
30
40
50
60
70
80
90
100
Fermentation time (h)
0,025 g NaOH/g Subst.
0,05 g NaOH/g Subst.
1% Glucose
1% Untreated Corn stalk

Fig. 4-10

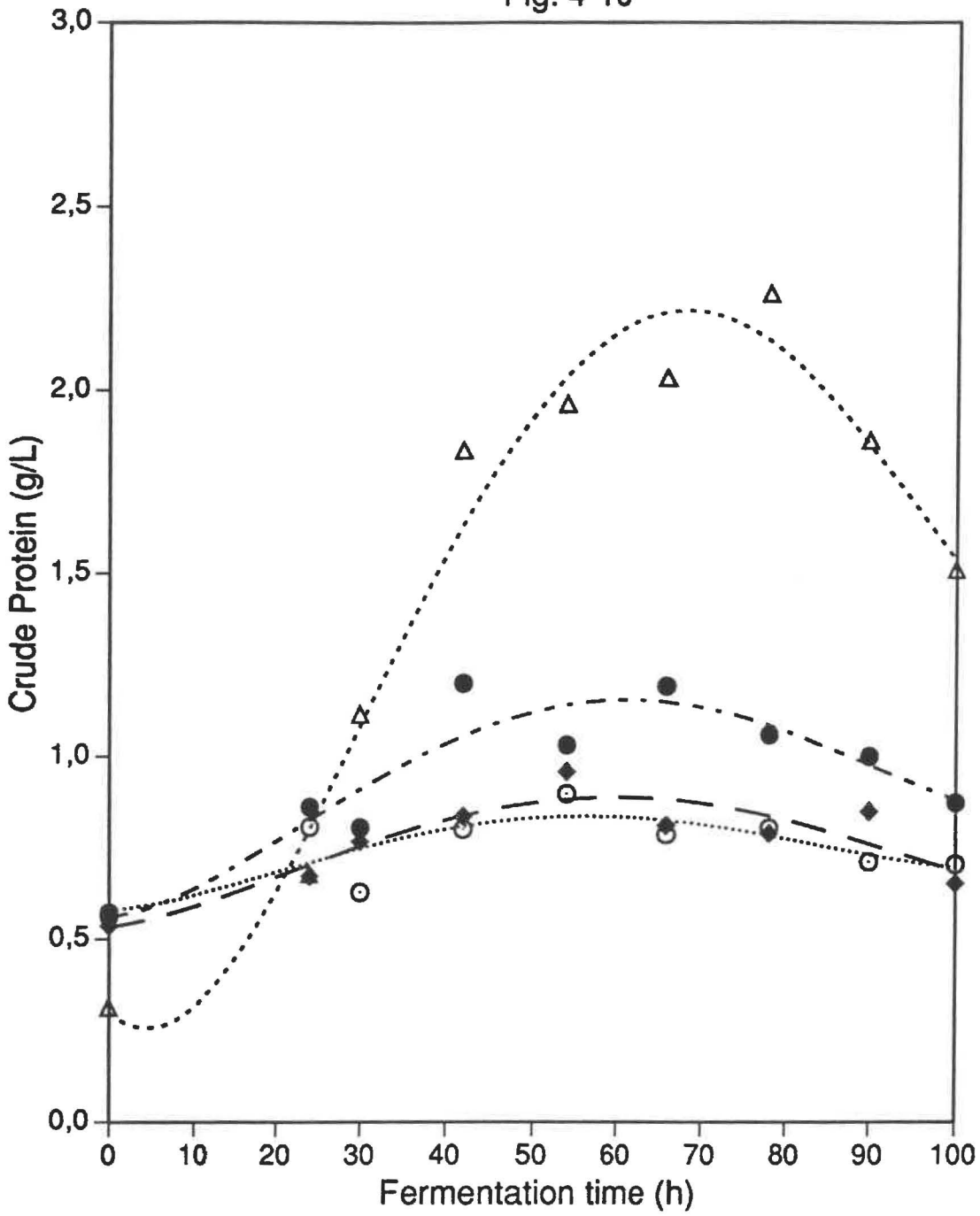

3,0
2,5
2,0
1,5
1,0
0,5
0,0
Crude Protein (g/L)
0
10
20
30
40
50
60
70
80
90
100
Fermentation time (h)
0,025 g NaOH/g Subst.
1% Glucose
0,05 g NaOH/g Subst.
1% Untreated Corn Stalk

FIGURE 4-11: Production of mycelial biomass by *Pleurotus sajor-caju* on 1% corn stalks treated with NaOH at 0,1 and 0,15 g/g substrate.

FIGURE 4-12: Crude protein content of mycelial biomass by *Pleurotus sajor-caju* on on 1% corn stalks treated with NaOH at 0,1 and 0,15 g/g substrate.

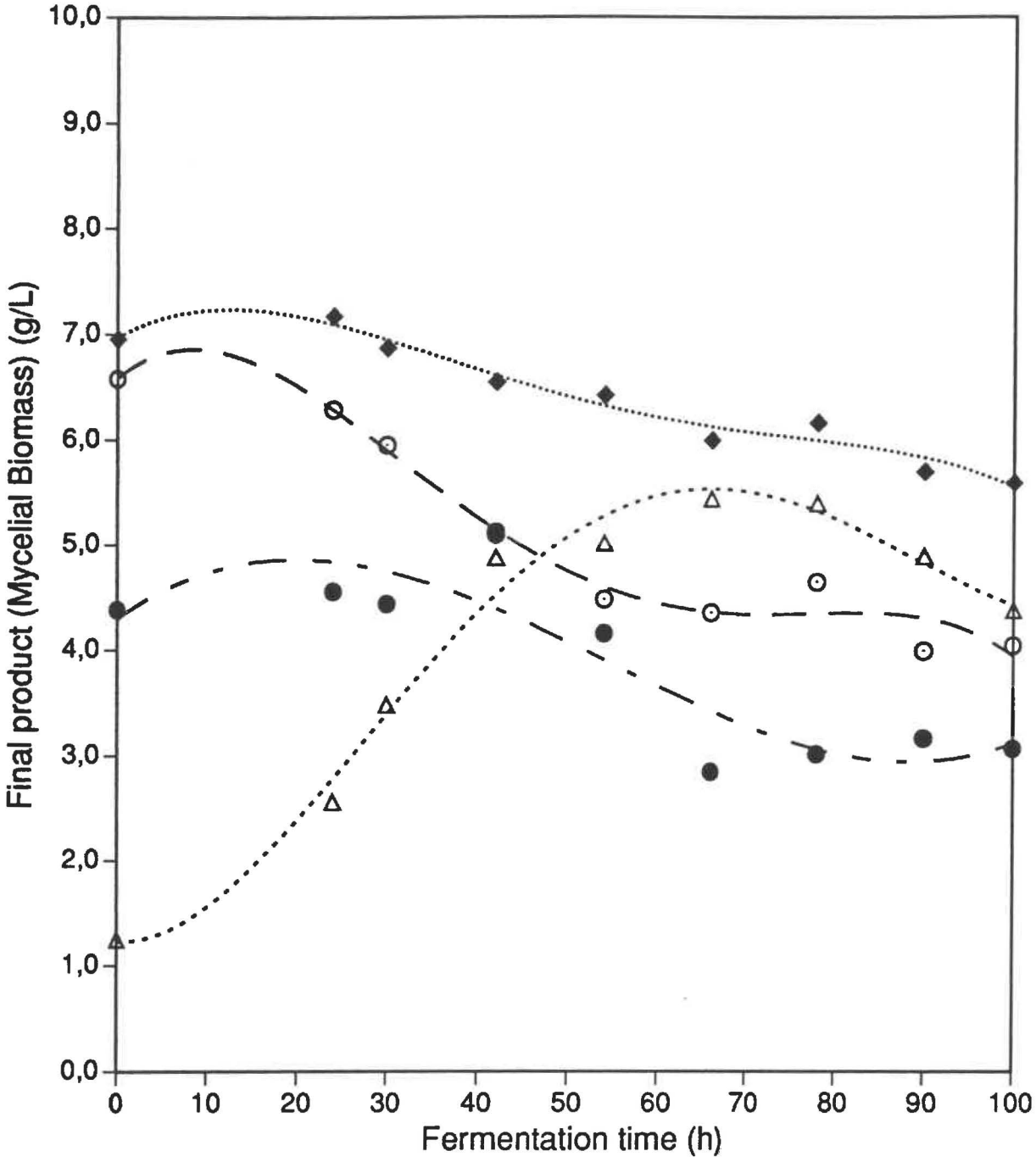
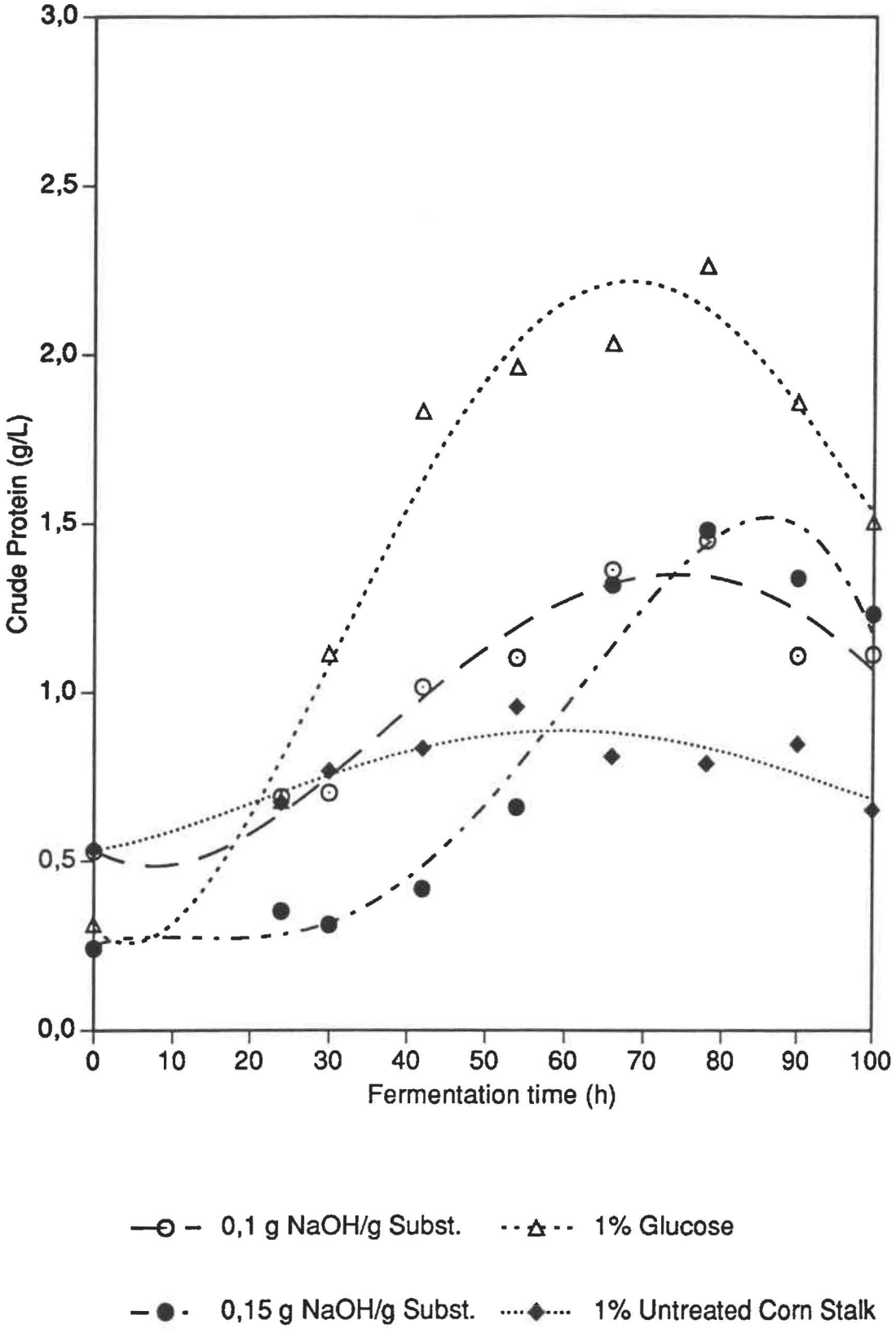

10,0
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
Final product (Mycelial Biomass) (g/L)
0
10
20
30
40
50
60
70
80
90
100
Fermentation time (h)

Fig. 4-12


3,0
2,5
2,0
1,5
1,0
0,5
0,0
Crude Protein (g/L)
0
10
20
30
40
50
60
70
80
90
100
Fermentation time (h)
0,1 g NaOH/g Subst.
1% Glucose
0,15 g NaOH/g Subst.
1% Untreated Corn Stalk

4.5. Combined effect of gamma (γ) irradiation and NaOH pretreatments on the production of mycelial biomass and protein with *Pleurotus sajor-caju.*

Physical pretreatments like irradiation can alter the crystallinity and surface area of substrate, whereas chemical pretreatments like alkali cause substantial delignification (Gharpuray et al.,1983). Therefore, a combination of such pretreatments might significantly alter the structure of the corn stalk, yielding a substrate that is highly susceptible to the lignocellulolytic enzyme system of *P. sajor-caju.* Furthermore, since dilute NaOH pretreatments have the effect of increasing the fiber saturation point and swelling capacity of lignocelluloses, it was thought that the combination pretreatments of NaOH followed by γ-irradiation may have the advantage of the latter for further modification of the exposed substrate with its high penetrating energy.

4.5.1. Combined effect of gamma irradiation (50 KGy) and NaOH (0,025-0,1 g/g substrate) pretreatments.

The results obtained from the production of protein-rich mycelial biomass of *P. sajor-caju* on 1% corn stalk pretreated with different NaOH concentrations and subjected to 50 KGy γ-irradiation, are presented in Tables 4-16 to 4-18 and corresponding Figures 4-13 to 4-15. When corn stalk was pretreated with 0,025 g NaOH/g substrate and subjected to γ-irradiation dose of 50 KGy, the optimum protein content in the final product was 9,6% (0,71 g protein L^{-1}) with

conversion efficiency of 12,9% and protein productivity of 1,7 mg $L^{-1}h^{-1}$. When the NaOH concentration was increased to 0,05 g NaOH/g corn stalk while maintaining the same radiation dose, the corresponding values increased to 11% (0,75 g protein L^{-1}), 15% and 2,6 mg $L^{-1}h^{-1}$. These values were further increased to 18,9% (0,9 g protein L^{-1}), 15,6%, and 2,1 mg $L^{-1}h^{-1}$ when the NaOH concentration was increased to 0,1 g NaOH/g corn stalk. The increase in NaOH concentration improved ($p \leq 0,05$) the protein synthesis at the 50 KGy irradiation level (appendix 2d).

It is apparent from Fig. 4-13 that increasing NaOH concentration and maintaining irradiation constant at 50 KGy, resulted in increased utilization of substrate during the course of the fermentation. This did not, however, result in any marked increase in protein synthesis as depicted in Fig. 4-13. The crude protein content in g/L for these pretreatments were similar to those obtained from untreated substrate. It may, therefore, be inferred that these combinations of pretreatments were not sufficient to enhance any gamma initiated radical formation of water molecules bound to the corn stalk matrix.

Table 4-16 : Combined effect of NaOH treatment (0,025 g/g substrate, 75% moisture) and irradiation (50 KGy) on the production of mycelial biomass and protein with *Pleurotus sajor-caju.*

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		GROWTH RATE dC/Cdt (1/h)	EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
		(%)	(g/L)			
0	7,800	6,614	0,516			
24	7,915	8,212	0,650			
36	7,760	8,166	0,634			
48	7,750	8,637	0,699			
62	8,250	8,202	0,677			
72	8,125	8,580	0,697			
84	7,210	8,545	0,619			
92	8,030	8,710	0,699			
114	7,405	9,573	0,709	0,006	12,867	1,693
132	7,335	9,394	0,707			

*FINAL PRODUCT The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *Pleurotus sajor-caju.*

Table 4-17 : Combined effect of NaOH treatment (0,05 g/g substrate, 75% moisture) and irradiation (50 KGy) on the production of mycelial biomass and protein with *Pleurotus sajor-caju.*

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		GROWTH RATE dC/Cdt (1/h)	EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
		(%)	(g/L)			
0	7,545	6,981	0,527			
24	7,790	9,398	0,732			
36	7,415	9,512	0,705			
48	7,590	8,708	0,661			
62	7,770	8,766	0,681			
72	7,945	8,831	0,702			
84	6,860	10,956	0,752	0,005	14,991	2,677
92	7,090	9,536	0,676			
114	7,030	10,497	0,738			
132	6,110	9,470	0,579			

*FINAL PRODUCT: The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *Pleurotus sajor-caju.*

Table 4-18 : Combined effect of NaOH treatment (0,05 g/g substrate, 75% moisture) and irradiation (50 KGy) on the production of mycelial biomass and protein with *Pleurotus sajor-caju.*

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (%)	PROTEIN CONTENT OF FINAL (C) (g/L)	GROWTH RATE dC/Cdt (1/h)	EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
0	6,915	9,545	0,660			
24	6,775	12,917	0,875			
36	6,345	14,039	0,891			
48	6,070	14,039	0,852			
62	5,340	16,517	0,882			
72	5,225	16,156	0,844			
84	4,910	17,914	0,880			
92	4,490	18,277	0,821			
114	4,735	18,875	0,894	0,006	15,580	2,050
132	5,345	17,416	0,931			

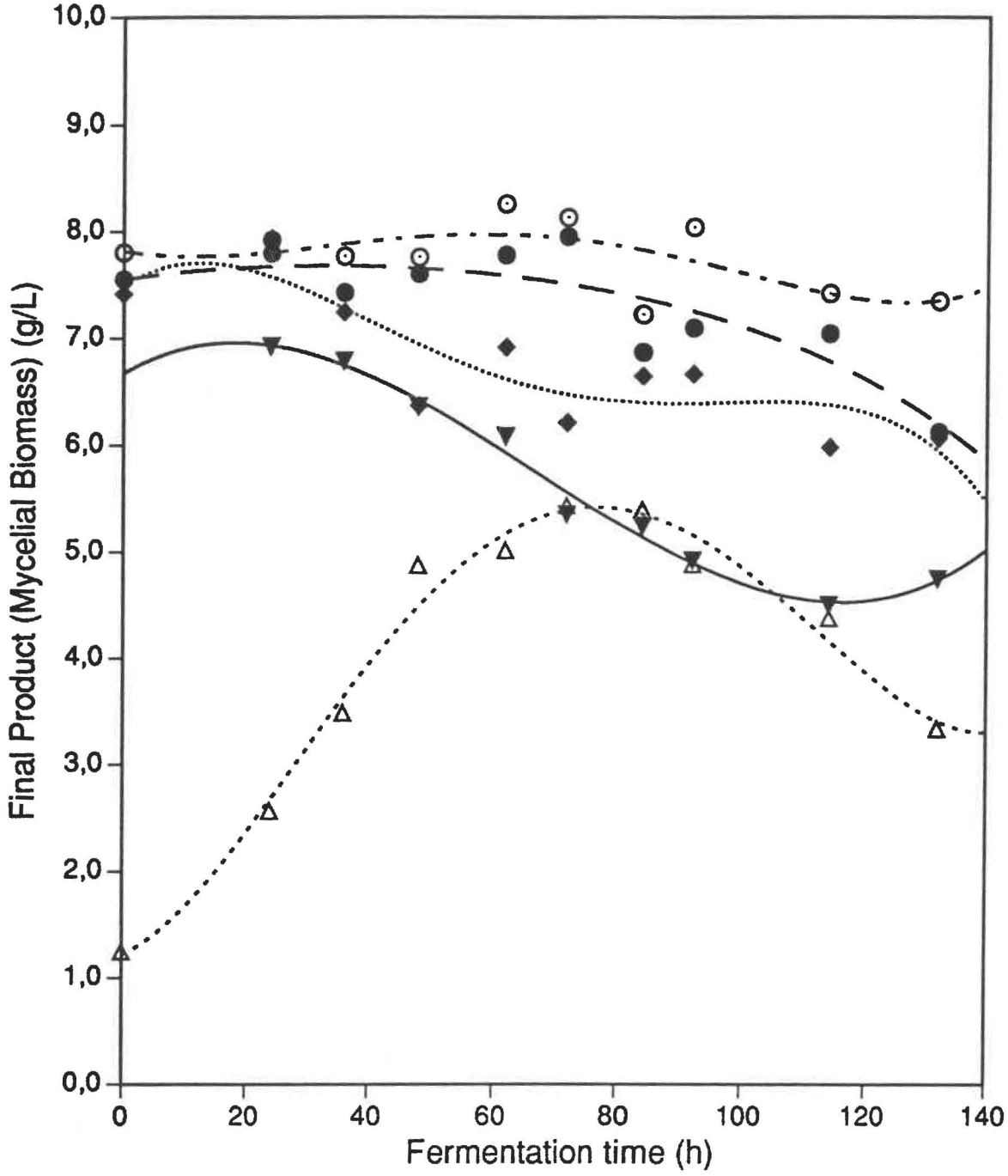
*FINAL PRODUCT: The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *Pleurotus sajor-caju.*

FIGURE 4-13: Combined effect of γ-irradiation (50 KGy) and NaOH treatment (0,025 - 0,1 g/g) of corn stalks for the production of mycelial biomass by *Pleurotus sajor-caju.*

FIGURE 4-14: Combined effect of γ-irradiation (50 KGy) and NaOH treatment (0,025 - 0,1 g/g) of corn stalks for the production of crude protein (g/L) by *Pleurotus sajor-caju.*

FIGURE 4-15: Combined effect of γ-irradiation (50 KGy) and NaOH treatment (0,025 - 0,1 g/g) of corn stalks for the production of crude protein (%) by *Pleurotus sajor-caju*

Fig. 4-13



0,025 g NaOH/g Subst. + 50 KGy

0,05 g NaOH/g Subst. + 50 KGy

0,1 g NaOH/g Subst. + 50 KGy

1% Glucose

1% Untreated Corn stalk

Fig. 4-14

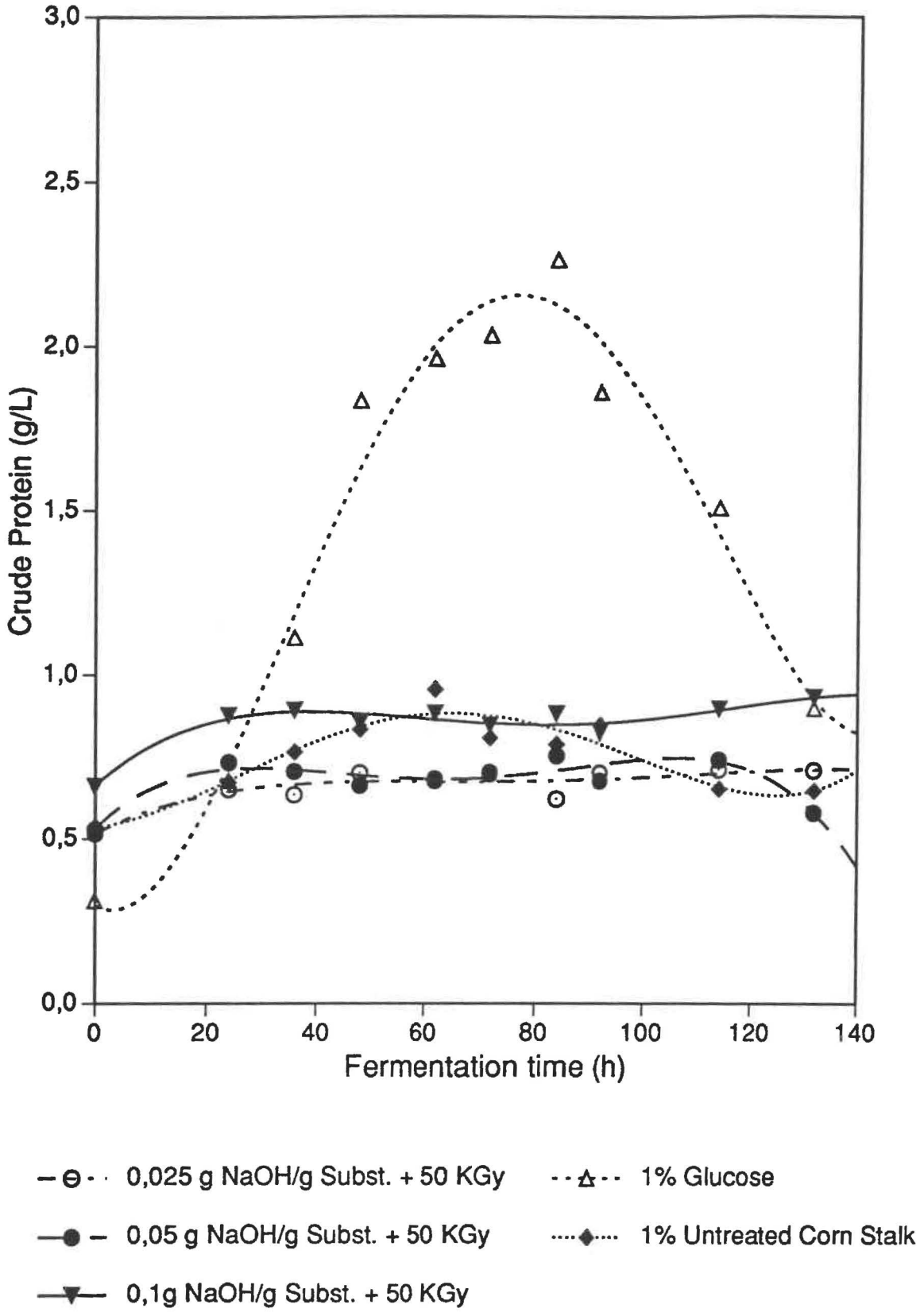
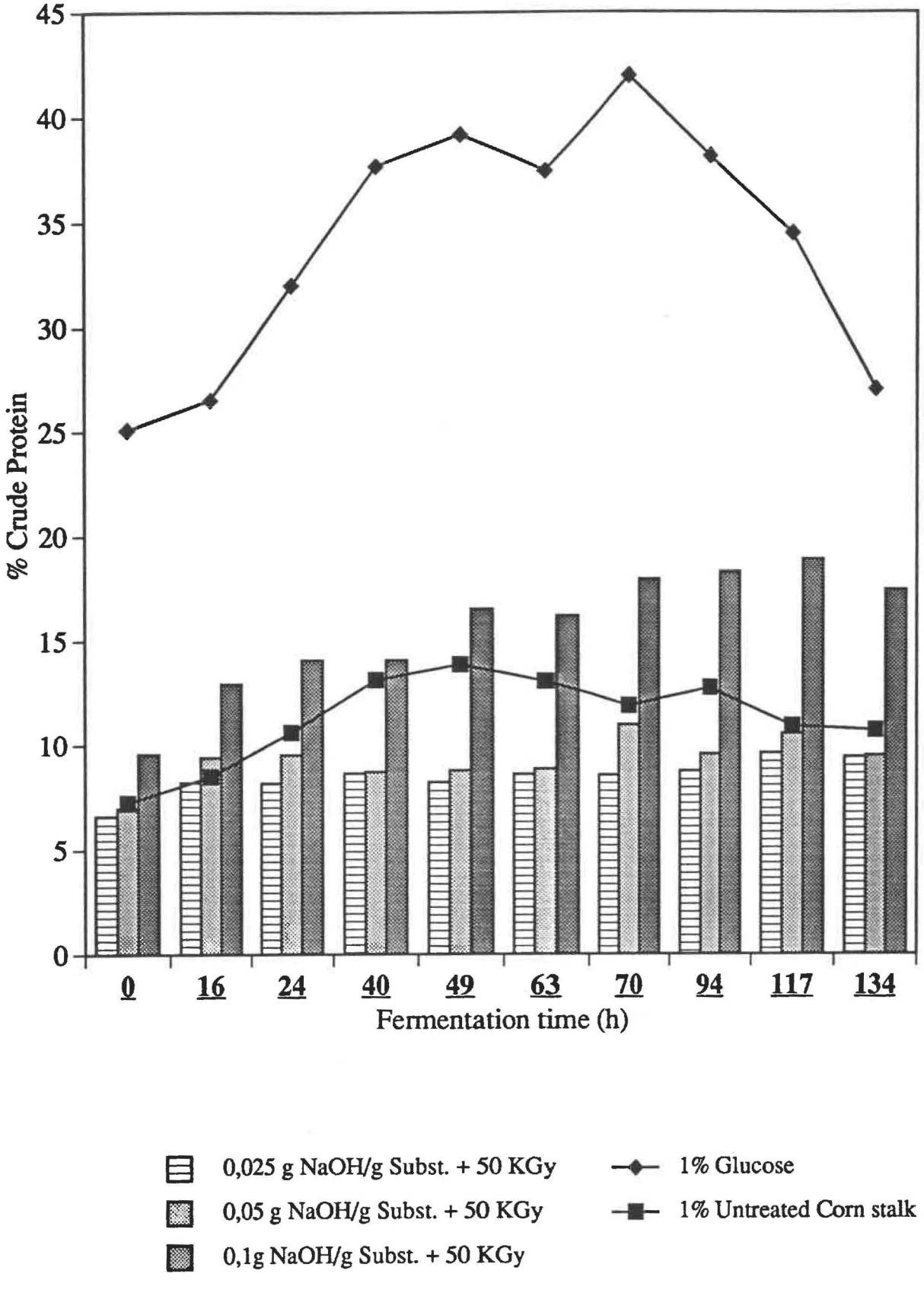

3,0
2,5
2,0
1,5
1,0
0,5
0,0
Crude Protein (g/L)
0
20
40
60
80
100
120
140
Fermentation time (h)
0,025 g NaOH/g Subst. + 50 KGy
1% Glucose
0,05 g NaOH/g Subst. + 50 KGy
1% Untreated Corn Stalk
0,1g NaOH/g Subst. + 50 KGy

Fig. 4-15


45
40
35
30
25
20
15
10
5
0
% Crude Protein
0
16
24
40
49
63
70
94
117
134
Fermentation time (h)
0,025 g NaOH/g Subst. + 50 KGy
0,05 g NaOH/g Subst. + 50 KGy
0,1g NaOH/g Subst. + 50 KGy
1% Glucose
1% Untreated Corn stalk

4.5.2. Combined effect of gamma irradiation (100 KGy) and NaOH (0,025-0,1 g/g substrate) pretreatments.

Tables 4-19 to 4-21 and Figures 4-16 to 4-18 show the results obtained from the production of mycelial biomass and protein with *P.sajor-caju* on 1% cornstalk pretreated with different concentrations of NaOH and 100 KGy γ-irradiation.

The combination treatment of 0,025 g NaOH/g corn stalk and 100 KGy resulted in a final product (mycelial biomass) with protein content of 14% (0,9 g protein L^{-1}) with conversion efficiency of 19% and protein productivity of 4,9 mg $L^{-1}h^{-1}$. When the NaOH concentration was increased to 0,05 g/g while maintaining the same irradiation dose, the protein content increased to 16% (1 g protein L^{-1}) with increased efficiency of 23% and protein productivity of 4,9 mg $L^{-1}h^{-1}$. At a higher NaOH concentration of 0,1 g/g substrate, the protein content doubled to 39% (1,6 g protein L^{-1}) with about 3 times increase in efficiency of conversion (63%) and protein productivity of 13,5 mg $L^{-1}h^{-1}$. There was however significant interaction ($p \leq 0,05$) with increase in NaOH concentration (appendix 2e). Fig. 4-17 clears shows that the interaction occured at the initial stages of the fermentation.

The trend here is similar to that recorded in section 4.5.1. The results indicate that increasing the concentration of NaOH at the γ-irradiation dose of 100 KGy, enhanced the susceptibility of the corn stalk polysaccharides for bioconversion into protein-rich mycelial biomass much more than using the same levels of NaOH concentration at the

lower irradiation dose of 50 KGy. The fact that increased NaOH concentration resulted in increased protein synthesis and also that when the NaOH concentration was constant and irradiation dose was increased, there was an increase in protein synthesis, shows that there was a synergistic effect of these pretreatments. This synergistic effect might have made the substrate more susceptible for its bioconversion into mycelial biomass having high protein content. Results obtained from the combination treatments were superior to those from their equivalent individual treatments. The results obtained from the combination pretreatment of 0,1 g NaOH/g and 100 KGy were comparable to results obtained from the single pretreatment of 0,15 g NaOH/g substrate.

The results support the view that in the presence of NaOH the concentration of soluble substances increases with irradiation. Duchacek and Bludusky (1979) reported a 50% increase in soluble substances when cellulose was irradiated in 0,1 N NaOH. In lignocelluloses, the increase in soluble matter may be a result of hemicelluloses breakdown. It has also been reported by Ehrlich and Han (1991) that in the presence of NaOH, gamma-initiated radical formation is enhanced.

It may be inferred from Fig. 4-16 that there was a faster decrease in the final product (mycelial biomass) as the concentration of NaOH was increased. This was evidently translated into higher protein synthesis as shown in Fig 4-16. An interesting trend beginning to appear in the protein profile is that as the quantities of combination treatments

increase, their protein profiles begin to resemble those obtained with glucose. Similar trends were observed in the single pretreatments but those for the combination pretreatments were more pronounced. Also, as the irradiation treatment increased, the lag phases also increased. This may be an indication of possible toxin production. However, the results seem to indicate that the oxidation products formed with the increased irradiation doses did not cause prolonged hindrance to the growth of *P. sajor-caju*. According to Duchacek and Bludusky (1979), malondialdehyde is one characteristic toxic product formed when irradiation is carried out under neutral or alkaline conditions.

Fig. 4-18 clearly shows that the percent protein at the combination pretreatments of 0,1 and 0,05 g/g NaOH and 100 KGy were superior to that of the untreated substrate and even to that of substrate treated with NaOH concentration of 0,1 g/g. In these cases, the crude protein levels were approaching those obtained from glucose. This is a marked improvement over the combination treatments of NaOH and 50 KGy irradiation.

Table 4-19 : Combined effect of NaOH treatment (0,025 g/g substrate, 75% moisture) and irradiation (100 KGy) on the production of mycelial biomass and protein with *Pleurotus sajor-caju.*

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C) (%)	(g/L)	GROWTH RATE dC/Cdt (1/h)	EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
0	7,655	8,322	0,637			
22	7,765	10,248	0,796			
30	7,145	11,008	0,787			
46	6,530	12,937	0,845			
58	6,615	13,948	0,923	0,006	19,044	4,925
70	6,455	13,395	0,865			
82	6,575	12,341	0,811			
94	6,070	12,212	0,741			
120	6,025	11,908	0,717			

*FINAL PRODUCT: The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *Pleurotus sajor-caju.*

Table 4-20 : Combined effect of NaOH treatment (0,05 g/g substrate, 75% moisture) and irradiation (100 KGy) on the production of mycelial biomass and protein with *Pleurotus sajor-caju.*

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (%)	PROTEIN CONTENT OF FINAL (C) (g/L)	GROWTH RATE dC/Cdt (1/h)	EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
0	7,805	8,408	0,656			
22	7,580	10,736	0,814			
30	7,220	12,137	0,876			
46	6,100	16,048	0,979			
58	6,215	16,252	1,010			
70	6,100	16,369	0,999	0,008	22,818	4,889
82	6,080	15,973	0,971			
94	5,995	15,367	0,921			
120	5,880	15,022	0,883			

*FINAL PRODUCT: The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *Pleurotus sajor-caju.*

Table 4-21 : Combined effect of NaOH treatment (0,1 g/g substrate, 75% moisture) and irradiation (100 KGy) on the production of mycelial biomass and protein with *Pleurotus sajor-caju.*

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT		GROWTH RATE dC/Cdt (1/h)	EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
		(%)	(C) (g/L)			
0	6,720	9,220	0,620			
22	6,520	14,984	0,977			
30	5,645	19,195	1,084			
46	3,820	33,789	1,291			
58	3,805	36,302	1,381			
70	4,030	38,909	1,568	0,013	63,230	13,549
82	3,725	34,495	1,285			
94	3,755	36,819	1,383			
120	3,565	30,414	1,084			

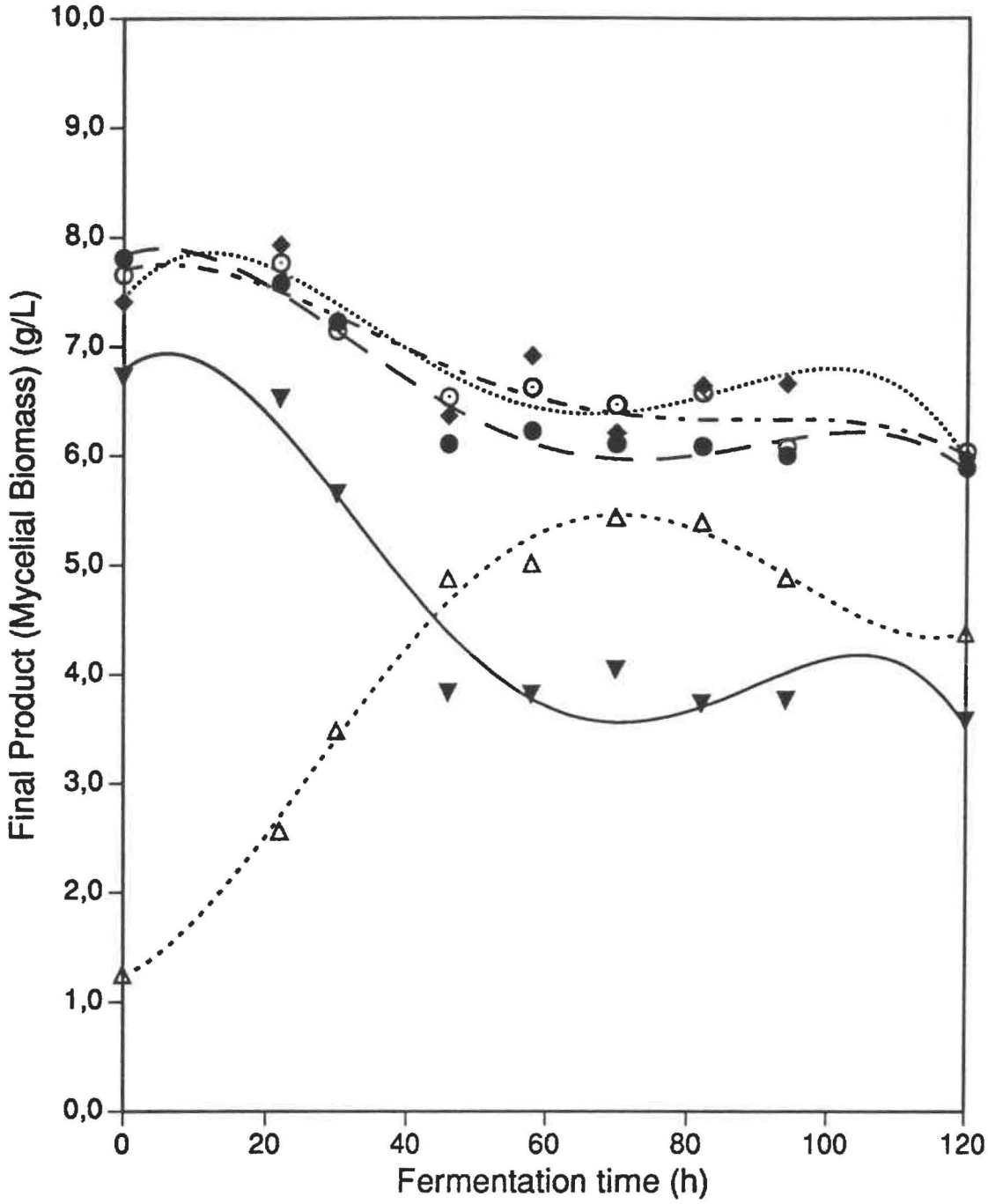
*FINAL PRODUCT: The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *Pleurotus sajor-caju.*

FIGURE 4-16: Combined effect of γ-irradiation (100 KGy) and NaOH treatment (0,025 - 0,1 g/g) of corn stalks for the production of mycelial biomass by *P. sajor-caju.*

FIGURE 4-17: Combined effect of γ-irradiation (100 KGy) and NaOH treatment (0,025 - 0,1 g/g) of corn stalks for the production of crude protein (g/L) by *P. sajor-caju.*

FIGURE 4-18: Combined effect of γ-irradiation (100 KGy) and NaOH treatment (0,025 - 0,1 g/g) of corn stalks for the production of crude protein (%) by *P. sajor-caju.*

Fig. 4-16



— ⊖ - 0,025 g NaOH/g Subst. + 100 KGy

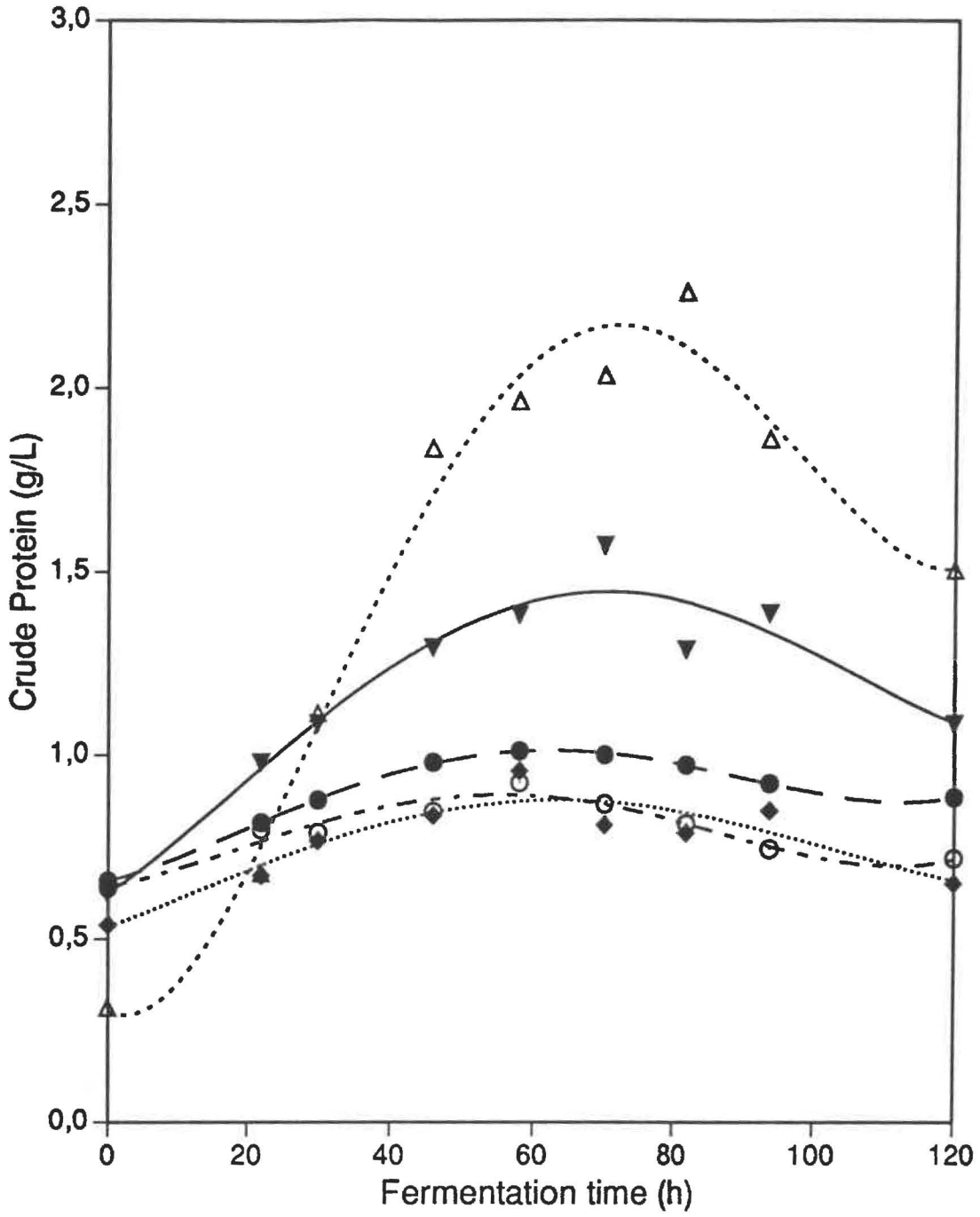
—● – 0,05 g NaOH/g Subst. + 100 KGy

—▼— 0,1 g NaOH/g Subst. + 100 KGy

- - △ - - 1% Glucose

····◆···· 1% Untreated Corn stalk

Fig. 4-17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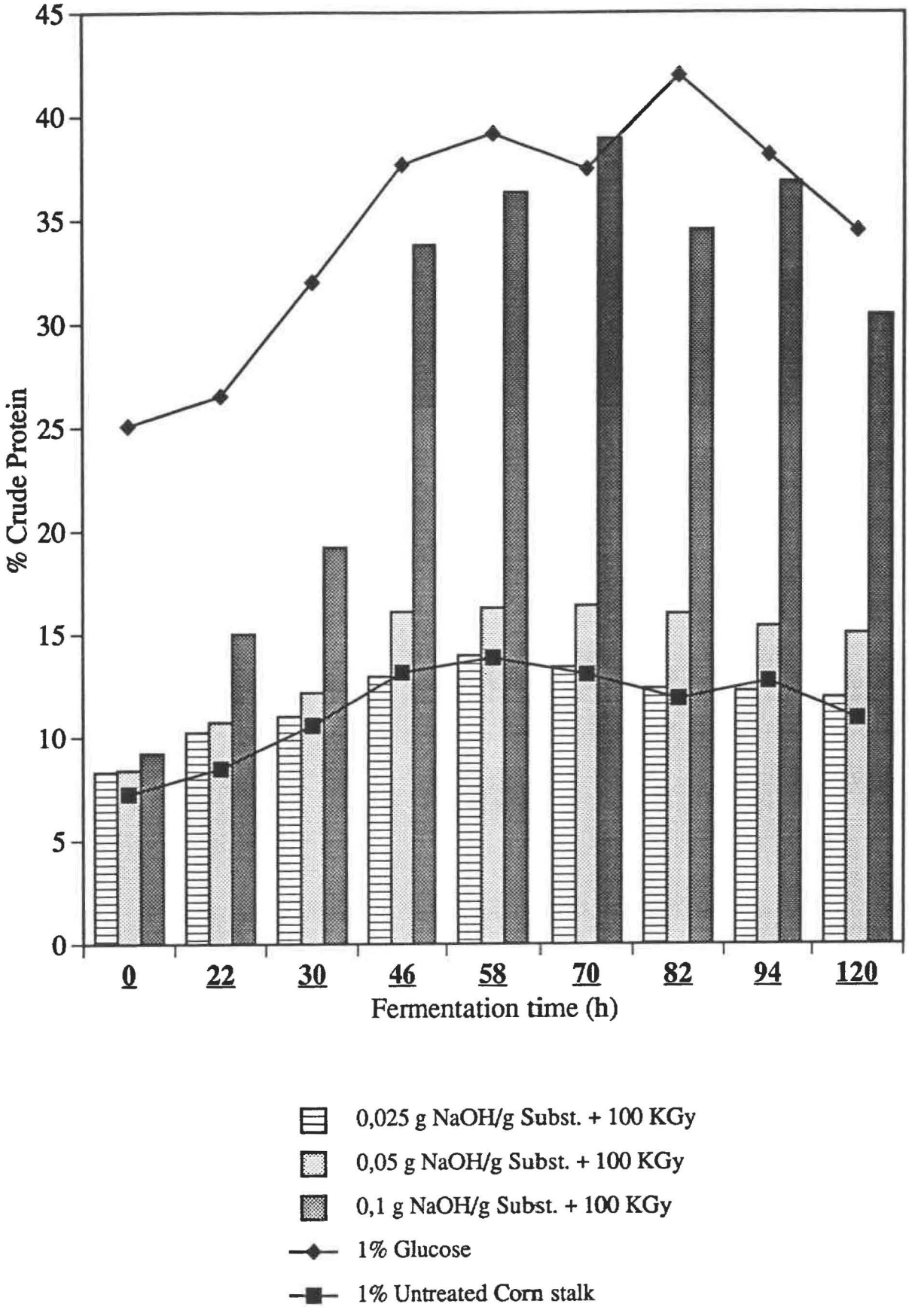
2,5
2,0
1,5
1,0
0,5
0,0
Crude Protein (g/L)
0
20
40
60
80
100
120
Fermentation time (h)
0,025 g NaOH/g Subst. + 100 KGy
0,05 g NaOH/g Subst. + 100 KGy
0,1 g NaOH/g Subst. + 100 KGy
1% Glucose
1% Untreated Corn Stalk

Fig. 4-18


45
40
35
30
25
20
15
10
5
0
% Crude Protein
0
22
30
46
58
70
82
94
120
Fermentation time (h)
0,025 g NaOH/g Subst. + 100 KGy
0,05 g NaOH/g Subst. + 100 KGy
0,1 g NaOH/g Subst. + 100 KGy
1% Glucose
1% Untreated Corn stalk

4.5.3. Combined effect of gamma irradiation (500 KGy) and NaOH (0,025-0,1 g/g substrate) pretreatments.

It is so far clear that combination treatments give superior protein yields than their individual treatments and that at gamma irradiation of 100 KGy and NaOH concentration of 0,05 g/g and above, protein yields from corn stalk begin to measure up to those obtained from glucose. Tables 4-22 to 4-24 and Figs. 4-19 to 4-21 indicate next what happens when the irradiation dose was increased to 500 KGy.

The combination pretreatment of 0,025 g NaOH/g corn stalk and 500 KGy produced final product (mycelial biomass) whose final product contained optimum protein of 17,5% (1,1 g protein L^{-1}) with a conversion efficiency of 29% and protein productivity of 7,3 mg $L^{-1}h^{-1}$. When the NaOH concentration was increased to 0,05 g/g substrate, the protein content was 40% (2,1 g protein L^{-1}) with conversion efficiency of 90,5% and protein productivity of 7,3 mg $L^{-1}h^{-1}$. These values were further increased respectively to 45% (2 g L^{-1}), 93% and 19,4 mg $L^{-1}h^{-1}$. It is remarkable to note here that at a NaOH concentration of 0,05 g/g substrate and above there was over 90% utilization of the corn stalk polysaccharides (Tables 4-23 and 4-24). The interaction ($p \leq 0,05$) (appendix 2f), shows that the observed effects were contributed largely by increase in radiation dose.

It indicated that irradiation pretreatment more than 100 KGy is required to make the lignocellulosic material susceptible for their bioconversion into protein. Aoki et al. (1977) reported that radiation

doses greater than 500 KGy cause a decrease in crystallinity in the cellulose molecules of lignocelluloses. Han and Ciegler (1982) also reported a decrease in crystallinity in sugarcane bagasse at radiation doses more than 100 KGy. In studying the effect of multiple pretreatments on the structural features of wheat straw, Gharpuray et al (1983) reported an increase in specific surface area, decrease in crystallinity index and increase in degree of delignification as a result of the multiple effects of caustic soda and mechanical milling. All these enhanced features of increased surface area, decreased crystallinity and delignification appear to be more pronounced at the combination treatment of 500 KGy and 0,1 g NaOH/g subtrate pretreatment.

Figs. 4-19 and 4-20 indicate that as the final product (mycelial biomass) rapidly decreased with fermentation time, the amount of protein synthesized increased. Fig. 4-21 clearly shows that at the combination treatment of 0,1 g NaOH/g and 500 KGy and fermentation time of at least 60h, the percent protein was more than that obtained from glucose.

Table 4-21 : Combined effect of NaOH treatment (0,1 g/g substrate, 75% moisture) and irradiation (100 KGy) on the production of mycelial biomass and protein with *Pleurotus sajor-caju.*

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		GROWTH RATE dC/Cdt (1/h)	EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
		(%)	(g/L)			
0	6,720	9,220	0,620			
22	6,520	14,984	0,977			
30	5,645	19,195	1,084			
46	3,820	33,789	1,291			
58	3,805	36,302	1,381			
70	4,030	38,909	1,568	0,013	63,230	13,549
82	3,725	34,495	1,285			
94	3,755	36,819	1,383			
120	3,565	30,414	1,084			

*FINAL PRODUCT: The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *Pleurotus sajor-caju.*

Table 4-23 : Combined effect of NaOH treatment (0,05 g/g substrate, 75% moisture) and irradiation (500 KGy) on the production of mycelial biomass and protein with *Pleurotus sajor-caju.*

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT		GROWTH RATE dC/Cdt (1/h)	EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
		(%)	(C) (g/L)			
0	7,550	9,300	0,702			
12	0,000	10,131	0,000			
24	3,575	12,225	0,437			
34	6,785	14,547	0,987			
48	6,325	19,294	1,220			
60	5,320	30,361	1,615			
72	5,165	31,633	1,634			
96	5,165	39,889	2,060	0,012	90,541	14,147
120	2,450	37,041	0,908			

*FINAL PRODUCT: The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *Pleurotus sajor-caju.*

Table 4-24 : Combined effect of NaOH treatment (0,1 g/g substrate, 75% moisture) and irradiation (500 KGy) on the production of mycelial biomass and protein with *Pleurotus sajor-caju.*

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		GROWTH RATE dC/Cdt (1/h)	EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
		(%)	(g/L)			
0	6,450	9,680	0,624			
12	6,753	11,964	0,808			
24	6,540	14,625	0,956			
34	5,882	17,998	1,059			
48	5,337	34,559	1,844			
60	4,507	41,052	1,850			
72	4,498	44,909	2,020	0,017	93,043	19,384
96	4,212	42,350	1,784			
120	3,835	41,376	1,587			

*FINAL PRODUCT: The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *Pleurotus sajor-caju.*

FIGURE 4-19: Combined effect of γ-irradiation (500 KGy) and NaOH treatment (0,025 - 0,1 g/g) of corn stalks for the production of mycelial biomass by *Pleurotus sajor-caju.*

FIGURE 4-20: Combined effect of γ-irradiation (500 KGy) and NaOH treatment (0,025 - 0,1 g/g) of corn stalks for the production of crude protein (g/L) by *Pleurotus sajor-caju.*

FIGURE 4-21: Combined effect of γ-irradiation (500 KGy) and NaOH treatment (0,025 - 0,1 g/g) of corn stalks for the production of per cent crude protein by *Pleurotus sajor-caju.*

Fig. 4-19

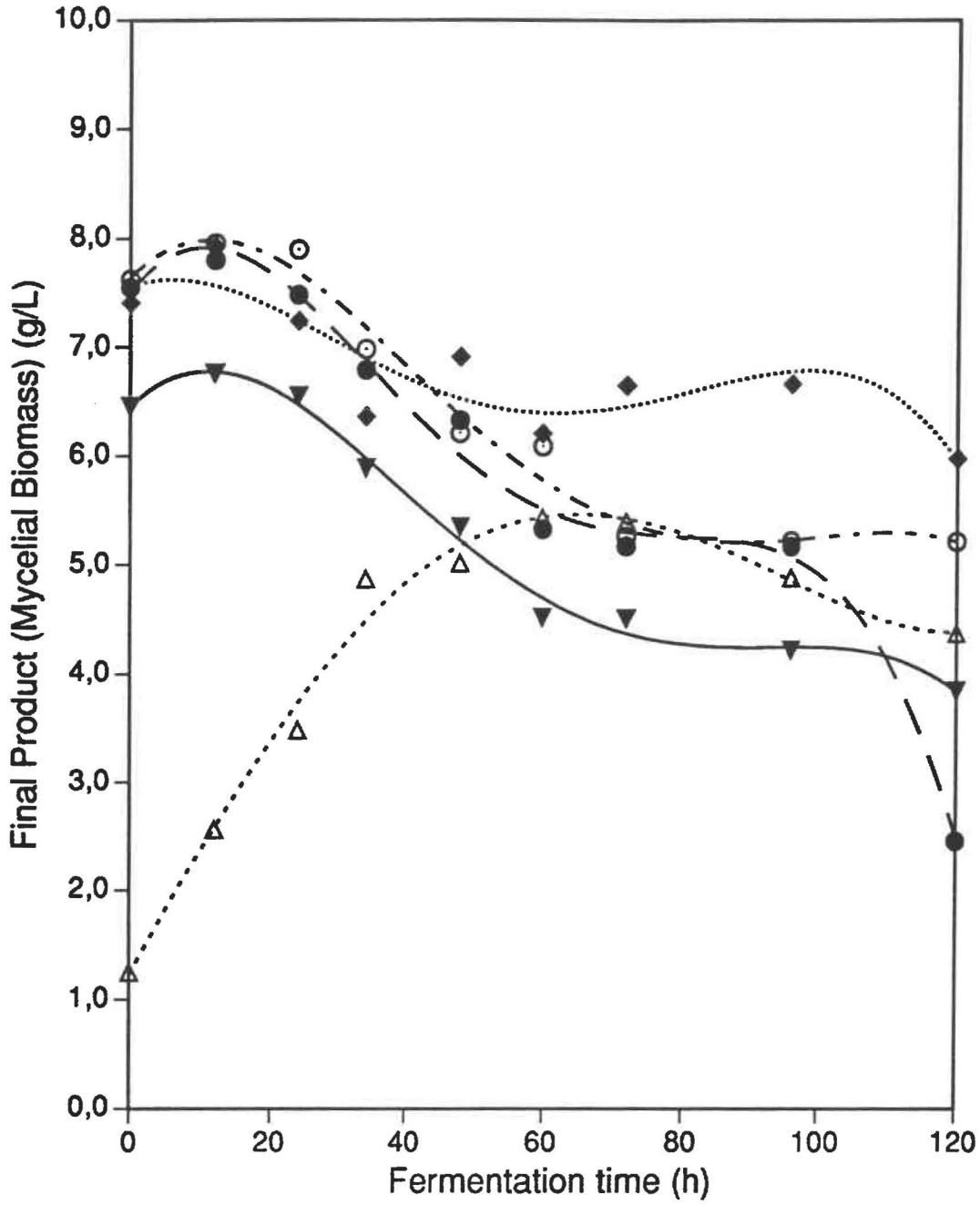
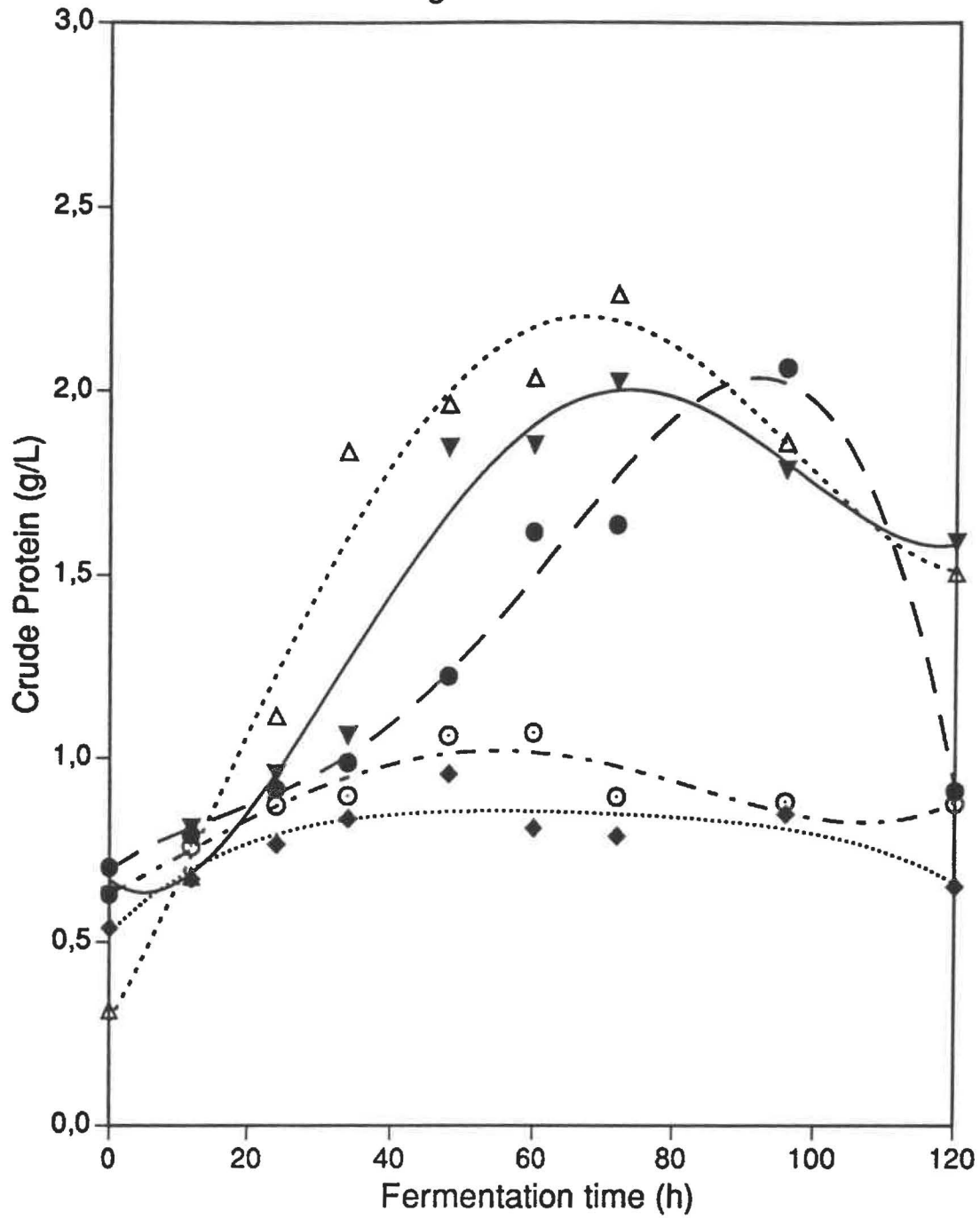

10,0
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
Final Product (Mycelial Biomass) (g/L)
0
20
40
60
80
100
120
Fermentation time (h)
0,025 g NaOH/g Subst. + 500 KGy
1% Glucose
0,05 g NaOH/g Subst. + 500 KGy
1% Untreated Corn stalk
0,1 g NaOH/g Subst. + 500 KGy

Fig. 4-20



0,025 g NaOH/g Subst. + 500 KGy

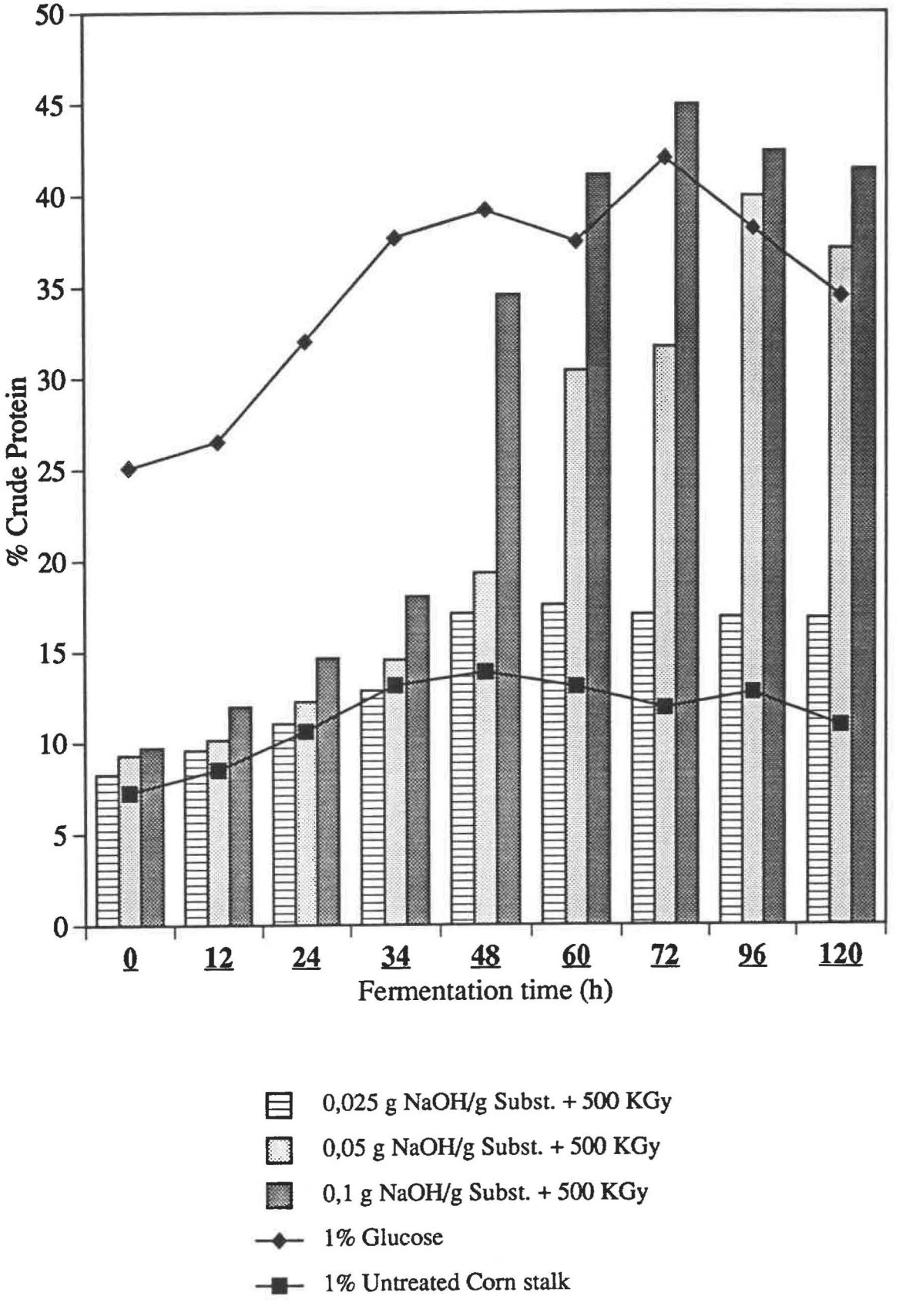
0,05 g NaOH/g Subst. + 500 KGy

0,1 g NaOH/g Subst. + 500 KGy

1% Glucose

1% Untreated Corn Stalk

Fig. 4-21


50
45
40
35
30
25
20
15
10
5
0
% Crude Protein
0
12
24
34
48
60
72
96
120
Fermentation time (h)
0,025 g NaOH/g Subst. + 500 KGy
0,05 g NaOH/g Subst. + 500 KGy
0,1 g NaOH/g Subst. + 500 KGy
1% Glucose
1% Untreated Corn stalk

4.5.4. Combined effect of gamma irradiation (1 MGy) and NaOH (0,01 and 0,05 g/g substrate) pretreatments.

This section was aimed at finding out to what extent the amount of sodium hyroxide can be reduced with high irradiation dose for the production of mycelial biomass and protein with *P. sajor-caju.*

The results shown on Tables 4-25 and 4-26 indicate that the combination of very low concentration of NaOH (0,01 g/g) and 1 MGy irradiation did not improve the production of mycelial biomass and protein as compared to that of the single treatment with irradiation at 1 MGy (Table 4-10). When the concentration of NaOH was increased to 0,05 g/g the results were just comparable to those obtained with the combination treatment of 500 KGy and 0,05 g/g NaOH (Table 4-23). There was significant interaction ($p \leq 0,05$) (appendix 2g) to suggest that at 1 MGy, increasing NaOH concentration becomes more meaningful from 60 - 80h fermentation time (Fig. 4-23).

These results may, therefore, seem to suggest that increasing γ-irradiation beyond 500 KGy with low concentrations of NaOH did not improve the production of mycelial biomass and protein by *P. sajor-caju.*

However, it is clear from Tables 4-25 and 4-26 and from their corresponding Figures 4-22 and 4-23 that when the concentration of NaOH was increased 5-fold from 0,01 to 0,05 g/g the optimum protein synthesized was increased nearly 3-fold. But when the results of the

combination treatment of 0,05 g/g NaOH and 1 MGy are compared to their individual treatments (tables 4-10 and 4-13), it was found that at their optimum protein synthesis levels, the per cent protein from the combination treatment was about twice that obtained from the individual treatments. This may go further to support the earlier suggestion that in using NaOH as a pretreatment, threshold values may exist and that there obviously was synergy between irradiation and NaOH treatments in the production of mycelial biomass of *P. sajor-caju.*

Table 4-25 : Combined effect of NaOH treatment (0,01 g/g substrate, 75% moisture) and irradiation (1 MGy) on the production of mycelial biomass and protein with *Pleurotus sajor-caju.*

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		GROWTH RATE dC/Cdt (1/h)	EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
		(%)	(g/L)			
0	6,790	7,744	0,526			
12	6,890	13,057	0,900			
24	6,510	13,275	0,864			
36	5,940	13,356	0,793			
48	5,810	13,344	0,775			
60	5,620	14,288	0,803	0,021	18,477	4,620
72	5,830	13,538	0,789			
96	5,700	14,138	0,806			
120	5,350	14,575	0,780			
144	5,270	14,194	0,748			

*FINAL PRODUCT The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *Pleurotus sajor-caju.*

Table 4-26 : Combined effect of NaOH treatment (0,05 g/g substrate, 75% moisture) and irradiation (1 MGy) on the production of mycelial biomass and protein with *Pleurotus sajor-caju.*

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		GROWTH RATE dC/Cdt (1/h)	EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
		(%)	(g/L)			
0	6,120	8,944	0,547			
24	5,990	9,294	0,557			
45	6,180	16,157	0,998			
51	4,400	20,038	0,882			
57	5,000	28,053	1,403			
69	5,140	32,103	1,650			
76	3,770	37,375	1,409			
93	4,350	40,669	1,769	0,014	81,449	13,140
117	3,700	37,800	1,399			

*FINAL PRODUCT: The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *Pleurotus sajor-caju.*

FIGURE 4-22: Combined effect of γ-irradiation (1 MGy) and NaOH treatment (0,01 and 0,05 g/g) of corn stalks for the production of mycelial biomass by *Pleurotus sajor-caju.*

FIGURE 4-23: Combined effect of γ-irradiation (1 MGy) and NaOH treatment (0,01 and 0,05 g/g) of corn stalks for the production of crude protein by *Pleurotus sajor-caju.*

Fig. 4-22

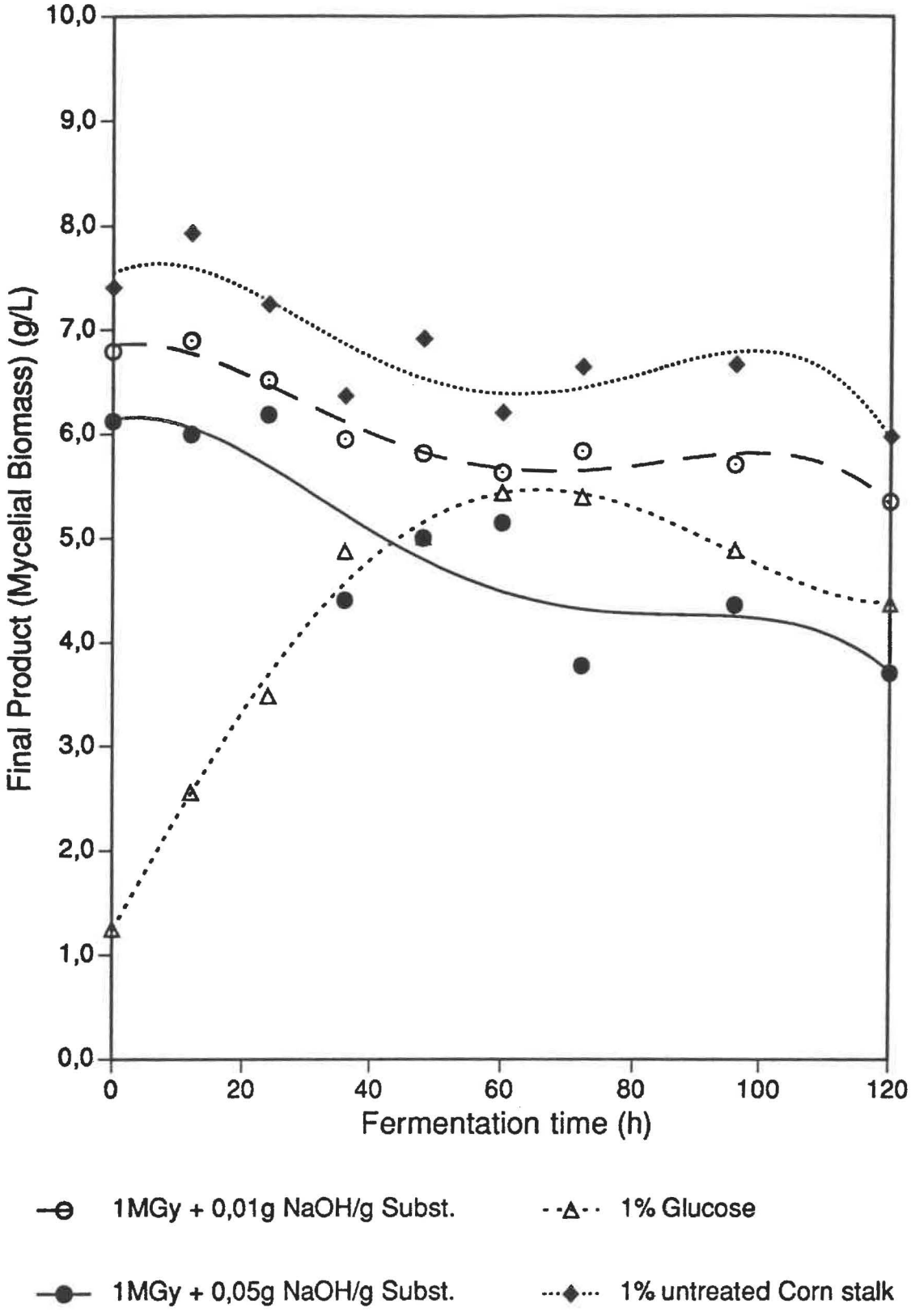
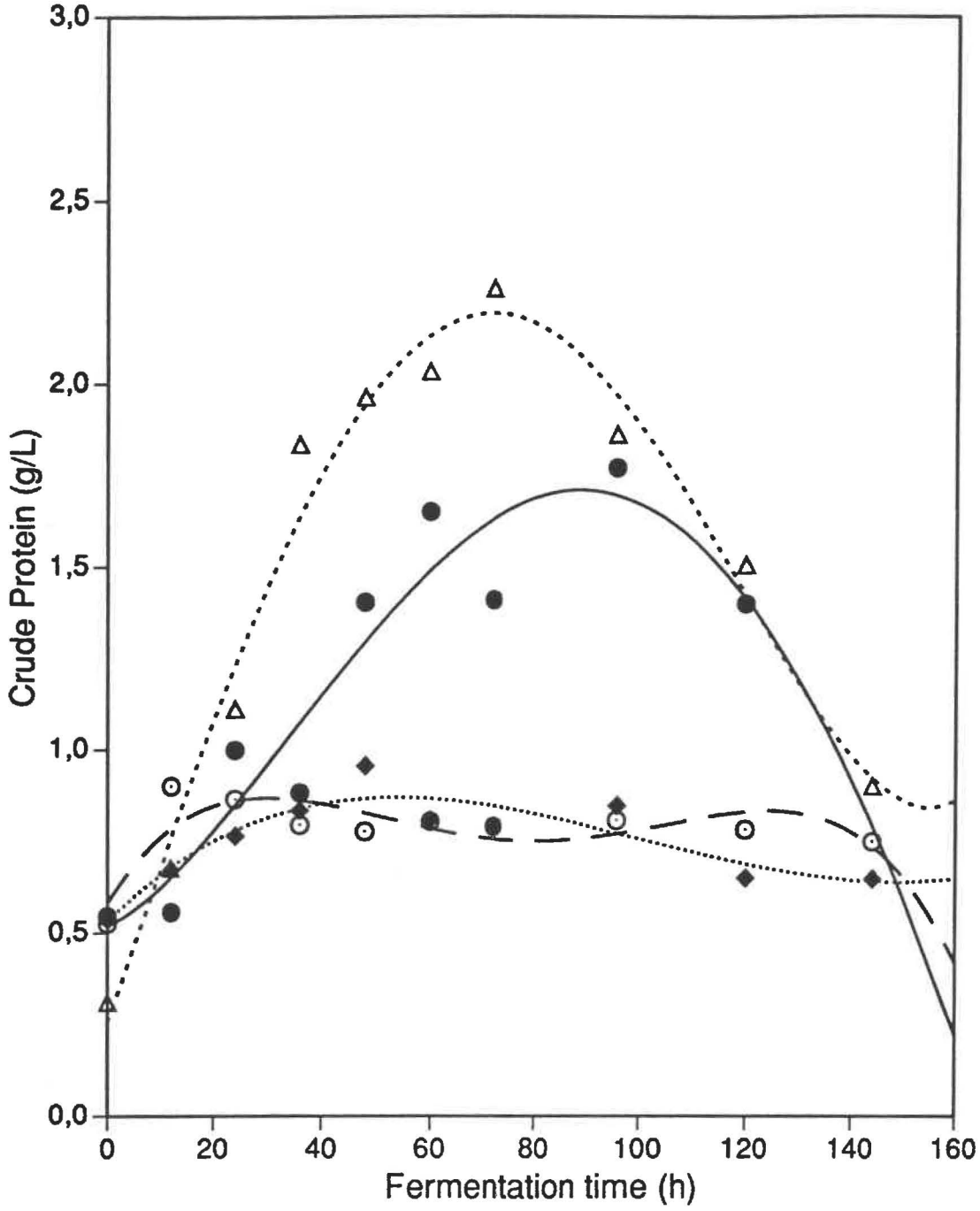

10,0
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
Final Product (Mycelial Biomass) (g/L)
0
20
40
60
80
100
120
Fermentation time (h)
1MGy + 0,01g NaOH/g Subst.
1% Glucose
1MGy + 0,05g NaOH/g Subst.
1% untreated Corn stalk

Fig. 4-23


3,0
2,5
2,0
1,5
1,0
0,5
0,0
Crude Protein (g/L)
0
20
40
60
80
100
120
140
160
Fermentation time (h)
1MGy + 0,01g NaOH/g Subst.
1% Glucose
1MGy + 0,05g NaOH/g Subst.
1% Untreated Corn stalk

4.5.5. Assessment of the overall effectiveness of the combination pretreatments for mycelial biomass and protein production by *Pleurotus sajor-caju.*

Combination pretreatments have been found here to enhance the susceptibility of corn stalk holocellulose for *in situ* enzymatic attack and bioconversion of *P. sajor-caju* into mycelial biomass rich in protein.

Although the structural features of the substrate like specific surface area, crystallinity index and lignin content were not measured to determine degree of pretreatments, an empirical reference can be drawn from the mycelial biomass and protein production. The progressive increase in the mycelial biomass content indicated that the structural parameters of the corn stalk were significantly modified at the different combination treatment conditions.

Our results would then suggest that the combination pretreatments of 500 KGy and 0,1 g NaOH/g or 100 KGy and 0,1 g NaOH/g substrate which gave good protein yields, effectively enhanced these parameters ($p \leq 0,05$) for high protein synthesis over the use of 0,15 g NaOH/g substrate alone.(appendix 2m). However, the efficiency of bioconversion was not significantly different between samples treated with 0,15 g NaOH/g substrate and those with the combination treatment of 100 KGy and 0,1 g NaOH/g substrate.

4.6. Effect of steam pretreatment of corn stalk on the production of mycelial biomass and protein with *Pleurotus sajor-caju.*

The results obtained from the production of mycelial biomass and protein with *P.sajor-caju* from steam pretreated substrate are presented in Tables 4-27 to 4-29 and corresponding Figures 4-24 and 4-25. Pretreatment of substrate with steam alone produced protein yields comparable to treatment with irradiation alone of the substrate at 1 MGy or at 0,05 g NaOH/g substrate (section 4.3 and 4.4) . However, when the steam treatment was combined with sodium sulphite or with sodium sulphite and NaOH, the amount of protein synthesized was comparable to any of the good pretreatments like the single treatment at 0,15 g NaOH/g substrate (section 4.4) or the combination treatment of 500 KGy γ-irradiation and 0,1 g NaOH/g substrate (section 4.5.3).

Tables 4-28 and 4-29 indicate that the amount of protein obtained from substrate treated with 8% Sodium sulphite and steam was lower than that obtained from 8% Sodium sulphite plus 0,5% sodium hydroxide and steam ($p \leq 0,05$) (appendix 2j). This trend is more clearly visualized in Figure 4-25. Though steaming substrate leads to increased pore size and increased enzymatic digestiblity (MacDonald and Mathews,1979), it is also now clear that there is much improvement in these features when cooking of substrate is initially

done with either sodium sulphite or with sodium sulphite and sodium hydroxide before the steaming process.

From Figure 4-25, it is also clear that the lag phases are increased to some extent with steam pretreatment. It has been suggested by Neese et al.,1977 that the residence time at high temperature during steam treatment be kept low to minimize reactions to reduce the production of inhibitory compounds. The exposure time of 4 minutes at 190°C used in this study seems not to produce many inhibitory compounds except for some increase in the initial adaptive phase for the growth of *P. sajor-caju.*

It may also be pointed out here that there was an increase in bulk density as a possible result of loss of integrity of cellulose fibres during the steaming process (Lyons and Kelsall, 1991).

Table 4-27 : Production of mycelial biomass and protein with *Pleurotus sajor-caju* on 1% corn stalks pretreated with steam only.

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
		(%)	(g/L)		
0	10,450	6,180	0,646		
22	10,990	6,737	0,740		
34	10,645	9,194	0,979		
47	9,120	10,216	0,932		
54	6,985	15,347	1,072		
72	6,820	17,969	1,225		
82	6,460	20,480	1,323	45,147	8,259
94	6,420	20,653	1,326		
106	3,575	16,618	0,594		
118	6,245	15,049	0,940		

*FINAL PRODUCT: The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass in the final product increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *P. sajor-caju*.

Table 4-28 : Production of mycelial biomass and protein with *Pleurotus sajor-caju* on 1% corn stalks pretreated with 8% sodium sulphite and steam only.

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
		(%)	(g/L)		
0	10,235	4,409	0,451		
22	8,075	7,314	0,591		
34	7,340	13,425	0,985		
47	6,245	23,698	1,480		
54	4,640	35,432	1,644		
72	4,220	42,252	1,783	88,785	18,497
82	3,995	36,567	1,461		
94	3,615	36,197	1,309		
106	3,525	36,524	1,287		
118	3,489	32,268	1,126		

*FINAL PRODUCTThe final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass in the final product increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *P. sajor-caju*.

Table 4-29 : Production of mycelial biomass and protein with *Pleurotus sajor-caju* on 1% corn stalks pretreated with 8% sodium sulphite and 0,5% NaOH and steam.

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		EFFICIENCY E = C-Co/So	PRODUCTIVITY P = C-Co/t-to
		(%)	(g/L)	(%)	(mg/L.h)
0	11,470	4,196	0,481		
22	8,615	6,707	0,578		
34	7,470	14,646	1,094		
47	6,970	24,639	1,717		
54	5,115	33,778	1,728		
72	5,060	37,062	1,875		
82	4,620	39,657	1,832		
94	4,575	42,487	1,944	91,065	14,532
106	3,770	36,382	1,372		
118	3,678	35,436	1,303		

*FINAL PRODUCT: The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass in the final product increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *P. sajor-caju.*

FIGURE 4-24: Production of mycelial biomass of *Pleurotus sajor-caju* on 1% substrate pretreated with steam only; 8% sodium sulphite + steam; and 8% sodium sulphite + 0,5% NaOH + steam.

FIGURE 4-25: Crude protein content of mycelial biomass of *Pleurotus sajor-caju* on 1% substrate pretreated with steam only; 8% sodium sulphite + steam; and 8% sodium sulphite + 0,5% NaOH + steam.

Fig. 4-24

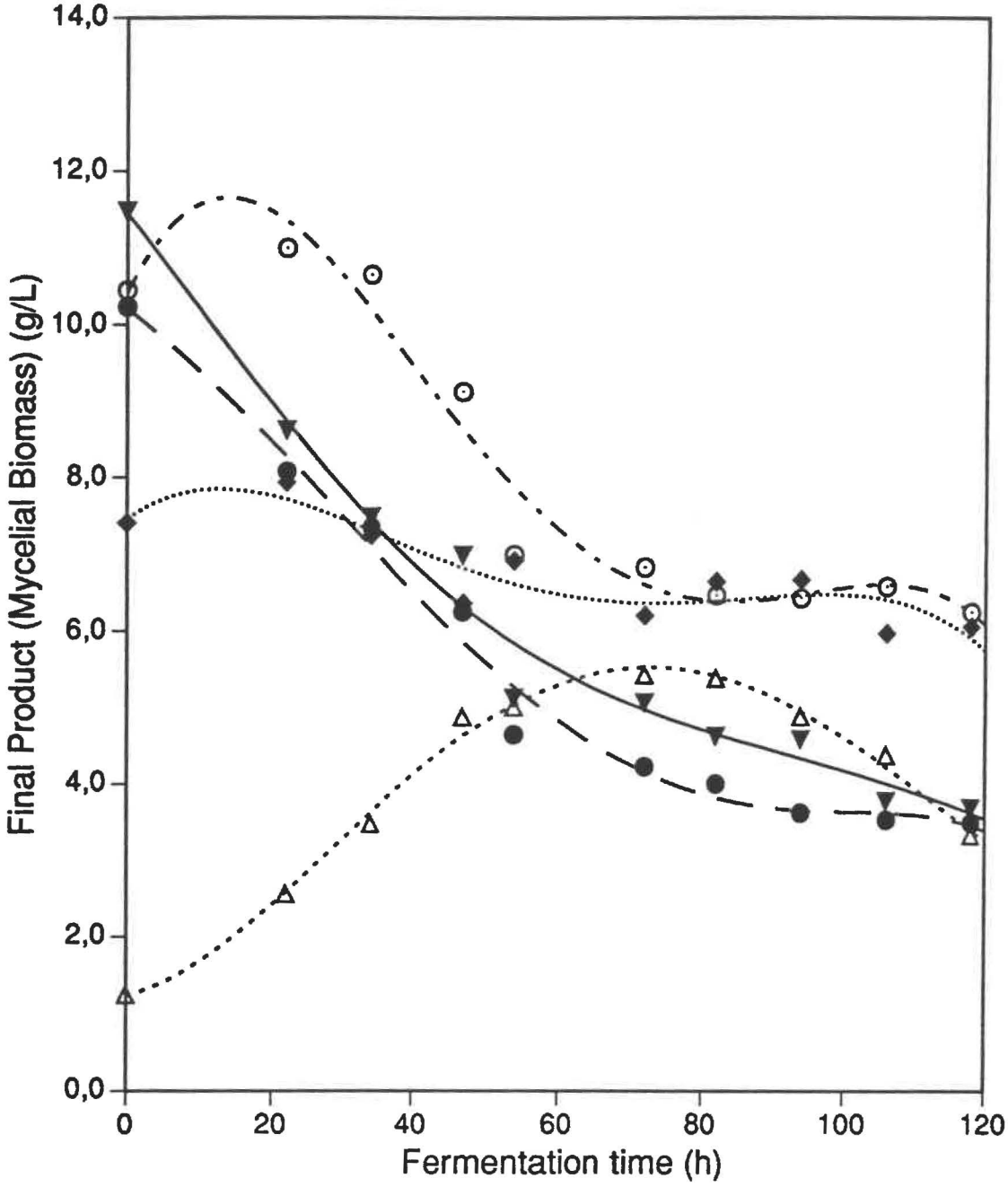
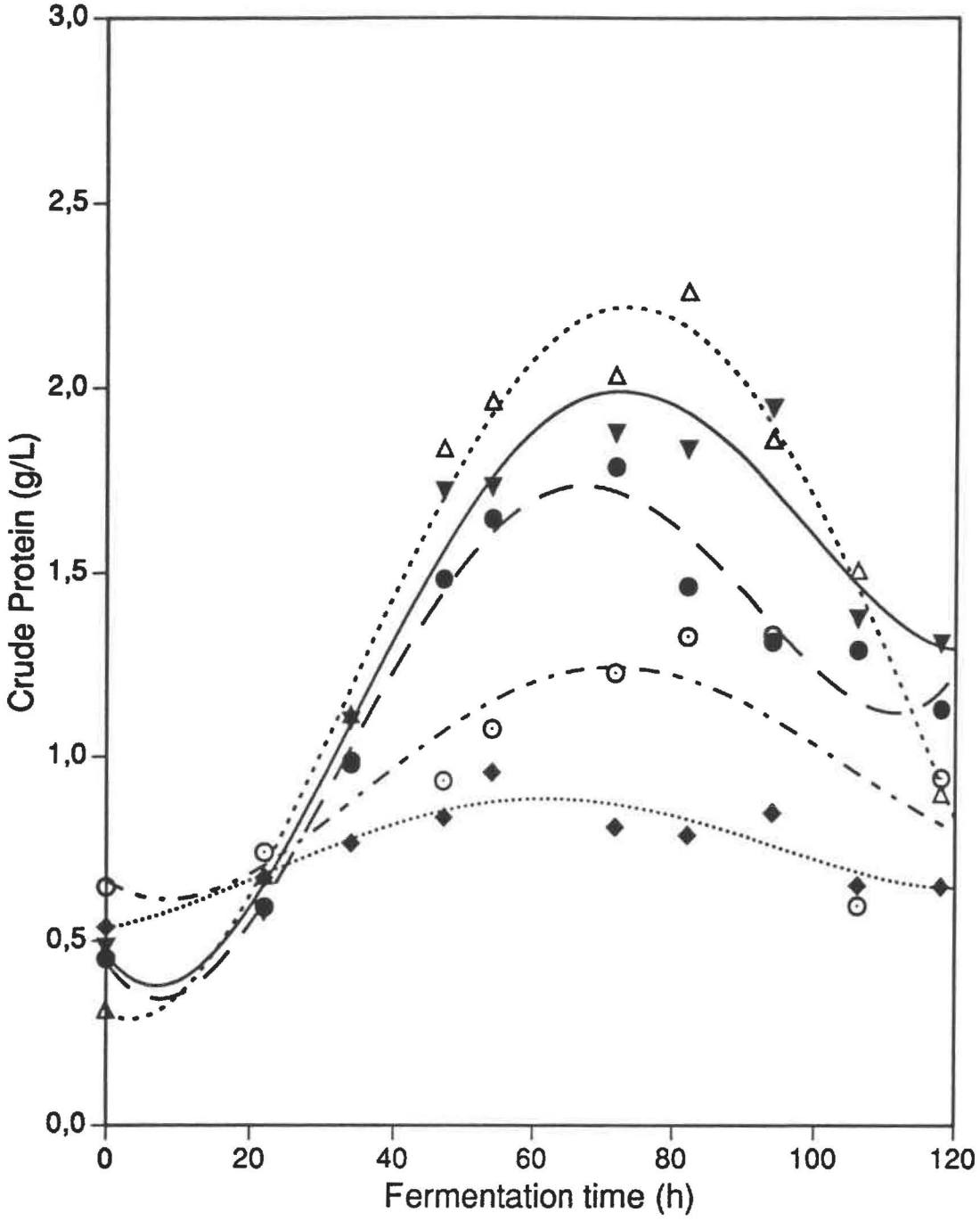

14,0
12,0
10,0
8,0
6,0
4,0
2,0
0,0
Final Product (Mycelial Biomass) (g/L)
0
20
40
60
80
100
120
Fermentation time (h)
steam only
8% Sodium sulphite and steam
8% Sodium sulphite + 0,5% NaOH + steam
1% Glucose
1% untreated Corn stalk

Fig. 4-25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2,5
2,0
1,5
1,0
0,5
0,0
Crude Protein (g/L)
0
20
40
60
80
100
120
Fermentation time (h)
Steam only
8% Sodium sulphite + Steam
8% Sodium sulphite + 0,5% NaOH + Steam
1% Glucose
1% Untreated Corn stalk

4.7. Effect of the different fractional components of corn stalk in the production of mycelial biomass and protein with *Pleurotus sajor-caju.*

Up to this point, our results have demonstrated the ability of *P.sajor-caju* to grow in the unfractionated mixture of cellulose, hemicelluloses and lignin of corn stalks after various pretreatments. This section assesses the performance of *P.sajor-caju* in the production of mycelial biomass and protein on the different components namely, cellulose and hemicelluloses, respectively.

Table 4-30 shows that up to 50% and 2,7 g L^{-1} protein with a conversion efficiency of over 95% and protein productivity of 27 mg $L^{-1}h^{-1}$ were obtained from the cellulose fraction of corn stalk. The corresponding results obtained from the hemicelluloses fraction were: 49%, 1,4 g L^{-1}; 32% and 14 mg $L^{-1}h^{-1}$ as presented in Table 4-31. For the protein synthesis and efficiency of conversion, there is significant interaction ($p \leq 0,05$) during the fermentation of these substrates. The productivity ($p \leq 0,05$) was evidently higher for the cellulose fraction than for the hemicelluloses fraction (appendix 2k).

Figures 4-26 to 4-28 illustrate the relationship between these different fractions and glucose in the production of mycelial biomass rich in protein. For instance, Figure 4-26 which presents the dry weight of the final product (mycelial biomass), demonstrates that in

the course of fermentation, the dry weight of hemicelluloses mycelial product increased whereas that for the cellulose decreased with the progress of growth of *P. sajor-caju*. This is due to the fact that hemicelluloses like glucose are low molecular weight compounds and are soluble in water. The low molecular weight soluble matter of hemicelluloses passes through filter paper and only mycelium used as inoculum is retained as residue when the samples of zero hours were filtered. This is translated as low initial weight of the final product (Fig. 4-23). During the course of fermentation, there is gain in weight due to the production of protein rich mycelial biomass. On the other hand, cellulose is crystalline, amorphous insoluble matter which is retained on filter paper to result in the high initial weight of the final product (Fig. 4-26). This insoluble matter (cellulose) is, however, converted into protein-rich mycelial biomass of *P. Sajor-caju* which results in continuous decrease in weight of the final product as explained in section 4.1.2.

From Figure 4-27, it is observed that the crude protein synthesized from cellulose far exceeds those of glucose and hemicelluloses. The lag phase for the cellulose substrate was longer than that on the hemicelluloses. This is reflected in the percent protein graph of Figure 4-28. In this presentation, protein production the early stages was higher for hemicelluloses than for cellulose which is understandable since the hemicelluloses are more soluble and hence much more readily utilizable than the cellulose. It is also further shown that after 66h of fermentation, both the hemicelluloses and

cellulose substrates produce higher per cent protein than glucose as substrate. The gradual decline of protein synthesis after 66h for hemicelluloses and after 100h for cellulose seems to be a result of depletion of substrate. Other factors which could have contributed to the decline include autolysis (Cochraine,1958)

A slight comparison of protein synthesis from the fractionated and non fractionated portions reveals that the per cent protein levels in the final product were comparable, whereas the efficiencies of bioconversion and productivities were different. Since the fractionated substrate was treated with NaOH 0,15 g/g substrate, the results are compared with those obtained from substrate treated with same concentration of NaOH. The comparison indicates that the efficiencies of substrate bioconversion into mycelial biomass protein and protein productivity were highest for cellulose followed by the unfractionated mixture (section 4.4) and then by hemicellulose. The trend makes sense since both the unfractionated mixture and the hemicellulose portion contain lignin whereas the cellulose fraction does not. Feist et al.,1970 and Wilson and Pidgen,1984, established that the lesser the lignin content of lignocelluloses, the higher the susceptibility to enzymatic hydrolysis and *in vitro* digestibility. The efficiencies and productivities were also slightly higher for the unfractionated mixture than for the hemicelluloses possibly because of binding of residual lignin to the hemicelluloses in the hemicelluloses and lignin fraction.

Table 4-30 : Production of mycelial biomass and protein with *Pleurotus sajor-caju* on 1% cellulose fraction of corn stalk.

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
		(%)	(g/L)		
0	10,610	3,237	0,343		
18	9,870	4,230	0,418		
42	7,200	17,052	1,228		
50	7,050	27,147	1,914		
66	6,160	43,912	2,705		
74	5,030	50,696	2,550		
90	5,490	49,793	2,734	95,608	26,558
100	5,010	50,713	2,541		
118	5,400	43,383	2,343		

*FINAL PRODUCT: The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *Pleurotus sajor-caju.*

Table 4-31 : Production of mycelial biomass and protein with *Pleurotus sajor-caju* on 1% hemicelluloses fraction of corn stalk.

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (mg/L.h)
		(%)	(g/L)		
0	1,540	42,831	0,660		
34	2,170	48,760	1,058		
46	2,380	48,352	1,151		
58	2,950	49,125	1,449	31,584	13,614
70	3,340	48,141	1,608		
82	3,740	44,966	1,682		
95	3,200	45,613	1,460		
106	3,910	44,968	1,758		
118	3,740	43,459	1,625		

*FINAL PRODUCT: The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *Pleurotus sajor-caju*.

FIGURE 4-26: Production of mycelial biomass of *Pleurotus sajor-caju* on 1% substrate of the fractional components of corn stalk.

FIGURE 4-27: Crude protein content of mycelial biomass of *Pleurotus sajor-caju* on 1% substrate of the fractional components of corn stalk.

FIGURE 4-28: Per cent crude protein content of mycelial biomass of *Pleurotus sajor-caju* on 1% substrate of the fractional components of corn stalk.

Fig. 4-26

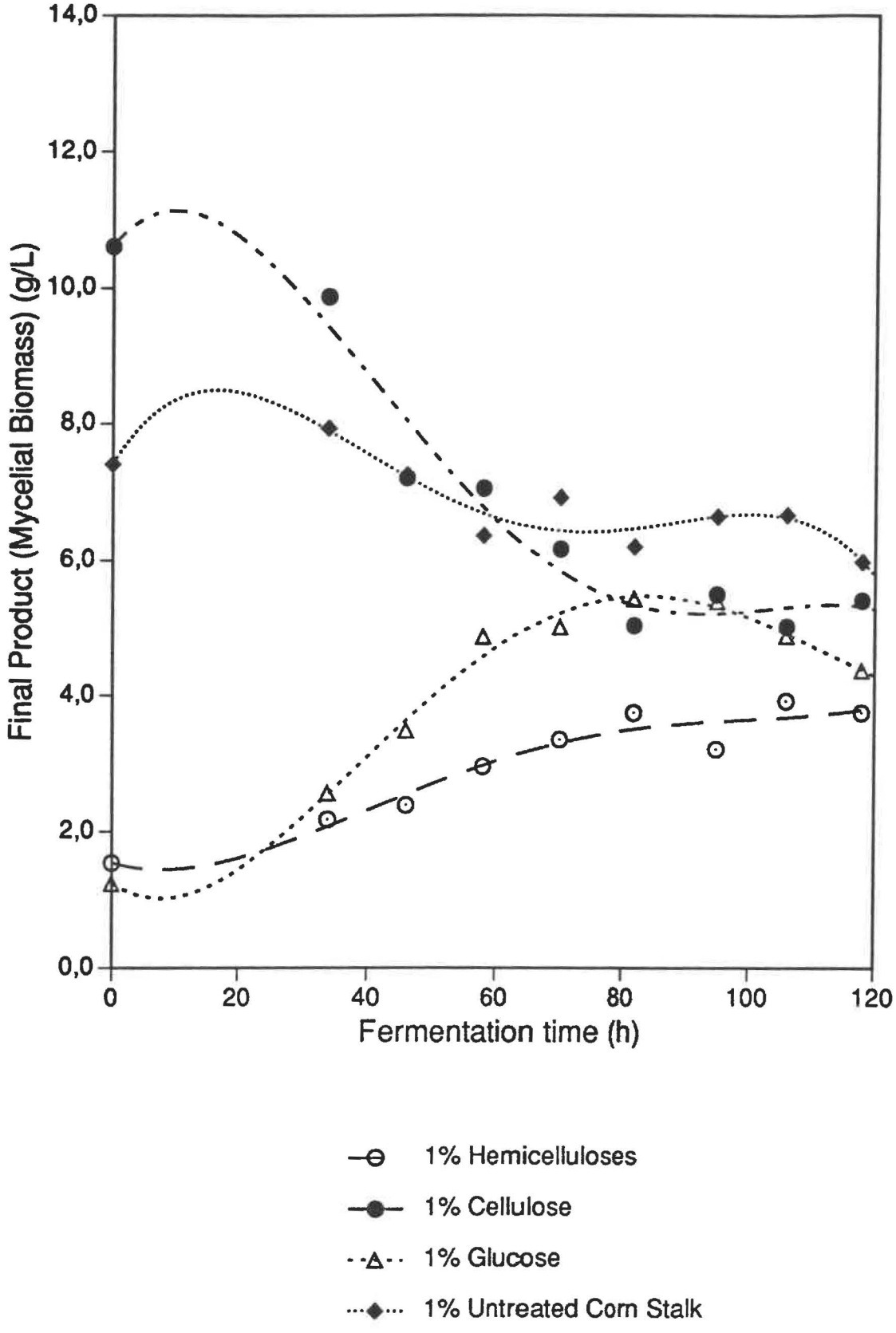
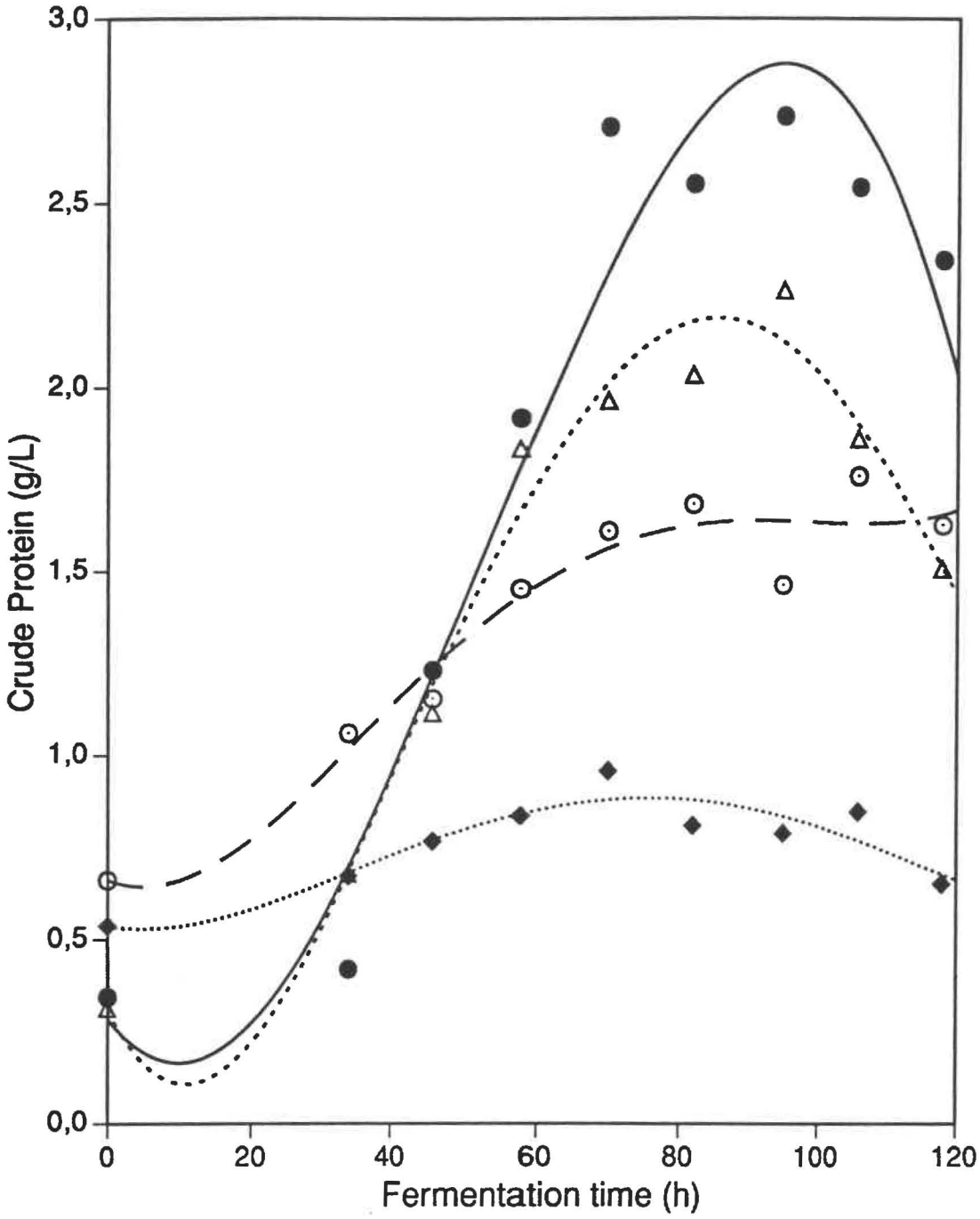

14,0
12,0
10,0
8,0
6,0
4,0
2,0
0,0
Final Product (Mycelial Biomass) (g/L)
0
20
40
60
80
100
120
Fermentation time (h)
1% Hemicelluloses
1% Cellulose
1% Glucose
1% Untreated Corn Stalk

Fig. 4-27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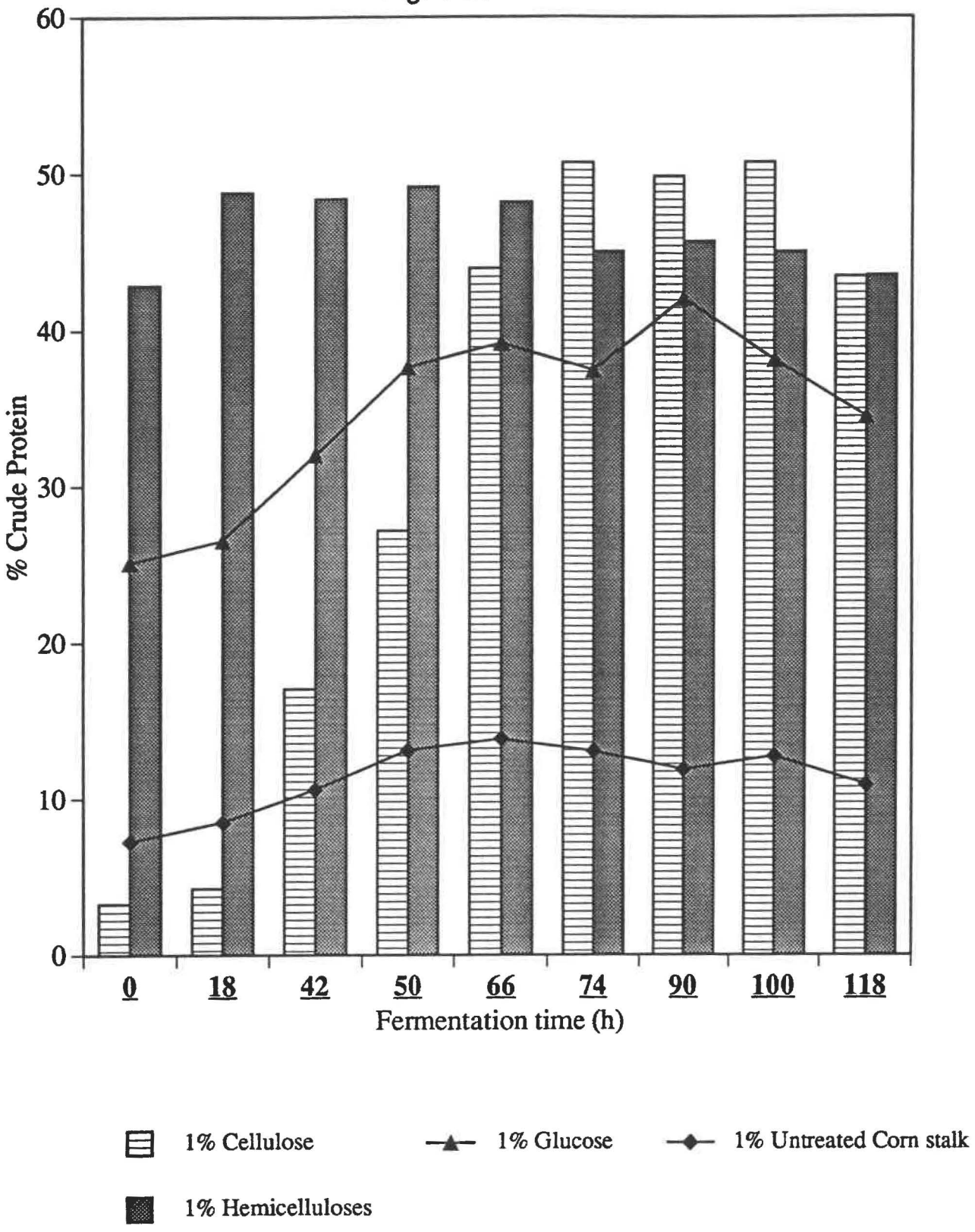
2,0
1,5
1,0
0,5
0,0
Crude Protein (g/L)
0
20
40
60
80
100
120
Fermentation time (h)
1% Hemicelluloses
1% Cellulose
1% Glucose
1% Untreated Corn Stalk

Fig. 4-28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50
40
30
20
10
0
% Crude Protein
0
18
42
50
66
74
90
100
118
Fermentation time (h)
1% Cellulose
1% Glucose
1% Untreated Corn stalk
1% Hemicelluloses

4.8. Production of mycelial biomass and protein in a scale-up fermentation of 10 Litres.

A one-stage scale up was attempted from 250 mL Erlenmeyer flasks to a 10-L fermentor in order to assess the possibility of large scale fermentation since the growth conditions like pH control, aeration and mass energy transfers in both systems do not always replicate themselves. One condition of irradiation treatment alone (1 MGy) and a combination treatment of irradiation and NaOH (I MGy and 0,5 g NaOH/g substrate) were selected for the assessment.

Tables 4-32 and 4-33 and Figures 4-29 and 4-30 represent the results from the 10-L fermentations. In Table 4-32, the optimum protein synthesized for single pretreatment of substrate with 1 MGy irradiation in the 10-L fermentor was slightly higher than that obtained from the same pretreated substrate in the 250 mL shake flasks experiments (section 4.3). Also, the efficiency of bioconversion and protein productivity were higher for the scale up fermentation. There was interaction between the irradiated and the combined irradiation and NaOH treatments ($p \leq 0,05$) (appendix 2h). This would suggest that the low NaOH concentration had no improvement in the protein synthesis in the 10-L fermentor. For instance, the protein content for the 10-L fermentation was 24% (1,6 g L^{-1}) while that of the 250 mL fermentation resulted in protein content of 16% (0,83 g protein L^{-1})(Table 4-10) . The efficiencies of conversion of substrate

into mycelial biomass and protein were 66% and 34% for the former and latter, respectively.

Similarly, when the results from the 10-L fermentation of substrate from the combined treatment of 1 MGy irradiation and 0,5% NaOH (Table 4-33) are compared with those obtained from substrate of same treatment in the 250 mL fermentation (Table 4-26), the protein contents were comparable. Though the efficiency of bioconversion was lower for the 10-L fermentation, it took a shorter time of fermentation to attain the same protein productivity.

It is relevant to note that comparable amounts of protein were produced in the scale up fermentation as was obtained from the 250 mL fermentation. This is encouraging if the end application of this process is to have a large scale production.

Table 4-32 : Production of mycelial biomass and protein with *Pleurotus sajor-caju* on 1% corn stalks irradiated with 1 MGy, in a 10-L fermentor.

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (g/L.h)
		(%)	(g/L)		
0	7,288	7,781	0,567		
24	6,324	12,558	0,794		
40	6,468	20,834	1,348		
49	6,464	24,109	1,558	66,088	0,020
64	5,268	26,753	1,409		
76	4,532	27,103	1,228		
89	4,300	27,187	1,169		
100	3,120	26,390	0,823		
114	2,192	24,012	0,526		

*FINAL PRODUCT: The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *Pleurotus sajor-caju*.

Table 4-33 : Production of mycelial biomass and protein with *Pleurotus sajor-caju* on 1% corn stalks treated with 1 MGy and NaOH (0,5%), in a 10-L fermentor.

TIME OF FERMENTATION (h)	DRY WT. OF FINAL PRODUCT* (MYCELIAL BIOMASS) (g/L)	PROTEIN CONTENT OF FINAL PRODUCT (C)		EFFICIENCY E = C-Co/So (%)	PRODUCTIVITY P = C-Co/t-to (g/L.h)
		(%)	(g/L)		
0	5,288	7,794	0,412		
24	5,768	11,368	0,656		
43	5,036	25,578	1,288		
50	3,928	27,684	1,087		
66	3,668	35,281	1,294	58,797	0,013
72	3,276	36,365	1,191		
92	2,844	34,819	0,990		
100	3,132	34,825	1,091		
116	2,812	33,600	0,945		
124	2,564	31,071	0,797		

*FINAL PRODUCT: The final product contains the newly synthesized mycelial biomass and the unutilized substrate. The proportion of mycelial biomass increases with the increase in the time of fermentation during the bioconversion of substrate into mycelial biomass with the growth of *Pleurotus sajor-caju.*

FIGURE 4-29: Production of mycelial biomass of *Pleurotus sajor-caju* on 1% substrate pretreated with 1 MGy ; and 1 MGy + 0,5% NaOH in a 10-L fermentor.

FIGURE 4-30: Crude protein content of mycelial biomass of *Pleurotus sajor-caju* on 1% substrate pretreated with 1 MGy ; and 1 MGy + 0,5% NaOH in a 10-L fermentor.

Fig. 4-29

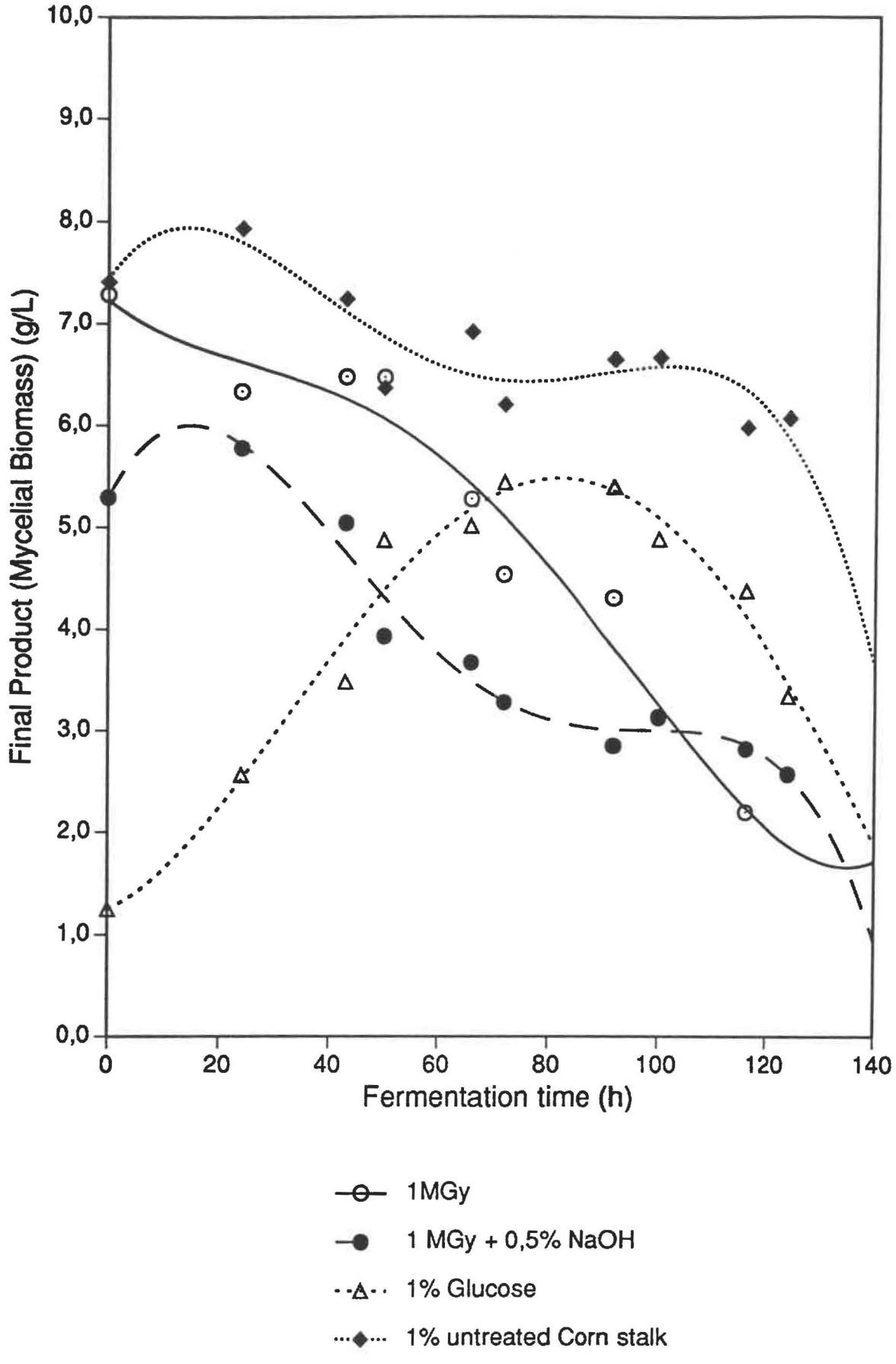
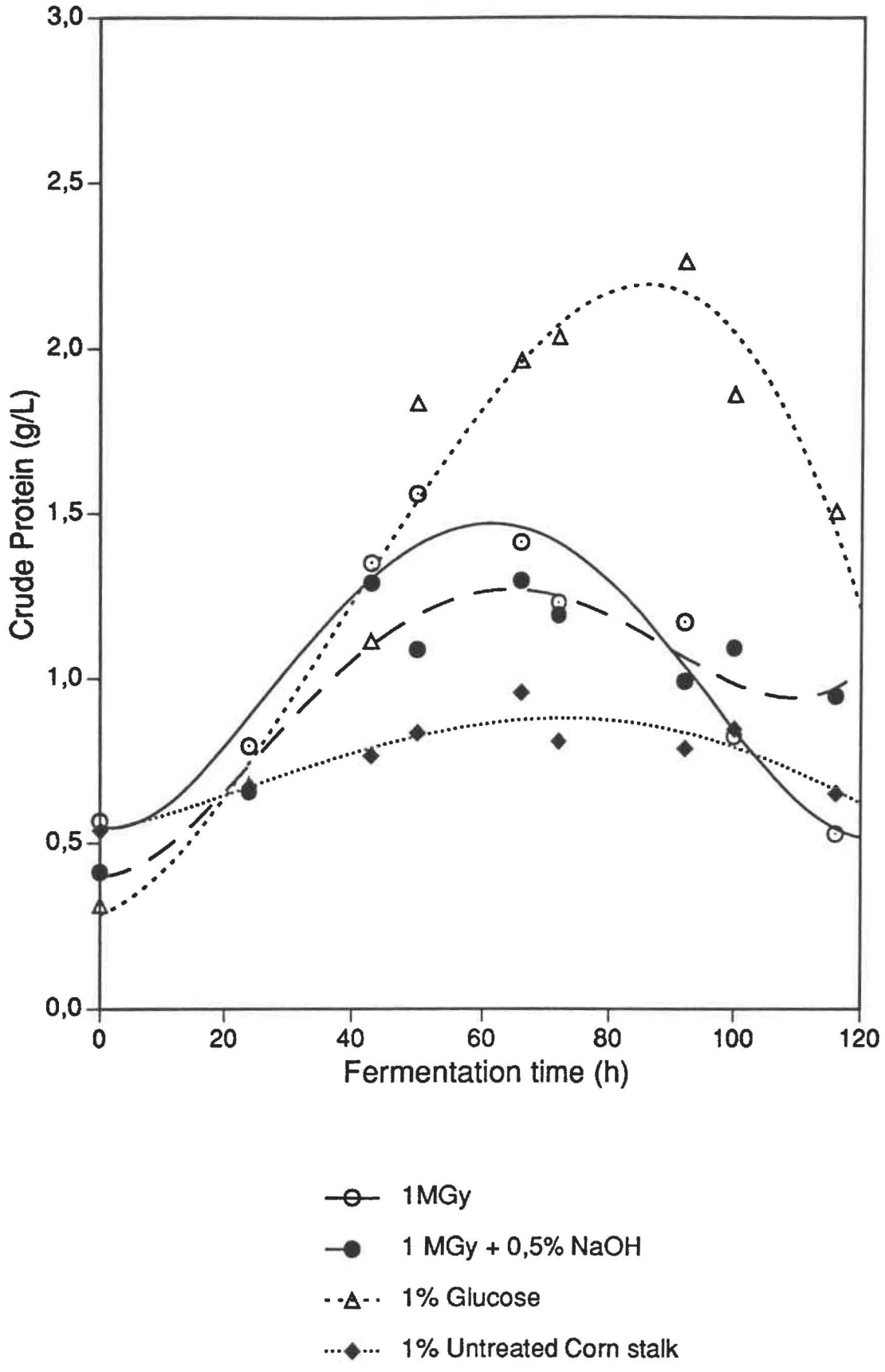

10,0
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
Final Product (Mycelial Biomass) (g/L)
0
20
40
60
80
100
120
140
Fermentation time (h)
1MGy
1 MGy + 0,5% NaOH
1% Glucose
1% untreated Corn stalk

Fig. 4-30


3,0
2,5
2,0
1,5
1,0
0,5
0,0
Crude Protein (g/L)
0
20
40
60
80
100
120
Fermentation time (h)
1MGy
1 MGy + 0,5% NaOH
1% Glucose
1% Untreated Corn stalk

4.9. Economic evaluation of protein productivity as a function of pretreatment cost.

Table 4-34 presents an assessment of the cost of protein production as a function of pretreatment cost. All other factors from cost of raw material handling to fermentation processing and harvesting are considered as constant and are not reflected in the cost of the final mycelial biomass product. This assessment was limited to a few pretreatments that gave high protein yields in order to give a sense of direction if these are considered for large scale production.

Common to all processes is the need for the highest productivity, least capital and running costs, high yield factor on the limiting substrate, near complete utilization of the substrate, ease of recovery and final work-up of the material by drying or formulation into final products (Solomons, 1983). The economics of fermentation processes and biomass production have been discussed by Litchifield (1977), Moo-Young (1977) and Solomons (1983). The total energy requirements for SCP production have been evaluated by Lewis (1976) and Datta (1981).

The cost of milling cellulose on a large scale is not well defined but estimates range from $53.00- $110.00 per metric ton of cellulose when reduced to less than 20 mesh size while the cost of 500 KGy of gamma irradiation is reported to be around $138.00- $160.00 per metric ton (Dunlap, 1975). The cost of 0,1- 0,15 g NaOH/g solid cellulosic material is between $20 and $30 i.e. $66-99 per ton of

cellulosic material (Dunlap 1975). Sterilization is about 4×10^6 Kcal/h for a plant processing 10 tons (dry)/h of lignocelluloses while power of 1 Kwh = 2500 Kcal (Datta, 1981). Based on these estimates, the selected pretreatments were assessed as presented in Table 4-34

The high cost of pretreatment attributed to the use of sodium hydroxide was largely contributed by the cost of sterilization. Lignocelluloses are normally subjected to 1 hour sterilization at 121^0C to eliminate all contaminating microorganisms before the fermentation medium is added. Where irradiation is used as a pretreatment , this step is eliminated since the irradiation doses used are sufficient to sterilize the lignocellulose substrate.

Assuming that minimal cost is incurred in the collection and transportation of lignocelluloses, it means that glucose may never be the substrate of choice for protein producton with *P. sajor-caju* as depicted in Table 4-34.

Since the protein synthesis levels from the effective pretreatments were comparable, it only becomes logical that in terms of economics of production, the choice of pretreatment would be the combination of 0,10 g NaOH/g substrate and 100 KGy irradiation.

As of August, 1993, the cost of soymeal, 48% protein, was $328,26 (Livestock Feed Bureau). When this was compared to the protein content of the combination pretreatment of 0,10 g NaOH/g substrate and 100 KGy irradiation (40% protein, $177, 50), the latter has a clear cost advantage even if additional cost from medium preparation

through fermentation to product storage are added. Moo-Young (1977) estimated the cost of supplies storage, medium preparation, inoculum preparation, fermentation, harvesting, drying and product storage of microfungi SCP from waste substrate to be \$99,90 ton^{-1}.

TABLE 4-34: Economic evaluation of protein productivity as a function of pretreatment cost.

PRETREATMENT per ton substrate	COST OF PRETREATMENT $ per ton	PROTEIN PRODUCTIVITY Kg Protein per ton per h	ECONOMIC PRODUCTIVITY Kg Protein per hour per $
0,10 g NaOH + sterilization	307,50	11,700	0,038
0,15 g NaOH + sterilization	340,50	15,470	0,045
0,10 g NaOH + 100 KGy	177,50	13,540	0,076
0,05 g NaOH + 500 KGy	263,50	14,150	0,054
0,10 g NaOH + 500 KGy	296,50	19,390	0,065
*Glucose	768,00	27,830	0,003

* The economic protein productivity of Glucose is based on the current cost of refined, commercial sugar (Redpath Ltd.)

Irradiation eliminates sterilization cost. This drastically reduces the cost of irradiation pretreatment for protein production.

5.0 CONCLUSION

The experimental data generated in this study provided information on the production of mycelial biomass of *P. sajor-caju* on treated and untreated corn stalk and glucose substrates from which the following conclusions have been drawn:

1. Single pretreatments of corn stalk with γ-irradiation require doses of at least 1 MGy or above for efficient protein synthesis by *Pleurotus sajor-caju.*

2. The NaOH pretreatments ($p \leq 0,05$) of 0,1 and 0,15 g/g substrate are superior to the highest irradiation dose of 1,7 MGy in the production of protein with *P. sajor-caju.*

3. A nitrogen content of 0,4 g L^{-1} was best for the production of mycelial biomass of *P. sajor-caju* on 1% glucose substrate in Mandel's medium.

4. Single pretreatments of corn stalk with 0,15 g NaOH/g substrate was the best individual treatment ($p \leq 0,05$) for protein synthesis of *P. sajor-caju.*

5. Combination treatments of NaOH and γ-irradiation reduced both the quantity of NaOH and doses of irradiation required to obtain optimum yields of protein.

6. Combination treatments ($p \leq 0,05$) of 500 KGy and 0,1 g NaOH/g substrate; 500 KGy and 0,05 g NaOH/g substrate; 1 MGy and 0,05 g NaOH/g substrate; 8% sodium sulphite plus 0,5% NaOH and

steam gave protein yields comparable to the best single pretreatment of 0,15 g NaOH/g corn stalk.

7. Corn stalk holocellulose could be as good as or better than glucose in the production of protein-rich mycelial biomass of *P. sajor-caju* when the above best selective pretreatment conditions were provided.

8. The cellulose and hemicelluloses fractions of corn stalk gave protein yields comparable to or better than glucose as substrates for protein-rich mycelial biomass of *P. sajor-caju.*

9. Protein yields from both the 250-mL shake flasks and 10-L fermentations were comparable, hence a good indicator for possible large scale production.

10. Cost of pretreatment assessment indicates a preference for the combination treatment of 0,1 g NaOH/g corn stalk and 100 KGy γ-irradiation on the basis of economic protein productivity (g protein synthesized $h^{-1}\$^{-1}$ of pretreatment.

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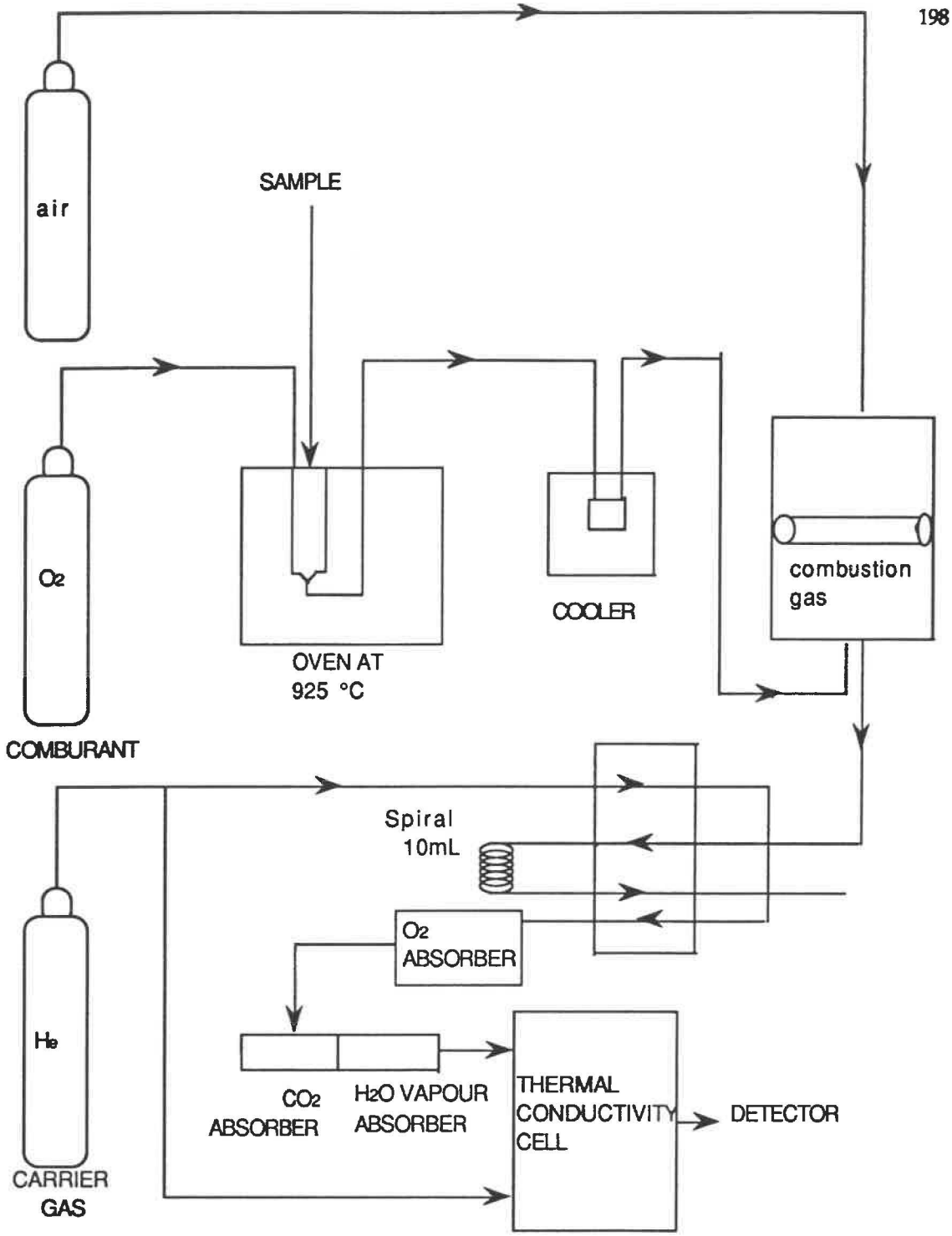

Appendix 1 NITROGEN ANALYSER

Appendix 2a: Covariance analysis of the effect of carbon: nitrogen ratio and fermentation time on the production of mycelial biomass protein

Protein content (%)

	C : N = 0	C : N = 0,05	C : N = 0,1	C : N = 0,125
C : N = 0,05	I	-	-	-
C : N = 0,1	I	I	-	-
C : N = 0,125	I	sd	sd	-
C : N = 0,15	I	I	sd	I

Protein content (g/L)

	C : N = 0	C : N = 0,05	C : N = 0,1	C : N = 0,125
C : N = 0,05	I	-	-	-
C : N = 0,1	I	sd	-	-
C : N = 0,125	I	I	sd	-
C : N = 0,15	I	sd	sd	I

Efficiency

	C : N = 0	C : N = 0,05	C : N = 0,1	C : N = 0,125
C : N = 0,05	I	-	-	-
C : N = 0,1	I	sd	-	-
C : N = 0,125	I	I	sd	-
C : N = 0,15	I	sd	sd	I

Productivity

	C : N = 0	C : N = 0,05	C : N = 0,1	C : N = 0,125
C : N = 0,05	I	-	-	-
C : N = 0,1	I	sd	-	-
C : N = 0,125	I	I	sd	-
C : N = 0,15	I	not sd	sd	I

sd = significant difference between the two treatments at $p \leq 0,05$ throughout the entire fermentation period.
Where there is significant difference between two treatments, the higher C:N ratio is superior as outlined in results
I = Interaction between the two treatments
Where interaction occurs, it means that no definite conclusion can be drawn since the superiority of one treatment over the other did not occur throughout the fermentation time. The detailed relationship between pretreatments and reasons supporting one treatment over another are explained in text.

Appendix 2b: Covariance analysis of the effect of irradiation and fermentation time on the production of mycelial biomass protein

Protein content (%)

	Untreated corn stalk	100 KGy	500 KGy	1 MGy
100 KGy	sd	-	-	-
500 KGy	sd	sd	-	-
1 MGy	sd	I	I	-
1,7 MGy	sd	sd	sd	sd

Protein content (g/L)

	Untreated corn stalk	100 KGy	500 KGy	1 MGy
100 KGy	sd	-	-	-
500 KGy	I	I	-	-
1 MGy	sd	sd	I	-
1,7 MGy	sd	sd	I	sd

Efficiency

	Untreated corn stalk	100 KGy	500 KGy	1 MGy
100 KGy	sd	-	-	-
500 KGy	sd	sd	-	-
1 MGy	sd	sd	I	-
1,7 MGy	sd	I	I	sd

Productivity

	Untreated corn stalk	100 KGy	500 KGy	1 MGy
100 KGy	I	-	-	-
500 KGy	sd	sd	-	-
1 MGy	sd	I	sd	-
1,7 MGy	I	I	I	sd

sd = significant difference between the two treatments at $p \leq 0,05$ throughout the entire fermentation period.
Where there is significant difference between two treatments, the higher radiation dose is superior as outlined in results
I = Interaction between the two treatments
Where interaction occurs, it means that no definite conclusion can be drawn since the superiority of one treatment over the other did not occur throughout the fermentation time. The detailed relationship between pretreatments and reasons supporting one treatment over another are explained in text.

Appendix 2c: Covariance analysis of the effect of NaOH treatment and fermentation time on the production of mycelial biomass protein

Protein content (%)

	Untreated corn stalk	NaOH, 0,025 g	NaOH, 0,05 g	NaOH, 0,1 g
NaOH, 0,025 g	sd	-	-	-
NaOH, 0,05 g	I	I	-	-
NaOH, 0,1 g	I	I	I	-
NaOH, 0,15 g	I	sd	sd	sd

Protein content (g/L)

	Untreated corn stalk	NaOH, 0,025 g	NaOH, 0,05 g	NaOH, 0,1 g
NaOH, 0,025 g	sd	-	-	-
NaOH, 0,05 g	I	I	-	-
NaOH, 0,1 g	I	I	sd	-
NaOH, 0,15 g	sd	sd	sd	sd

Efficiency

	Untreated corn stalk	NaOH, 0,025 g	NaOH, 0,05 g	NaOH, 0,1 g
NaOH, 0,025 g	sd	-	-	-
NaOH, 0,05 g	I	sd	-	-
NaOH, 0,1 g	I	I	I	-
NaOH, 0,15 g	I	I	sd	sd

Productivity

	Untreated corn stalk	NaOH, 0,025 g	NaOH, 0,05 g	NaOH, 0,1 g
NaOH, 0,025 g	sd	-	-	-
NaOH, 0,05 g	sd	sd	-	-
NaOH, 0,1 g	I	I	I	-
NaOH, 0,15 g	I	I	I	sd

sd = significant difference between the two treatments at $p \leq 0,05$ throughout the entire fermentation period.
Where there is significant difference between two treatments, the higher NaOH conc. is superior as outlined in results.
I = Interaction between the two treatments.
Where interaction occurs, it means that no definite conclusion can be drawn since the superiority of one treatment over the other did not occur throughout the fermentation time. The detailed relationship between pretreatments and reasons supporting one treatment over another are explained in text.

Appendix 2d: Covariance analysis of the effect of combination treatment (NaOH & irradiation) and fermentation time on the production of mycelial biomass protein

Protein content (%)

	Untreated corn stalk	0,025 g NaOH + 50 KGy	0,05 g NaOH + 50 KGy
0,025g NaOH + 50 KGy	I	-	-
0,05 g NaOH + 50 KGy	I	sd	-
0,1 g NaOH + 50 KGy	sd	I	I

Protein content (g/L)

	Untreated corn stalk	0,025 g NaOH + 50 KGy	0,05 g NaOH + 50 KGy
0,025 g NaOH + 50 KGy	I	-	-
0,05 g NaOH + 50 KGy	I	sd	-
0,1 g NaOH + 50 KGy	I	sd	sd

Efficiency

	Untreated corn stalk	0,025 g NaOH + 50 KGy	0,05 g NaOH + 50 KGy
0,025g NaOH + 50 KGy	I	-	-
0,05 g NaOH + 50 KGy	I	I	
0,1 g NaOH + 50 KGy	I	sd	I

Productivity

	Untreated corn stalk	0,025 g NaOH + 50 KGy	0,05 g NaOH + 50 KGy
0,025g NaOH + 50 KGy	sd	-	-
0,05 g NaOH + 50 KGy	I	I	-
0,1 g NaOH + 50 KGy	I	I	sd

sd = significant difference between the two treatments at $p \leq 0,05$ throughout the entire fermentation period.
Where there is significant difference between two treatments, the higher NaOH conc. is superior as outlined in results.
I = Interaction between the two treatments.
Where interaction occurs, it means that no definite conclusion can be drawn since the superiority of one treatment over the other did not occur throughout the fermentation time. The detailed relationship between pretreatments and reasons supporting one treatment over another are explained in text.

Appendix 2e: Covariance analysis of the effect of combination treatment (NaOH & irradiation) and fermentation time on the production of mycelial biomass protein

Protein content (%)

	Untreated corn stalk	0,025 g NaOH + 100 KGy	0,05 g NaOH + 100 KGy
0,025g NaOH + 100 KGy	sd	-	-
0,05 g NaOH + 100 KGy	sd	I	-
0,1 g NaOH + 100 KGy	I	I	I

Protein content (g/L)

	Untreated corn stalk	0,025 g NaOH + 100 KGy	0,05 g NaOH + 100 KGy
0,025 g NaOH + 100 KGy	sd	-	-
0,05 g NaOH + 100 KGy	sd	I	-
0,1 g NaOH + 100 KGy	I	I	I

Efficiency

	Untreated corn stalk	0,025 g NaOH + 100 KGy	0,05 g NaOH + 100 KGy
0,025g NaOH + 100 KGy	sd	-	-
0,05 g NaOH + 100 KGy	sd	sd	-
0,1 g NaOH + 100 KGy	I	I	I

Productivity

	Untreated corn stalk	0,025 g NaOH + 100 KGy	0,05 g NaOH + 100 KGy
0,025g NaOH + 100 KGy	sd	-	-
0,05 g NaOH + 100 KGy	sd	I	-
0,1 g NaOH + 100 KGy	I	I	I

sd = significant difference between the two treatments at $p \leq 0,05$ throughout the entire fermentation period.
Where there is significant difference between two treatments, the higher NaOH conc. is superior as outlined in results.
I = Interaction between the two treatments.
Where interaction occurs, it means that no definite conclusion can be drawn since the superiority of one treatment over the other did not occur throughout the fermentation time. The detailed relationship between pretreatments and reasons supporting one treatment over another are explained in text.

Appendix 2f: Covariance analysis of the effect of combination treatment (NaOH & irradiation) and fermentation time on the production of mycelial biomass protein

Protein content (%)

	Untreated corn stalk	0,025 g NaOH + 500 KGy	0,05 g NaOH + 500 KGy
0,025g NaOH + 500 KGy	sd	-	-
0,05 g NaOH + 500 KGy	sd	I	-
0,1 g NaOH + 500 KGy	sd	I	I

Protein content (g/L)

	Untreated corn stalk	0,025 g NaOH + 500 KGy	0,05 g NaOH + 500 KGy
0,025 g NaOH + 500 KGy	sd	-	-
0,05 g NaOH + 500 KGy	sd	sd	-
0,1 g NaOH + 500 KGy	I	I	I

Efficiency

	Untreated corn stalk	0,025 g NaOH + 500 KGy	0,05 g NaOH + 500 KGy
0,025g NaOH + 500 KGy	sd	-	-
0,05 g NaOH + 500 KGy	sd	sd	-
0,1 g NaOH + 500 KGy	I	I	I

Productivity

	Untreated corn stalk	0,025 g NaOH + 500 KGy	0,05 g NaOH + 500 KGy
0,025g NaOH + 500 KGy	sd	-	-
0,05 g NaOH + 500 KGy	sd	I	-
0,1 g NaOH + 500 KGy	I	I	I

sd = significant difference between the two treatments at $p \leq 0,05$ throughout the entire fermentation period.
Where there is significant difference between two treatments, the higher NaOH conc. is superior as outlined in results.
I = Interaction between the two treatments.
Where interaction occurs, it means that no definite conclusion can be drawn since the superiority of one treatment over the other did not occur throughout the fermentation time. The detailed relationship between pretreatments and reasons supporting one treatment over another are explained in text.

Appendix 2g: Covariance analysis of the effect of combination treatment (NaOH & irradiation) and fermentation time on the production of mycelial biomass protein

Protein content (%)

	Untreated corn stalk	0,01g NaOH + 1 MGy
0,01g NaOH + 1 MGy	I	-
0,05 g NaOH + 1 MGy	I	I

Protein content (g/L)

	Untreated corn stalk	0,01g NaOH + 1 MGy
0,01 g NaOH + 1 MGy	I	-
0,05 g NaOH + 500 KGy	I	I

Efficiency

	Untreated corn stalk	0,01g NaOH + 1 MGy
0,01g NaOH + 1 MGy	I	-
0,05 g NaOH + 1 MGy	I	I

Productivity

	Untreated corn stalk	0,01g NaOH + 1 MGy
0,01g NaOH + 1 MGy	I	-
0,05 g NaOH + 1 MGy	I	I

sd = significant difference between the two treatments at $p \leq 0,05$ throughout the entire fermentation period.
Where there is significant difference between two treatments, the higher NaOH conc. is superior as outlined in results.
I = Interaction between the two treatments.
Where interaction occurs, it means that no definite conclusion can be drawn since the superiority of one treatment over the other did not occur throughout the fermentation time. The detailed relationship between pretreatments and reasons supporting one treatment over another are explained in text.

Appendix 2h: Covariance analysis of the effect of combination treatment (NaOH & irradiation) and fermentation time on the production of mycelial biomass protein in a 10-L fermentor

Protein content (%)

	Untreated corn stalk	0,05g NaOH + 1 MGy
0,05g NaOH + 1 MGy	I	-
1 MGy	I	I

Protein content (g/L)

	Untreated corn stalk	0,05g NaOH + 1 MGy
0,05 g NaOH + 1 MGy	I	-
1 MGy	I	I

Efficiency

	Untreated corn stalk	0,05g NaOH + 1 MGy
0,05g NaOH + 1 MGy	I	-
1 MGy	I	I

Productivity

	Untreated corn stalk	0,01g NaOH + 1 MGy
0,01g NaOH + 1 MGy	I	-
1 MGy	I	sd

sd = significant difference between the two treatments at $p \leq 0,05$ throughout the entire fermentation period.
Where there is significant difference between two treatments, the NaOH conc. is superior as outlined in results.
I = Interaction between the two treatments.
Where interaction occurs, it means that no definite conclusion can be drawn since the superiority of one treatment over the other did not occur throughout the fermentation time. The detailed relationship between pretreatments and reasons supporting one treatment over another are explained in text.

Appendix 2j: Covariance analysis of the effect of steam and fermentation time on the production of mycelial biomass protein

Protein content (%)

	Untreated corn stalk	Steam	8% Sodium sulphite
Steam	sd	-	-
Steam + 8% Sodium sulphite	I	I	-
Steam + 8% Sodium sulphite + 0,5% NaOH	I	I	not sd

Protein content (g/L)

	Untreated corn stalk	Steam	8% Sodium sulphite
Steam	sd	-	-
Steam + 8% Sodium sulphite	I	I	-
Steam + 8% Sodium sulphite + 0,5% NaOH	I	sd	I

Efficiency

	Untreated corn stalk	Steam	8% Sodium sulphite
Steam	I	-	-
Steam + 8% Sodium sulphite	I	sd	-
Steam + 8% Sodium sulphite + 0,5% NaOH	I	I	sd

Productivity

	Untreated corn stalk	Steam	8% Sodium sulphite
Steam	I	-	-
Steam + 8% Sodium sulphite	I	sd	-
Steam + 8% Sodium sulphite + 0,5% NaOH	I	I	sd

sd = significant difference between the two treatments at $p \leq 0,05$ throughout the entire fermentation period.
Where there is significant difference between two treatments, the higher NaOH conc. is superior as outlined in results.
I = Interaction between the two treatments.
Where interaction occurs, it means that no definite conclusion can be drawn since the superiority of one treatment over the other did not occur throughout the fermentation time. The detailed relationship between pretreatments and reasons supporting one treatment over another are explained in text.

Appendix 2k: Covariance analysis of the effect of fractionation (cellulose and hemicelluloses) and fermentation time on the production of mycelial biomass protein.

Protein content (%)

	Untreated corn stalk	Hemicellulose
Hemicellulose	sd	-
Cellulose	I	I

Protein content (g/L)

	Untreated corn stalk	Hemicellulose
Hemicellulose	I	-
Cellulose	I	I

Efficiency

	Untreated corn stalk	Hemicellulose
Hemicellulose	I	-
Cellulose	I	I

Productivity

	Untreated corn stalk	Hemicellulose
Hemicellulose	I	-
Cellulose	I	sd

sd = significant difference between the two treatments at $p \leq 0,05$ throughout the entire fermentation period.
Where there is significant difference between two treatments, the fractionation is superior as outlined in results.
I = Interaction between the two treatments.
Where interaction occurs, it means that no definite conclusion can be drawn since the superiority of one treatment over the other did not occur throughout the fermentation time. The detailed relationship between pretreatments and reasons supporting one treatment over another are explained in text.

Appendix 2m: Covariance analysis of the best pretreatments and glucose with fermentation time on the production of mycelial biomass protein.

Protein content (%)

	1,7 MGy	0,15 g NaOH	0,1g NaOH + 50 KGy	0,1g NaOH + 100 KGy	0,1g NaOH + 500 KGy	Glucose
0,15 g NaOH	sd	–	–	–	–	–
0,1g NaOH + 50 KGy	sd	I	–	–	–	–
0,1g NaOH + 100 KGy	I	sd	I	–	–	–
0,1g NaOH + 500 KGy	I	sd	I	sd	–	–
Steam + 8% Sodium sulphite + 0,5% NaOH	I	not sd	I	sd	sd	I
Glucose	I	sd	I	I	I	–

Protein content (g/L)

	1,7 MGy	0,15 g NaOH	0,1g NaOH + 50 KGy	0,1g NaOH + 100 KGy	0,1g NaOH + 500 KGy	Glucose
0,15 g NaOH	sd	–	–	–	–	–
0,1g NaOH + 50 KGy	I	I	–	–	–	–
0,1g NaOH + 100 KGy	I	I	I	–	–	–
0,1g NaOH + 500 KGy	I	sd	I	I	–	–
Steam + 8% Sodium sulphite + 0,5% NaOH	I	sd	I	sd	I	I
Glucose	I	I	I	I	I	–

Appendix 2m: Covariance analysis of the best pretreatments and glucose with fermentation time on the production of mycelial biomass protein.

Efficiency

	1,7 MGy	0,15 g NaOH	0,1g NaOH + 50 KGy	0,1g NaOH + 100 KGy	0,1g NaOH + 500 KGy	Glucose
0,15 g NaOH	I		–	–	–	–
0,1g NaOH + 50 KGy	I	I		–	–	–
0,1g NaOH + 100 KGy	I	not sd	I		–	–
0,1g NaOH + 500 KGy	I	I	I	I		–
Steam + 8% Sodium sulphite + 0,5% NaOH	I	sd	I	I	I	I
Glucose	I	not sd	I	I	I	

Productivity

	1,7 MGy	0,15 g NaOH	0,1g NaOH + 50 KGy	0,1g NaOH + 100 KGy	0,1g NaOH + 500 KGy	Glucose
0,15 g NaOH	I		–	–	–	–
0,1g NaOH + 50 KGy	I	I		–	–	–
0,1g NaOH + 100 KGy	sd	I	I		–	–
0,1g NaOH + 500 KGy	I	I	I	sd		–
Steam + 8% Sodium sulphite + 0,5% NaOH	I	sd	I	I	I	I
Glucose	sd	sd	I	not sd	I	

sd = significant difference between the two treatments at $p \leq 0,05$ throughout the entire fermentation period.
Where there is significant difference between two treatments, the supporting evidence is outlined in results section.
I = Interaction between the two treatments.
Where interaction occurs, it means that no definite conclusion can be drawn since the superiority of one treatment over the other did not occur throughout the fermentation time. The detailed relationship between pretreatments and reasons supporting one treatment over another are explained in text.

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